Acetylpyrene-labelled 7-methylguanine nucleotides: unusual fluorescence properties and application to Decapping Scavenger activity monitoring

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## I. Tables

**Table 1.** Spectroscopic characterization of compounds 1–3

<table>
<thead>
<tr>
<th></th>
<th>1a</th>
<th>1b</th>
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<td><strong>Absorption $\lambda_{\text{max}}$ (nm)</strong></td>
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<tr>
<td><strong>$\varepsilon_{\text{max}} (M^{-1}cm^{-1})$</strong>[a]</td>
<td>12680 ± 53</td>
<td>12630 ± 43</td>
<td>12560 ± 93</td>
<td>-</td>
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<td>12330 ± 47</td>
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<td>13500 ± 100</td>
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<td><strong>$\tau, \langle \tau \rangle$ (ns)</strong>[b]</td>
<td>0.15 (79)</td>
<td>0.38 (21)</td>
<td>4.29 (&lt;1)</td>
<td>0.15 (&lt;1)</td>
<td>0.10 (25)</td>
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<td>0.41 (69)</td>
<td>0.72 (6)</td>
<td>&lt;0.20&gt;</td>
<td>&lt;0.35&gt;</td>
<td>0.42 (11)</td>
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<td>0.968</td>
<td>0.944</td>
<td>4.29 (11)</td>
<td>0.968</td>
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[a] $\varepsilon_{\text{max}}$ values were determined in different buffers: 0.1 M phosphate buffer pH 6.0 (black) and pH 7.0 (red) and 50 mM Tris buffer pH 7.6 (blue) at 30°C for pyrene absorption maximum (369 nm for 1a, 1b and 2b and 359 nm for 3).

[b] $\tau$ values were determined in 0.1 M acetate buffer pH 5.0 at 30°C (1μM, $\langle \tau \rangle$ amplitude weighted average lifetime) for excitation and emission wavelength 375 nm and 470 nm, respectively. Fractional amplitudes (in %) are shown in brackets.
Table 2. Selected $^1$H NMR data for 1b, m$^7$GTP and m$^7$GpppG at 0.2 mM concentration.

<table>
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<tr>
<th>Compound</th>
<th>Temperature (°C)</th>
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* overlapped with HDO
Fig. S1. Synthesis of thio-substituted nucleotide analogs. (A) Synthesis of nucleotide analogs containing a phosphorothioate group at the terminal position of oligophosphate chain;¹ (B) Synthesis of a nucleotide analog containing the methylene group at the α,β position and the phosphorothioate group at the γ position of oligophosphate chain;

¹ Experimental details are provided in the Supporting Information.
Fig. S2. Fluorescence intensity of compounds 1–3 as a function of concentration. Measurements were carried out in 50 mM Tris buffer pH 7.6 for excitation at 282 nm (left column) or 369 nm (right column) for: 1a (blue), 1b (red), 2b (pink) and 3 (black). A and B represent the data before, whereas C and D after inner filter effect (IFE) correction. IFE corrections were carried out as described previously.²
Fig. S3. Temperature effects in emission spectra of compounds 1b, 2b and 3. Fluorescence emission spectra (excitation at 369 nm) were measured in 50 mM Tris pH 7.6 buffer at temperatures 15–70°C for 14.5 μM 1b (A), 3 (B) and 2b (C). (D) Fluorescence intensities at different temperatures for each compound normalized to the fluorescence at 15 °C: 1b (black), 2b (red) and 3 (blue).
Fig. S4. Influence of pH on spectroscopic properties of AcPy-labelled nucleotides. (A) Absorption spectra of 18 μM 1b measured in 0.1 M KH₂PO₄ adjusted to the desired pH value with aqueous KOH; (B) protonation states of guanosine; (C) fluorescence spectra of 14.5 μM 2b in 0.1 M NaH₂PO₄ solutions (ex. 369 nm); (D) dependence of fluorescence at 471 nm (ex. 369 nm) of 14.5 μM 2b on buffer pH, in 0.1 M H₃PO₄ or KH₂PO₄ solutions adjusted to the desired pH values with aqueous KOH.
Fig. S5 Titration of 3 with m^7GMP and GMP monitored by absorption and emission spectroscopy. Absorption spectra of 2.4 μM 3 measured upon titration with (A) m^7GMP in acetate buffer pH 5.0, (B) m^7GMP in 50 mM Tris buffer pH 7.6, (C) GMP in acetate buffer pH 5.0, (D) GMP in 50 mM Tris buffer pH 7.6. Fluorescence spectra of 2.4 μM 3 measured upon titration with (E) GMP in phosphate buffer pH 5.0, (F) m^7GMP in phosphate buffer pH 5.0.
Fig. S6 DcpS catalyzed hydrolysis of 1b monitored by absorption and emission spectroscopy.

