Electronic Supplementary data for

Copper-mediated arylsulfanylations and arylselanylations of pyrimidine or 7-deazapurine nucleosides and nucleotides

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Experimental part

Materials and instrumentation

All chemicals were purchased from commercial sources and were used without further purification. Copper powder (spheroidal), 14-25 µm was purchased from Sigma-Aldrich®. Dry DMF was used as received from supplier, CHCl₃ was distilled prior column chromatography. PO(OMe)₃ was dried, distilled and POCl₃ distilled using conventional methods prior use. All compounds were fully characterized by NMR spectroscopy and the spectra were recorded on a Bruker Avance-IIHD 600 (¹H at 600.1 MHz, and ¹³C at 150.9 MHz) or on a Bruker Avance-IIHD 500 (500.0 MHz for ¹H, 125.7 MHz for ¹³C, 202.3 MHz for ³¹P and 95.4 MHz for ⁷⁷Se) spectrometer. ¹H and ¹³C resonances were assigned using H,H-COSY, H,C-HSQC and H,C-HMBC 2D NMR spectra. The samples were measured in DMSO-dge or in D₂O and chemical shifts (δ-scale, in ppm) were referenced to residual solvent signal (DMSO (δ (¹H) = 2.50 ppm, δ (¹³C) = 39.70 ppm) or to 1,4-dioxane as external standard in the case of D₂O solutions (δ (¹H) = 3.75 ppm, δ (¹³C) = 69.30 ppm)); ³¹P spectra were referenced to H₃PO₄ as an external standard (δ (³¹P) = 0 ppm) and ⁷⁷Se spectra were referenced to Me₂Se as an external standard (δ (⁷⁷Se) = 0 ppm). Coupling constants (J) are given in Hz. The following abbreviations (or a combination of thereof) were used to explain signal multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. The purity of substances and the courses of the reactions were monitored by thin layer chromatography using TLC aluminium sheets with Silica gel 60 F₂₅₄ (Merck) and analysed at 254 and/or 365 nm. Column chromatography was performed using Silica gel Geduram 60 F₂₅₄ (Merck, particle size 0.063–0.200 mm). Reversed-phase high-performance flash chromatography (RP-HPFC) purifications were performed with Biotage SP1 apparatus on KP-C18-HS 25+M and 40+M columns. Semipreparative separations of 2'-deoxyribonucleosides 5'-O-triphosphates (dNTPs), 2'-deoxyribonucleosides 5'-O-monophosphates (dNMPs) or 2'-deoxyribonucleosides (dNs) were performed using HPLC on a column packed with 10 µm C-18 reverse phase [Phenomenex, Luna C18 (2)]. All reverse phase columns were treated/regenerated with sufficient amount of DMSO and aq. EDTA disodium salt solution after each purification of a Cu-mediated reaction mixture. High resolution mass spectra were measured on a LTQ Orbitrap XL (Thermo Fisher Scientific) spectrometer using ESI ionization technique.
General procedure for copper-mediated arylsulfanylations of arylselenylations of nucleosides (dN\textsubscript{I}) (Method A):

To a U-shaped microwave vial sealable with a Teflon cap were added dN\textsubscript{I} (0.255 mmol, 1 equiv.), Cu powder (16.2 mg, 0.255 mmol, 1 equiv.), 2,2'-bipyridyl (9.9 mg, 0.063 mmol, 0.25 equiv.) and corresponding diselenide or disulfide (0.140 mmol, 0.55 equiv.). The vessel was capped and then evacuated and backfilled with Ar three times. DMF (2.1 ml, degassed) was injected via a syringe (liquid dimethyl diselenide was added via a Hamilton syringe at this stage) and the vessel was evacuated and backfilled with Ar and the contents were sonicated. The reaction mixture was heated to 80 °C or 110 °C, vigorously stirred and the consumption of starting nucleoside was carefully monitored by TLC on SiO\textsubscript{2} using CHCl\textsubscript{3}/MeOH = 5/1 or 10/1 v/v as an eluent (the colour changes during the reaction to dark red). The reaction was quenched by addition of MeOH (5 ml), the precipitate was filtered off and the filtrate evaporated to dryness in vacuo. The solid residue was either loaded on SiO\textsubscript{2} by co-evaporation from CHCl\textsubscript{3}/MeOH, and purified by column chromatography (SiO\textsubscript{2}, CHCl\textsubscript{3}/MeOH = 5/1 or 10/1 v/v) or reverse phase HPFC (C-18, 0 → 100% MeOH in water; solid residue was dissolved in approx. 5 ml DMSO/H\textsubscript{2}O=1/1 v/v and the suspension filtered through a syringe filter prior injection) afforded the chalcogenated product as an amorphous solid.