Hydrolysis of 19 μM 1b catalyzed by 10 nM DcpS enzyme monitored by UV-VIS. (A) Reaction progress monitored in 1 min intervals; (B) Spectra of the reaction mixture before (red) and after (black) enzymatic cleavage; (C), (D), and (E) Hydrolysis reaction progress of 16 μM 1b catalyzed by 10 nM DcpS enzyme monitored by emission changes with excitation wavelength 282 nm, 369 nm or 374 nm, respectively; (F) Fluorescence intensity changes at 471 nm for excitation at 369 nm.
Fig. S7. Monitoring DcpS activity in the presence of various inhibitors using 1b as an activity-based probe. Compound 1b at 5 μM was incubated with DcpS enzyme in the presence of half-log dilution series of 8 different inhibitors. The reactions were carried out in a 96-well plate and fluorescence read-outs (ex. 369 nm em. 471 nm) were taken at 1.5 min intervals using a microplate reader.
(A) Depicts reaction progress with increasing concentrations of m⁷GDP (inhibitor) and (B) shows reaction progress in the presence of 15.8 μM of different inhibitors. Control is a probe of 5 μM 1b with denaturated DcpS enzyme.
I. Experimental procedures

1. General information

The reactions involving microwave irradiation were conducted in heavy-walled glass 10 ml pressurized vials and CE ’s proprietary “snap-on” caps. The microwave heating was performed in a CEM Discover single-mode microwave cavity, using dynamic power mode with maximum power of 10 W and at 45 ± 1 °C and at 2450 Hz. The reaction mixtures were stirred with a magnetic stir bar during the irradiation. The temperature, pressure and power were monitored during the course of reactions using the provided software (standard infrared temperature sensor).

Synthesized nucleotides were purified by ion-exchange chromatography on DEAE-Sephadex A-25 (HCO₃⁻ form) column. A column was loaded with reaction mixture and washed through with excess of water to remove metal (II) salt/EDTA complex. Then, the nucleotides were eluted using a linear gradient of triethylammonium bicarbonate (TEAB) in deionized water. After evaporation under reduced pressure with repeated additions of ethanol to decompose TEAB, compounds were isolated as triethylammonium (TEA) salts. Yields were calculated on the basis of either sample weight or (preferably) optical milliunits (opt.mu) of the product. Optical unit measurements were performed in 0.1 M phosphate buffer (pH 7 or pH 6 for m⁷G nucleotides) at 260 nm.

Analytical HPLC was performed on Agilent Tech. Series 1200 using (RP)Supelcosil LC-18-T HPLC column (4.6 x 250 mm, flow rate 1.3 mL/min) with a linear gradient 0–50% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 7.5 min or 0–25% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 15 min, UV-detection at 260 nm and fluorescence detection (excitation at 280 nm and detection at 337 nm or 450 nm). Semi-preparative HPLC was performed on the same apparatus equipped with Discovery RP Amide C-16 HPLC column (25cm x 21.2 mm, 5μm, flow rate 5.0 mL/min) with linear gradients 0-100 % of acetonitrile in 0.05 M ammonium acetate buffer (pH 5.9) in 120 min, UV-detection at 260 nm and fluorescence detection (excitation at 280 nm and detection at 450 nm).

The structure and homogeneity of each final product was confirmed by RP HPLC, high resolution mass spectrometry HRMS (ESI-) and ¹H NMR and ³¹P NMR spectroscopy. Intermediate products were characterized by low resolution MS (ESI-) or NMR. Mass spectra were recorded on Thermo Scientific LTQ Orbitrap Velos (high resolution) and AB Sciex API 3200 (low resolution) spectrometers. Routine ¹H NMR and ³¹P NMR spectra were run in pure D₂O at 25 °C and nucleotide concentration of 1–5 mM on a Varian UNITYplus spectrometer at 399.94 MHz and 161.9 MHz, respectively, except for compound 3, the spectrum of which was recorded in deuterated DMSO-d₆. Routine two dimensional NMR spectra, gradient TOCSY, COSY, NOESY, ROESY, and ¹H,¹³C-HSQC, of compound 1b (at ~10 mM) were run in ²H₂O on a Varian UNITYplus spectrometer at 500.60 MHz for ¹H and 125.88 MHz for ¹³C, at 25°C. ¹H NMR spectra of compounds 1b, m⁷GpppG and m⁷GTP at various temperatures, from 2 °C to 52 °C, at concentration of 0.2 mM in 0.05 M phosphate buffer in ²H₂O, pD 5.6, were recorded on a Bruker AVANCE III spectrometer at 500.24 MHz. The two dimensional
gradient COSY spectrum of 1b at 1 mM concentration was run in 0.05 M phosphate buffer in D2O, pD 5.6 on a Bruker AVANCE III spectrometer at 500.24 MHz. The 1H chemical shifts were determined relative to internal standard sodium 3-trimethylsilyl-[2,2,3,3-2H4]-propionate (TSP), and the 31P chemical shifts relative to external standard 20% 2H3PO4.

2. Chemical synthesis

2.1. Starting materials and reagents

Solvents and chemical reagents were purchased from Sigma-Aldrich and used without any pre-treatment unless otherwise stated. 5’ GMP disodium salt and 1-(bromoacetyl)pyrene were purchased from Sigma-Aldrich. Triethylammonium thiophosphate and nucleotide analogues containing phosphorothioate moiety at the terminal position of the phosphate chain (m7GDPβS-pyrene, m7GTPαS-pyrene, GDPβS-pyrene and GTPβS-pyrene) were synthesized as described previously. S-(β-cyanoethyl)thiophosphate was also synthesized using published formula. m7GpCH2p was synthesized as described previously.

Putative DcpS inhibitors used for IC50 parameters determination by fluorescence method were synthesized in our laboratory: GpCH2p, m7GMP, m7GDP, m7GpCH2p, m7GpNHp, m7GDPαBH3 D1 and D2, m7GpCH2ppG.

2.2. m7GpCH2ppS

Triethylammonium salt of m7GpCH2p (85 mg, 0.130 mmol) was mixed with S-(β-cyanoethyl)thiophosphate (87 mg, 0.390 mmol) and MgCl2 (99 mg, 1.04 mmol) in DMF (1.3 ml). After 31 h deprotection of sulphur atom was carried out by adding to the reaction mixture DBU (20 % of the reaction volume) and DTT (20 mg for each 100 μl of DBU) and heating under microwaves for 40 min. The reaction was quenched by addition of 10 volumes of 1% aqueous acetic acid and washed repeatedly with ethyl acetate to remove DBU. Then ion-exchange chromatography was performed. Obtained eluate was evaporated under reduced pressure and washed with 96 %, 99.8 % ethanol and acetonitrile. The collected eluate was lyophilized repeatedly to afford 13 mg (0.015 mmol). Yield: 12 %.
2.3. m7GDP β S-AcPy (1a)

Triethylammonium salt of m7GDP β S (144 mg, 0.214 mmol) was mixed with 1-(bromoacetyl)pyrene (198 mg, 0.613 mmol) in DMSO (2 ml). The reaction was carried out at room temperature for 50 min and stopped by adding 20 ml of water. The precipitate containing unreacted 1-(bromoacetyl)pyrene was removed by centrifugation. The reaction product was purified using semi-preparative RP HPLC. The collected eluate was lyophilized repeatedly to afford 60 mg (0.084 mmol) of 1a as a yellow solid. Yield: 39 %.