General procedure for arylsulfanylation of nucleosides (dN\textsubscript{I}) using copper(I) thiophenolate (Method B):

To a U-shaped microwave vial sealable with a Teflon cap were added dN\textsubscript{I} (0.255 mmol, 1 equiv.), PhSCu (0.281 mmol, 1.1 equiv.), 2,2'-bipyridyl (9.9 mg, 0.063 mmol, 0.25 equiv.). The vessel was capped and then evacuated and backfilled with Ar three times. DMF (2.1 ml, degassed) was injected via a syringe and the vessel was evacuated and backfilled with Ar and the contents were sonicated. The reaction mixture was heated to 110 °C, vigorously stirred and the consumption of starting nucleoside was carefully monitored by TLC on SiO\textsubscript{2} (CHCl\textsubscript{3}/MeOH = 5/1 v/v). The reaction was quenched by addition of MeOH (5 ml), the precipitate was filtered off and the filtrate evaporated to dryness in vacuo. The solid residue was either loaded on SiO\textsubscript{2} by co-evaporation from CHCl\textsubscript{3}/MeOH, and purified by column chromatography (SiO\textsubscript{2}, CHCl\textsubscript{3}/MeOH = 5/1 v/v) or reverse phase HPFC (C-18, 0 → 100% MeOH in water; solid residue was dissolved in approx. 5 ml DMSO/H\textsubscript{2}O=1/1 v/v and the
suspension filtered through a syringe filter prior injection) afforded the unsymmetrical sulphide as an amorphous solid.

5-Phenylsulfanyl-2'-deoxycytidine (dCPhS)

Method A - starting from (PhS)₂ (31 mg), conditions: 110 °C, 6 hours. Yield: 50 mg (58 %). White foam.

Method B - starting from PhSCu (48.6 mg), conditions: 110 °C, 1.5 hours. Yield: 48 mg (56 %). White foam.

1H NMR (500.0 MHz, DMSO-d₆): 2.06 (dt, 1H, J₆₂,₁ = J₂₂,₃ = 6.3, H-2'b); 2.21 (ddd, 1H, J₆₂,₁ = 13.1, J₂₂,₃ = 6.3, H-2'a); 3.54 (ddd, 1H, J₆₂,OH = 4.9, J₆₂,₄ = 3.4, H-5'b); 3.62 (ddd, 1H, J₆₂,OH = 11.8, J₆₂,₄ = 3.4, H-5'a); 3.81 (q, 1H, J₆₂,₄ = 3.4, H-4'); 4.22 (m, 1H, H-3'); 5.09 (t, 1H, J₆₂,OH = 4.9, OH-5'); 5.24 (d, 1H, J₆₂,OH = 4.3, OH-3'); 6.11 (t, 1H, J₆₂,OH = 6.3, H-1'); 6.93 (bs, 1H, NH₆H₆); 7.18 (m, 2H, H-o-Ph); 7.19 (m, 1H, H-p-Ph); 7.32 (m, 2H, H-m-Ph); 7.69 (bs, 1H, NH₆H₆); 8.43 (s, 1H, H-6).

13C NMR (125.7 MHz, DMSO-d₆): 41.47 (CH₂-2'); 61.30 (CH₂-5'); 70.38 (CH-3'); 86.11 (CH-1'); 87.95 (CH-4'); 94.42 (C-1'); 126.47 (CH-2'); 126.85 (CH-5'); 129.67 (CH-3'); 136.59 (C-4'); 149.66 (CH-6); 154.75 (C-2); 165.41 (C-4).


5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine (dCNO₃P)

Method A - starting from (4-NO₂-PhS)₂ (43.2 mg), conditions: 80 °C, 4.5 hours. Yield: 48.5 mg (50 %). Yellowish microcrystals.

1H NMR (600.1 MHz, DMSO-d₆): 2.09 (dt, 1H, J₆₂,₁ = J₂₂,₃ = 6.2, H-2'b); 2.23 (ddd, 1H, J₆₂,₁ = 13.3, J₂₂,₃ = 6.2, J₂₂,₄ = 4.1, H-2'a); 3.52 (ddd, 1H, J₆₂,₁ = 11.8, J₂₂,OH = 5.0, J₂₂,₄ = 3.5, H-5'b); 3.60 (ddd, 1H, J₆₂,₁ = 11.8, J₂₂,OH = 5.0, J₂₂,₄ = 3.5, H-5'a); 3.81 (q, 1H, J₆₂,₄ = 3.5, H-4'); 4.21 (ddd, 1H, J₆₂,₁ = 6.2, 4.1, J₆₂,₄ = 4.4, J₂₂,₄ = 3.5, H-3'); 5.03 (t, 1H, J₆₂,OH = 5.0, OH-5'); 5.22 (d, 1H, J₆₂,OH = 4.4, OH-3'); 6.10 (t, 1H,
$J_{1',2'} = 6.2$, H-1'); 7.07 (bs, 1H, NH$_2$H$_b$); 7.34 (m, 2H, H-$o$-C$_6$H$_4$NO$_2$); 7.70 (bs, 1H, NH$_a$H$_b$); 8.15 (m, 2H, H-$m$-C$_6$H$_4$NO$_2$); 8.46 (s, 1H, H-6).

$^{13}$C NMR (150.9 MHz, DMSO-$d_6$): 41.23 (CH$_2$-2'); 60.85 (CH$_2$-5'); 69.89 (CH-3'); 86.01 (CH-1'); 87.72 (CH-4'); 91.45 (C-5); 124.30 (CH-$m$-C$_6$H$_4$NO$_2$); 125.82 (CH-$o$-C$_6$H$_4$NO$_2$); 145.25 (C-$p$-C$_6$H$_4$NO$_2$); 147.05 (C-$i$-C$_6$H$_4$NO$_2$); 150.24 (CH-6); 154.34 (C-2); 164.83 (C-4).

MS-ESI (C$_{15}$H$_{16}$O$_4$N$_4$S) m/z (% int.) calcd: 403.4 [M + Na]$^+$. Found: 403.1 [M + Na]$^+$ (100), 783.3 [2M + Na]$^+$ (30).


5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine (dCMOPS)

Method A - starting from (4-MeO-PhS)$_2$ (39.1 mg), conditions: 110 °C, 8 hours. Yield: 20 mg (21%). White foam.

$^1$H NMR (600.1 MHz, DMSO-$d_6$): 2.05 (ddd, 1H, $J_{gem} = 13.1$, $J_{2b,1'} = 6.7$, $J_{2b,3'} = 5.9$, H-2'b); 2.20 (ddd, 1H, $J_{gem} = 13.1$, $J_{2a,1'} = 6.0$, $J_{2a,3'} = 3.9$, H-2'a); 3.57, 3.64 (2 × ddd, 2 × 1H, $J_{gem} = 11.8$, $J_{5',OH} = 4.8$, $J_{5',5'} = 3.5$, H-5'); 3.72 (s, 3H, CH$_3$O); 3.82 (q, 1H, $J_{4',3'} = 3.5$, H-4'); 4.23 (m, 1H, H-3'); 5.12 (t, 1H, $J_{OH,5'} = 4.8$, OH-5'); 5.22 (d, 1H, $J_{OH,3'} = 4.3$, OH-3'); 6.10 (dd, 1H, $J_{1',2'} = 6.7$, 6.0, H-1'); 6.89 (bs, 1H, NH$_2$H$_b$); 6.91 (m, 2H, H-$m$-C$_6$H$_4$OMe); 7.28 (m, 2H, H-$o$-C$_6$H$_4$OMe); 7.67 (bs, 1H, NH$_a$H$_b$); 8.43 (s, 1H, H-6).

$^{13}$C NMR (150.9 MHz, DMSO-$d_6$): 41.15 (CH$_2$-2'); 55.44 (CH$_3$O); 61.10 (CH$_2$-5'); 70.15 (CH-3'); 85.77 (CH-1'); 87.67 (CH-4'); 96.71 (C-5); 115.10 (CH-$m$-C$_6$H$_4$OMe); 126.12 (C-$i$-C$_6$H$_4$OMe); 130.46 (CH-$o$-C$_6$H$_4$OMe); 148.50 (CH-6); 154.43 (C-2); 158.74 (C-$p$-C$_6$H$_4$OMe); 165.02 (C-4).

MS-ESI (C$_{16}$H$_{20}$O$_5$N$_3$S) m/z (% int.) calcd: 388.2 [M + Na]$^+$. Found: 388.1 [M + Na]$^+$ (100), 753.3 [2M + Na]$^+$ (85).

HRMS-ESI (C$_{16}$H$_{20}$O$_5$N$_3$S) m/z (% int.) calcd: 366.11182 [M + H]$^+$. Found: 366.11198 [M + H]$^+$. 

55
5-[(2,4-Dinitrophenyl)sulfanyl]-2'-deoxycytidine (dC^{DNPS})

Method A - starting from (2,4-NO$_2$-PhS)$_2$ (50.7 mg), conditions: 85 °C, 2 hours. Yield: 30 mg (28 %). Yellow microcrystals.