HRMS (-)ESI m/z found: 714.0826, calc. for C29H26N5O11P2S- : 714.0830; 1H NMR (400 MHz, D2O) δ = 8.03 (m, 3H), 7.89 (m, 3H), 7.82 (d, 2H, J = 12.0 Hz), 7.57 (m, 3H), 4.66 (s, 1H), 4.49 (m, 1H), 4.36 (m, 2H), 4.12 (m, 1H), 4.03, (m, 1H), 3.92 (t, 1H, J = 4.0 Hz), 3.63 (m, 1H), 3.12 (s, 3H); 31P NMR (162 MHz, D2O) δ = 7.22 (dt, 1P, J1 = 12.15 Hz, J2 = 28.08 Hz), -12.07 (d, 1P, J = 28.08 Hz).

2.4. m7GTP γ S-AcPy (1b)

Triethylammonium salt of m7GTP γ S (38 mg, 0.045 mmol) was mixed with 1-(bromoacetyl)pyrene (43 mg, 0.133 mmol) in DMSO (3 ml). The reaction was carried out at room temperature for 50 min and stopped by adding 30 ml of water. The precipitate containing unreacted 1-(bromoacetyl)pyrene was removed by centrifugation. The reaction product was purified using semi-preparative RP HPLC. The collected eluate was lyophilized repeatedly to afford 9.4 mg (0.012 mmol) of 1b as a yellow solid. Yield: 27 %.

HRMS (-)ESI m/z found: 794.0488, calc. for C29H27N5O14P3S- : 794.0494; 1H NMR (400 MHz, D2O) δ = 8.36 (d, 1H, J = 5.0 Hz), 8.17 (d, 1H, J = 10.0 Hz), 8.06 (m, 4H), 7.94 (d, 1H, J = 5 Hz), 7.81 (d, 1H, J = 10.0 Hz), 7.68 (d, 1H, J = 5.0 Hz), 7.67 (d, 1H, J = 5.0 Hz), 4.84 (s, 1H), 4.72 (m, 1H), 4.46 (dd, 1H, J1 = 3.8 Hz, J2 = 7.6 Hz), 4.20 (d, 1H, J = 7.6 Hz), 4.12 (dd, 1H, J1 = 3.8 Hz, J2 = 7.6 Hz), 3.98 (t, 1H, J = 5.0 Hz), 3.73 (m, 1H), 3.24 (s, 3H); 31P NMR (162 MHz, D2O) δ = 7.58 (d, 1P, J = 24.30 Hz), -11.56 (d, 1P, J = 17.82 Hz), -23.25 (m, 1P).
2.5. \( m^7 \text{GpCH}_{2} \text{ppS-AcPy (1c)} \)

Triethylammonium salt of \( m^7 \text{GTP}_{\alpha,\beta} \text{CH}_{2} \gamma \text{S} \) (13 mg, 0.015 mmol) was mixed with 1-(bromoacetyl)pyrene (14 mg, 0.043 mmol) in DMSO (1 ml). Reaction was carried out at room temperature and stopped after 60 minutes by adding 10 ml of water. The precipitate containing unreacted 1-(bromoacetyl)pyrene was removed by centrifugation. The reaction product was purified using semi-preparative RP HPLC. The collected eluate was lyophilized repeatedly to afford 2 mg (0.002 mmol) of 1c as a yellow solid. Yield: 16%.