$^1$H NMR (500.0 MHz, DMSO-d$_6$): 2.12 (dt, 1H, $J_{gem} = 13.3$, $J_{2b,1'} = J_{2b,3'} = 6.2$, H-2'b); 2.25 (ddd, 1H, $J_{gem} = 13.3$, $J_{2a,1'} = 6.2$, $J_{2a,3} = 4.3$, H-2'a); 3.53 (ddd, 1H, $J_{gem} = 11.9$, $J_{5b,OH} = 5.0$, $J_{5b,4'} = 3.5$, H-5'b); 3.62 (ddd, 1H, $J_{gem} = 11.9$, $J_{5,a,OH} = 5.0$, $J_{5,a,4'} = 3.5$, H-5'a); 3.82 (q, 1H, $J_{4,3'} = 3.9$, H-3'); 5.07 (t, 1H, $J_{3',2'} = 6.2$, 4.3, $J_{3',OH} = 4.4$, $J_{3',4'} = 3.5$, H-3'); 5.07 (t, 1H, $J_{OH,5'} = 5.0$, OH-5'); 5.24 (d, 1H, $J_{OH,3'} = 4.4$, OH-3'); 6.11 (t, 1H, $J_{1',2'} = 6.2$, H-1'); 7.14 (bs, 1H, NH$_2$H$_6$); 7.47 (d, 1H, $J_{6.5} = 9.0$, H-6-C$_6$H$_3$(NO$_2$)$_2$); 7.67 (bs, 1H, NH$_2$H$_6$); 8.40 (dd, 1H, $J_{5.6} = 9.0$, $J_{5.3} = 2.5$, H-5-C$_6$H$_3$(NO$_2$)$_2$); 8.53 (s, 1H, H-6); 8.88 (d, 1H, $J_{3.5} = 2.5$, H-3-C$_6$H$_3$(NO$_2$)$_2$).

$^{13}$C NMR (125.7 MHz, DMSO-d$_6$): 41.27 (CH$_2$-2'); 60.81 (CH$_2$-5'); 69.80 (CH-3'); 86.12 (CH-1'); 87.76 (CH-4'); 91.37 (C-5); 121.31 (CH-3-C$_6$H$_3$(NO$_2$)$_2$); 127.82 (CH-5-C$_6$H$_3$(NO$_2$)$_2$); 128.63 (CH-6-C$_6$H$_3$(NO$_2$)$_2$); 144.68 (C-4-C$_6$H$_3$(NO$_2$)$_2$); 145.13 (C-1-C$_6$H$_3$(NO$_2$)$_2$); 145.32 (C-2-C$_6$H$_3$(NO$_2$)$_2$); 150.50 (CH-6); 154.36 (C-2); 164.18 (C-4).

MS-ESI (C$_{15}$H$_{15}$N$_5$O$_8$S) m/z (% int.) calcld: 448.4 [M + Na]$^+$. Found: 448.1 [M + Na]$^+$ (100), 873.1 [2M + Na]$^+$ (55).

HRMS-ESI (C$_{15}$H$_{15}$N$_5$O$_8$S) m/z (% int.) calcld: 448.05335 [M + Na]$^+$. Found: 448.05347 [M + Na]$^+$.

5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine (dC$^{ThS}$)

Method A - starting from bis(2-thienyl)disulfide (32.3 mg), conditions: 90 °C, 2 hours. Yield: 40 mg (46 %). White foam.

$^1$H NMR (500.0 MHz, DMSO-d$_6$): 2.00 (dt, 1H, $J_{gem} = 13.1$, $J_{2b,1'} = J_{2b,3'} = 6.2$, H-2'b); 2.19 (ddd, 1H, $J_{gem} = 13.1$, $J_{2a,1'} = 6.2$, $J_{2a,3} = 3.9$, H-2'a); 3.59 (ddd, 1H, $J_{gem} = 11.8$, $J_{5b,OH} = 4.8$, $J_{5b,4'} = 3.5$, H-5'b); 3.66 (ddd, 1H, $J_{gem} = 11.8$, $J_{5a,OH} = 4.8$, $J_{5,a,4'} = 3.5$, H-5'a); 3.83 (q, 1H, $J_{4,3'} = J_{4,5'} = 3.5$, H-4'); 4.23 (dddd, 1H, $J_{3,2'} = 6.2$, 3.9, $J_{3',OH} = 4.3$, $J_{3',4'} = 3.5$, H-3'); 5.17 (t, 1H, $J_{OH,5'} = 4.8$, OH-5'); 5.25 (d, 1H, $J_{OH,3'} = 4.3$, OH-3'); 6.08 (t, 1H,
$J_{1',2'} = 6.2$, H-1'); 7.01 (dd, 1H, $J_{4,5} = 5.3$, $J_{4,3} = 3.6$, H-4-thienyl); 7.16 (bs, 1H, NH$_a$H$_b$); 7.32 (dd, 1H, $J_{3,4} = 3.6$, $J_{3,5} = 1.3$, H-3-thienyl); 7.58 (dd, 1H, $J_{5,4} = 5.3$, $J_{5,3} = 1.3$, H-5-thienyl); 7.83 (bs, 1H, NH$_a$H$_b$); 8.50 (s, 1H, H-6).

$^{13}$C NMR (125.7 MHz, DMSO-$d_6$): 41.26 (CH$_2$-2'); 61.12 (CH$_2$-5'); 70.13 (CH-3'); 85.91 (CH-1'); 87.73 (CH-4'); 98.74 (br, C-5); 128.11 (CH-4-thienyl); 130.21 (CH-5-thienyl); 132.46 (CH-3-thienyl); 134.44 (C-2-thienyl); 148.41 (CH-6); 154.18 (C-2); 164.58 (C-4).

MS-ESI (C$_{13}$H$_{15}$O$_4$N$_3$S$_2$) $m/z$ (% int.) calcd: 364.4 [M + Na]$^+$. Found: 364.2 [M + Na]$^+$ (100), 705.5 [2M + Na]$^+$ (80).

HRMS-ESI (C$_{13}$H$_{15}$O$_4$N$_3$S$_2$) $m/z$ (% int.) calcd: 364.03962 [M + Na]$^+$. Found: 364.03967 [M + Na]$^+$.  

5-Phenylselanyl-2'-deoxycytidine (dC$^{PhSe}$)

Method A - starting from (PhSe)$_2$ (39.9 mg), conditions: 80 °C, 1.5 hours. Yield: 54 mg (50 %). White foam, which was recrystallized from water/MeOH.

$^1$H NMR (500.0 MHz, DMSO-$d_6$): 2.05 (dt, 1H, $J_{gem} = 13.2$, $J_{2'b,1'} = J_{2'b,3'} = 6.3$, H-2'b); 2.21 (ddd, 1H, $J_{gem} = 13.2$, $J_{2'a,1'} = 6.3$, $J_{2'a,3'} = 3.7$, H-2'a); 3.55 (ddd, 1H, $J_{gem} = 11.8$, $J_{5'b,OH} = 4.9$, $J_{5'b,4'} = 3.7$, H-5'b); 3.62 (ddd, 1H, $J_{gem} = 11.8$, $J_{5'a,OH} = 4.9$, $J_{5'a,4'} = 3.7$, H-5'a); 3.82 (q, 1H, $J_{4',3'} = J_{4',5'} = 3.7$, H-4'); 4.23 (m, 1H, H-3'); 5.08 (t, 1H, $J_{OH,5'} = 4.9$, OH-5'); 5.23 (d, 1H, $J_{OH,3'} = 4.3$, OH-3'); 6.13 (t, 1H, $J_{1',2'} = 6.3$, H-1'); 6.74 (bs, 1H, NH$_a$H$_b$); 7.24 (m, 1H, H-p-Ph); 7.30 (m, 2H, H-m-Ph); 7.34 (m, 2H, H-o-Ph); 7.71 (bs, 1H, NH$_a$H$_b$); 8.45 (s, 1H, H-6).

$^{13}$C NMR (125.7 MHz, DMSO-$d_6$): 41.15 (CH$_2$-2'); 61.11 (CH$_2$-5'); 70.20 (CH-3'); 85.76 (CH-1'); 87.67 (CH-4'); 91.40 (C-5); 126.93 (CH-p-Ph); 129.45 (CH-o-Ph); 129.64 (CH-m-Ph); 131.44 (C-i-Ph); 149.79 (CH-6); 154.18 (C-2); 165.18 (C-4).

$^{77}$Se NMR (95.4 MHz, DMSO-$d_6$): 283.80.

MS-ESI (C$_{15}$H$_{17}$O$_4$N$_3$Se) $m/z$ (% int.) calcd: 406.0 [M + Na]$^+$. Found: 406.1 [M + Na]$^+$ (100), 787.2 [2M + Na]$^+$ (10).