HRMS (\(-\))ESI m/z found: 792.0712, calc. for \( C_{30}H_{29}N_{5}O_{13}P_{3}S^- \): 792.0701; \(^1\)H NMR (500 MHz, \( D_{2}O \)) \( \delta = 8.34 \text{ (s, 1H), 8.28 \text{ (d, 1H, } J = 8.0 \text{ Hz), 8.12 \text{ (d, 1H, } J = 4.0 \text{ Hz), 8.09 \text{ (d, 1H, } J = 8.0 \text{ Hz), 8.04 \text{ (d, 1H, } J = 4.0 \text{ Hz), 7.99 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.93 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.78 \text{ (d, 1H, } J = 12.0 \text{ Hz), 7.74 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.69 \text{ (d, 1H, } J = 8.0 \text{ Hz), 4.91 \text{ (s, 1H), 4.34 \text{ (m, 1H), 4.14 \text{ (m, 1H), 4.08 \text{ (m, 1H), 3.97 \text{ (t, 1H, } J = 4.0 \text{ Hz), 3.76 \text{ (t, 1H, } J = 4.0 \text{ Hz), 3.18 \text{ (s, 3H), 2.42 \text{ (m, 2H); } ^{31}P \text{ NMR (162 MHz, } D_{2}O \)) } \delta = 16.96 \text{ (m, 1P), 7.54 \text{ (m, 2P).} } \)

2.6. \( \text{GDP}_{\beta}S\text{-AcPy (2a)} \)

Triethylammonium salt of \( \text{GDP}_{\beta}S \) (70 mg, 0.107 mmol) was mixed with 1-(bromoacetyl)pyrene (104 mg, 0.321 mmol) in DMSO (3 ml). The reaction was carried out at room temperature for 50 minutes and stopped by adding 30 ml of water. The precipitated unreacted 1-(bromoacetyl)pyrene was removed by centrifugation. The reaction product was purified using semi-preparative RP HPLC. The eluate was lyophilized repeatedly to afford 15.2 mg (0.022 mmol) of 2a as a yellow solid. Yield: 21%.

HRMS (-)ESI m/z found: 700.0658, calc. for \( C_{28}H_{24}N_{5}O_{11}P_{2}S^- \): 700.0674; \(^1\)H NMR (400 MHz, \( D_{2}O \)) \( \delta = 8.12 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.99 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.92 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.87 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.79 \text{ (d, 2H, } J = 8.0 \text{ Hz), 7.64 \text{ (d, 1H, } J = 8.0 \text{ Hz), 7.52 \text{ (d, 2H, } J = 8.0 \text{ Hz), 7.46 \text{ (d, 1H, } J = 8.0 \text{ Hz), 4.77 \text{ (s, 1H), 4.49 \text{ (m, 2H), 4.35 \text{ (d, 1H, } J = 8.0 \text{ Hz), 4.01 \text{ (t, 1H, } J = 6.0 \text{ Hz), 3.71 \text{ (m, 1H); } ^{31}P \text{ NMR (162 MHz, } D_{2}O \)) } \delta = 7.17 \text{ (d, 1P, } J = 29.16 \text{ Hz), -11.85 \text{ (d, 1P, } J = 29.16 \text{ Hz).} } \)

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2.7. GTP\textsubscript{γ}S-AcPy (2b)

Triethylammonium salt of GTP\textsubscript{γ}S (50 mg, 0.060 mmol) was mixed with 1-(bromoacetyl)pyrene (78 mg, 0.243 mmol) in DMSO (3 ml). Reaction was carried out at room temperature and stopped after 60 minutes by adding 30 ml of water. The precipitate containing unreacted 1-(bromoacetyl)pyrene was removed by centrifugation. The reaction product was purified using semi-preparative RP HPLC. The collected eluate was lyophilized repeatedly to afford 12.3 mg (0.015 mmol) of 2b as a yellow solid. HPLC purification yield: 81%; Reaction yield: 25%.