S7
5-Methylselanyl-2’-deoxycytidine (dCMeSe)

Method A - starting from (MeSe)2 (1.05 equiv., 50 mg, 25 µl), conditions: 85 °C, 3 hours. The compound was separated using column chromatography and HPLC (C-18, 0 → 100% MeOH in water). The compound was freeze-dried from H2O. Yield: 15 mg (18 %). White microcrystals.

1H NMR (500.0 MHz, DMSO-d6): 2.00 (ddd, 1H, Jgem = 13.1, J2b,1’ = 7.0, J2b,3’ = 6.0, H-2’b); 2.11 (s, 3H, CH3Se); 2.13 (ddd, 1H, Jgem = 13.1, J2a,1’ = 6.1, J2a,3’ = 3.7, H-2’a); 3.54 (dd, 1H, Jgem = 11.8, J5b,4’ = 3.7, H-5’b); 3.61 (dd, 1H, Jgem = 11.8, J5a,4’ = 3.7, H-5’a); 3.78 (q, 1H, J4’,3’ = J4’,5’ = 3.7, H-3’); 4.21 (dt, 1H, J3’,2’ = 6.0, 3.7, J3’,4’ = 3.7, H-3’); 6.10 (dd, 1H, J1’,2’ = 7.0, 6.1, H-1’); 6.78, 7.69 (2 × bs, 2 × 1H, NH2); 8.20 (s, 1H, H-6).

13C NMR (125.7 MHz, DMSO-d6): 9.41 (CH3Se); 40.94 (CH2-2’); 61.24 (CH2-5’); 70.33 (CH-3’); 85.42 (CH-1’); 92.35 (C-5); 147.26 (CH-6); 154.72 (C-2); 165.18 (C-4).

77Se NMR (95.4 MHz, DMSO-d6): 89.74 (q, JSe,H = 10.9).


5-Phenylsulfanyl-2’-deoxyuridine (dUPhS)S1

Method B - starting from PhSCu (48.3 mg), conditions: 85 °C, 1.5 hours. Yield: 40.5 mg (47 %). White foam.

The compound is described ref. S1 and the data are in accordance with the literature.

1H NMR (500.0 MHz, DMSO-d6): 2.16 (ddd, 1H, Jgem = 13.2, J2b,1’ = 6.5, J2b,3’ = 4.5, H-2’b); 2.20 (ddd, 1H, Jgem = 13.2, J2a,1’ = 6.7, J2a,3’ = 5.4, H-2’a); 3.54, 3.60 (2 × bdd, 2 × 1H, Jgem = 11.8, J5’,4’ = 3.4, H-5’); 3.81 (q, 1H, J4’,3’ = J4’,5’ = 3.4, H-4’); 4.24 (bm, 1H, H-3’); 5.09 (bs, 1H, OH-5’); 5.26 (bs, 1H, OH-3’); 6.14 (dd, 1H, J1’,2’ = 6.7, 6.5, H-1’); 7.16 (m, 1H, H-p-Ph); 7.19 (m, 2H, H-o-Ph); 7.29 (m, 2H, H-m-Ph); 8.44 (s, 1H, H-6); 11.65 (bs, 1H, NH).
13C NMR (125.7 MHz, DMSO-d6): 40.50 (CH2-2'); 61.03 (CH2-5'); 70.19 (CH-3'); 85.21 (CH-1'); 87.80 (CH-4'); 103.62 (C-5); 125.84 (CH-p-Ph); 126.62 (CH-o-Ph); 129.18 (CH-m-Ph); 136.54 (C-i-Ph); 146.94 (CH-6); 150.45 (C-2); 161.49 (C-4).


5-[(2-Thienyl)sulfanyl]-2'-deoxyuridine (dUThS)

Method A - starting from bis(2-thienyl)disulfide (32.3 mg), conditions: 85 °C, 1.5 hours. Yield: 22.5 mg (26 %). White foam.

1H NMR (500.0 MHz, DMSO-d6): 2.08 (ddd, 1H, J_gem = 13.4, J_2b,1' = 7.0, J_2b,3' = 5.9, H-2'b); 2.14 (ddd, 1H, J_gem = 13.4, J_2a,1' = 6.2, J_2a,3' = 3.6, H-2'a); 3.51 (bdd, 1H, J_gem = 11.7, J_5',4' = 3.6, H-5'a); 3.56 (bdd, 1H, J_gem = 11.7, J_5',4' = 3.6, H-5'a); 3.80 (q, 1H, J_4',3' = J_4',5' = 3.6, H-4'); 4.23 (dt, 1H, J_3',2' = 5.9, 3.6, J_3',4' = 3.6, H-3'); 5.09 (bs, 1H, OH-5'); 5.25 (bs, 1H, OH-3'); 6.09 (dd, 1H, J_1',2' = 7.0, 6.2, H-1'); 7.00 (dd, 1H, J_4,5, = 5.3, J_4,3, = 3.6, H-4-thienyl); 7.22 (dd, 1H, J_3,4, = 3.6, J_3,5, = 1.3, H-3-thienyl); 7.60 (dd, 1H, J_5,4, = 5.3, J_5,3, = 1.3, H-5-thienyl); 8.25 (s, 1H, H-6); 11.64 (bs, 1H, NH).

13C NMR (125.7 MHz, DMSO-d6): 40.35 (CH2-2'); 61.18 (CH2-5'); 70.31 (CH-3'); 85.12 (CH-1'); 87.78 (CH-4'); 108.15 (C-5); 128.08 (CH-4-thienyl); 130.46 (CH-5-thienyl); 132.75 (C-2-thienyl); 133.31 (CH-3-thienyl); 143.90 (CH-5-thienyl); 150.14 (C-2); 161.18 (C-4).

MS-ESI (C13H14O3N3S2) m/z (% int.) calcd: 365.4 [M + Na]+. Found: 365.0 [M + Na]+ (100), 707.0 [2M + Na]+ (90).

**5-Phenylselanyl-2'-deoxyuridine (dU^PhSe)** S2

Method A - starting from (PhSe)$_2$ (43.6 mg), conditions: 85 °C, 1.5 hours. Yield: 23.5 mg (24 %). White foam.

The compound is described in ref. S2 however, the compound was not characterized sufficiently.

$^1$H NMR (500.0 MHz, DMSO-$d_6$): 2.12 (ddd, 1H, $J_{gem} = 13.3, J_{2'b,1'} = 7.0, J_{2'b,3'} = 5.7, H$-2'b); 2.15 (ddd, 1H, $J_{gem} = 13.3, J_{2'a,1'} = 6.3, J_{2'a,3'} = 4.0, H$-2'a); 3.47, 3.52 (2 × bdd, 2 × 1H, $J_{gem} = 11.8, J_{5',4'} = 3.5, H$-5'); 3.79 (q, 1H, $J_{4',3'} = J_{4',5'} = 3.5, H$-4'); 4.20 (ddd, 1H, $J_{3',2'} = 5.7, 4.0, J_{3',4'} = 3.5, H$-3'); 5.03 (bs, 1H, OH-5'); 5.24 (bs, 1H, OH-3'); 6.13 (dd, 1H, $J_{1',2'} = 7.0, 6.3, H$-1'); 7.24 (m, 1H, H-p-Ph); 7.29 (m, 2H, H-m-Ph); 7.36 (m, 2H, H-o-Ph); 8.28 (s, 1H, H-6); 11.61 (bs, 1H, NH).

$^{13}$C NMR (125.7 MHz, DMSO-$d_6$): 40.33 (CH$_2$-2'); 61.15 (CH$_2$-5'); 70.34 (CH-3'); 85.01 (CH-1'); 87.73 (CH-4'); 101.36 (C-5); 126.86 (CH-p-Ph); 129.51 (CH-m-Ph); 130.20 (CH-o-Ph); 131.04 (C-i-Ph); 146.07 (CH-6); 150.56 (C-2); 161.67 (C-4).

$^{77}$Se NMR (95.4 MHz, DMSO-$d_6$): 310.06.

MS-ESI (C$_{15}$H$_{16}$O$_5$N$_2$Se) $m/z$ (% int.) calcd: 407.0 [M + Na]$^+$. Found: 407.1 [M + Na]$^+$ (100), 791.0 [2M + Na]$^{2+}$ (40).


**5-Methylselanyl-2'-deoxyuridine (dU^MeSe)** S3

Method A - starting from (MeSe)$_2$ (1.1 equiv., 52 mg, 26 µl), conditions: 100 °C, 6 hours. The compound was separated using HPFC and HPLC (C-18, 0 → 100% MeOH in water). The compound was freeze-dried from H$_2$O. Yield: 9.3 mg (11 %). White microcrystals.

The compound is described in ref. S3 and the data are in accordance with the literature.