HRMS (−)ESI m/z found: 780.0332, calc. for C\textsubscript{28}H\textsubscript{24}N\textsubscript{5}O\textsubscript{14}P\textsubscript{3}S: 780.0337; \textsuperscript{1}H NMR (400 MHz, D\textsubscript{2}O) δ = 8.32 (d, 1H, \textit{J} = 8 Hz), 8.07 (d, 1H, \textit{J} = 8.0 Hz), 8.01 (d, 1H, \textit{J} = 8.0 Hz), 7.94 (m, 2H), 7.86 (d, 1H, \textit{J} = 12.0 Hz), 7.76 (d, 1H, \textit{J} = 12.0 Hz), 7.56 (m, 2H), 7.43 (s, 1H), 4.81 (d, 1H, \textit{J} = 8.0 Hz), 4.65 (d, 2H, \textit{J} = 8.0 Hz), 4.33 (m, 2H), 4.11 (m, 2H), 4.04 (t, 1H, \textit{J} = 4.0 Hz), 3.73 (t, 1H, \textit{J} = 4.0 Hz); \textsuperscript{31}P NMR (162 MHz, D\textsubscript{2}O) δ = 7.90 (d, 1P, \textit{J} = 26.46 Hz), -11.20 (d, 1P, \textit{J} = 18.36 Hz), -23.34 (dd, 1P, \textit{J}_1 = 26.46 Hz, \textit{J}_2 = 18.36 Hz).

2.8. PS-AcPy (3)

Triethylammon thiophosphate (54 mg, 0.242 mmol) was mixed with 1-(bromoacetyl)pyrene (88 mg, 0.270 mmol) and triethylamine (37.8 μl, 0.242 mmol, 1 eq.) in DMSO (3 ml). The reaction was carried out at room temperature for 40 min and stopped by adding 30 ml of water. The precipitate containing unreacted 1-(bromoacetyl)pyrene was removed by centrifugation. The reaction product was purified using semi-preparative RP HPLC. Then the eluate was lyophilized repeatedly to afford 40 mg (0.108 mmol) of 3 as a yellow solid. Yield: 44%.

HRMS (−)ESI m/z found: 355.0202, calc. for C\textsubscript{18}H\textsubscript{12}O\textsubscript{4}PS: 355.0199; \textsuperscript{1}H NMR (400 MHz, D\textsubscript{2}O) δ = 8.78 (d, 1H, \textit{J} = 12.0 Hz), 8.67 (d, 1H, \textit{J} = 8.0 Hz), 8.39 – 8.27 (m, 5H), 8.21 (d, 1H, \textit{J} = 12.0 Hz), 8.14 (d, 1H, \textit{J} = 8.0 Hz), 4.28 (d, 3H, 12.0 Hz); \textsuperscript{31}P NMR (162 MHz, D\textsubscript{2}O) δ = 10.45 (s, 1P).
3. UV-VIS and fluorescence measurements

General information

Absorption spectra, unless otherwise stated, were measured using Cary 100 UV-VIS (Agilent) with Dual Cell Peltier holder for temperature controlling. Titration experiments of 3 with m7GMP and GMP were performed on Shimadzu UV-1800 with CPS-100 holder for temperature controlling.

Emission and excitation spectra were measured on spectrofluorimeter Cary Eclipse (Agilent) equipped with xenon lamp under thermostated conditions using a quartz cuvette 10x4 mm. Before each measurement the buffer was degassed and for some experiments involving 3, which was particularly sensitive to oxygen presence, additionally rinsed by argon.

Basic measurements

Absorption spectra at different compound concentrations (Fig. S2) were measured in 50 mM Tris buffer (50 mM Tris, 200 mM KCl, 0.5 mM EDTA, adjusted to pH 7.6 with HCl) at 30 °C in 10x2 mm quartz cuvette (optical path length 10 mm).

Emission spectra were recorded for excitation 369 nm in 50 mM Tris buffer at 30 °C in 10x4 mm quartz cuvette (Fig. 1) or 10x10 mm quartz cuvette (Fig. S2).

Excitation spectra were recorded for emission at 471 nm and 385 nm in 50 mM Tris buffer at 30 °C in 10x4 mm quartz cuvette.

pH dependence measurements

pH dependence experiments (Fig. 3, S4) were carried out in 0.1 M H3PO4 or KH2PO4 solutions adjusted to the desired pH values with aqueous KOH at 30 °C in 10x2 mm quartz cuvette (optical path length 10 mm) for absorption measurements and 10x4 mm quartz cuvette for emission measurements.

Titration with GMP and m7GMP

Titration of 3 with m7GMP and GMP (Fig. 3, S5) were carried out at 30 °C in 0.1 M acetate buffer, pH 5.0 or 50 mM Tris buffer, pH 7.6.

Fluorescence intensity at 471 nm was plotted versus pH to obtain a titration curve. The pKₐ values were extracted from the curves by fitting to Henderson – Hasselbalch equation [1] as described previously:⁸

\[ F(471\,nm) = S_A + \frac{S_{HA} - S_A}{1 + 10^{(pH - pK_a)}}. \]  

Temperature dependence measurements

Emission spectra at different temperatures for compounds 1b, 2b and 3 were measured in 50 mM Tris buffer with excitation at 369 nm (only AcPy absorption) or 282 nm (both AcPy and nucleotide absorption).

Corrections for inner filter effect

Absorption spectra for inner filter effect correction were measured in 10x10 mm quartz cuvettes. Raw fluorescence spectra were corrected for the inner filter effect as described².
Corrected fluorescence value can be calculated using the equation [2].

\[ F_{\text{corrected}} = I_F \ast F_{\text{observed}}, \]  

Where IFE are so called Inner Filter Effect corrections, calculated from absorption spectra, by following equation:

\[ I_F = 10^{(A_{\text{exc}} + A_{\text{em}})}, \]  

\[ A_{\text{exc}} \text{ and } A_{\text{em}} \text{ are absorbance values at excitation (369 nm or 282 nm) and emission (471 nm) wavelength, respectively.} \]

**Time-resolved fluorescence**

For fluorescence lifetime measurements samples were excited at 375 nm. The excitation source was a LDH-P-C-375B picosecond pulsed diode laser head (375 nm, optical pulse duration: 56 ps FWHM) driven by a PDL800-D driver. Intensity decays were collected by a time-domain technique using a FluoTime 200 lifetime fluorometer (PicoQuant, GmbH) equipped with an R3809U-50 microchannel plate photomultiplier (MCP-PMT, Hamamatsu), and a PicoHarp300 TCSPC module. The fluorescence was observed through a 100 mm focal length single grating emission monochromator (ScienceTech 9030). All the samples measured were freshly prepared and the recordings were taken at temperature 30°C. The fluorescence lifetimes were calculated using the FluoFit software package (version 4.6.6). The analysis involved iterative reconvolution fitting of a sum of exponentials to the experimentally recorded decays:

\[ I(t) = \sum a_i \exp \left( -\frac{t}{\tau_i} \right), \]  

where \( I(t) \) is the intensity at time \( t \), \( a_i \) is the amplitude of a single exponential component \( i \), \( \tau_i \) is the lifetime of the component.

### 4. DcpS reaction monitoring and IC\(_{50}\) parameters determination

Human DcpS was expressed and purified as described previously\(^9\). The enzyme was stored at -80 °C in 50 mM Tris HCl, 200 mM NaCl, 1 mM DTT, pH 7.6, containing 10 % glycerol. The concentration of protein was 9.1 μM (monomer).

Enzymatic reactions were carried out for 14.5 μM \( 1b \) and 10 nM DcpS in 50 mM Tris buffer (50 mM Tris, 200 mM KCl, 0.5 mM EDTA, adjusted to pH 7.6 with HCl) at 30 °C and monitored by either absorption or emission spectroscopy as described above.

For kinetic parameters determination of enzymatic hydrolysis of \( 1b \) by DcpS enzyme plate reader Infinite 200®PRO from Tecan was used. All measurements were carried out at 30 °C in 50 mM Tris buffer, using black 96-wells plates, for excitation and emission wavelength 369 nm and 471 nm respectively. Each well contained \( 1b \) at 5 μM, and DcpS at 18 nM in total
volume of 150 μl. The reactions were monitored either without inhibitor or in the presence of half-log dilution series of each inhibitor. The initial rate values were determined as slopes of the curves in the linear reaction range and plotted against logarithm of inhibitor concentration. The IC$_{50}$ parameters were determined by fitting the following equation [5] to the experimental data$^{10}$.

\[ y = A1 + \frac{A2 - A1}{1 + 10^{(x-\log IC_{50})}} \], \hspace{1cm} [5]

5. Supporting references