$^1$H NMR (500.0 MHz, DMSO-$d_6$): 2.10 (ddd, 1H, $J_{gem} = 13.3, J_{2'b,1'} = 6.3, J_{2'b,3'} = 3.6, H$-2'b); 2.13 (s, 3H, CH$_3$Se); 2.15 (ddd, 1H, $J_{gem} = 13.3, J_{2'a,1'} = 7.2, J_{2'a,3'} = 5.7, H$-2'a); 3.57, 3.61 (2 ×
dd, 2 × 1H, J_{gem} = 11.8, J_{5',4'} = 3.2, H-5'); 3.80 (q, 1H, J_{4',3'} = J_{4',5'} = 3.2, H-4'); 4.26 (ddd, 1H, J_{3',4'} = 5.6, 3.6, J_{3',5'} = 3.2, H-3'); 5.11, 5.25 (2 × bs, 2 × 1H, OH-3',5'); 6.18 (dd, 1H, J_{1',2'} = 7.2, 6.3, H-1'); 7.83 (s, 1H, H-6); 11.52 (bs, 1H, NH).

^{13}C NMR (125.7 MHz, DMSO-d_6): 5.42 (CH_3Se); 40.24 (CH_2-2'); 61.26 (CH_2-5'); 70.57 (CH-3'); 84.73 (CH-1'); 87.70 (CH-4'); 104.21 (C-5); 137.75 (CH-6); 150.23 (C-2); 161.87 (C-4).

^{77}Se NMR (95.4 MHz, DMSO-d_6): 125.98 (q, J_{Se,H} = 11.4).

MS-ESI (C_{10}H_4OsN_2Se) m/z (% int.) calcd: 345.0 [M + Na]^+. Found: 345.0 [M + Na]^+ (100), 667.0 [2M + Na]^+ (35).

HRMS-ESI (C_{10}H_4OsN_2Se) m/z (% int.) calcd: 344.99601 [M + Na]^+. Found: 344.99612 [M + Na]^+.

7-Phenylsulfanyl-7-deaza-2'-deoxyadenosine (dA_{PhS})

Method B - starting from PhSCu\(^1\) (45.7 mg), conditions: 110 °C, 1.5 hours. Yield: 34 mg (40 %). White foam.

\(^{1}H\) NMR (500.0 MHz, DMSO-d_6): 2.23 (ddd, 1H, J_{gem} = 13.1, J_{2b,1'} = 6.0, J_{2b,3'} = 2.8, H-2'b); 2.54 (ddd, 1H, J_{gem} = 13.1, J_{2a,1'} = 8.0, J_{2a,3'} = 5.8, H-2'a); 3.53 (ddd, 1H, J_{gem} = 11.8, J_{5b,OH} = 5.9, J_{5b,4'} = 4.2, H-5'b); 3.60 (ddd, 1H, J_{gem} = 11.8, J_{5a,OH} = 5.2, J_{5a,4'} = 4.4, H-5'a); 3.85 (ddd, 1H, J_{4',5'} = 4.4, 4.2, J_{4',3'} = 2.5, H-4'); 4.37 (ddddd, 1H, J_{3',2'} = 5.8, 2.8, J_{3',OH} = 4.1, J_{3',4'} = 2.5, H-3'); 5.09 (dd, 1H, J_{OH,5'} = 5.9, 5.2, OH-5'); 5.29 (d, 1H, J_{OH,3'} = 4.1, OH-3'); 6.55 (dd, 1H, J_{1',2'} = 8.0, 6.0, H-1'); 7.12 (m, 2H, H-o-Ph); 7.15 (m, 1H, H-p-Ph); 7.29 (m, 2H, H-m-Ph); 7.88 (s, 1H, H-6); 8.14 (s, 1H, H-2).

\(^{13}C\) NMR (125.7 MHz, DMSO-d_6): 40.17 (CH_2-2'); 62.01 (CH_2-5'); 71.10 (CH-3'); 83.58 (CH-1'); 87.77 (CH-4'); 99.37 (C-5); 103.26 (C-4a); 125.90 (CH-p-Ph); 126.00 (CH-o-Ph); 129.47 (CH-m-Ph); 129.82 (CH-6); 138.29 (C-i-Ph); 150.81 (C-7a); 152.75 (CH-2); 157.60 (C-4).

MS-ESI (C_{17}H_{18}O_3NS) m/z (% int.) calcd: 381.1 [M + Na]^+. Found: 381.2 [M + Na]^+ (100).

HRMS-ESI (C_{17}H_{18}O_3NaN_4S) m/z (% int.) calcd: 381.09918 [M + Na]^+. Found: 381.09932 [M + Na]^+.

S11
7-Phenylselanyl-7-deaza-2'-deoxyadenosine (dAPhSe)

Method A - starting from (PhSe)₂ (37.4 mg), conditions: 90 °C, 1.5 hours. Yield: 31 mg (32 %). White foam.

$^1$H NMR (500.0 MHz, DMSO-d$_6$): 2.22 (ddd, 1H, $J_{gem} = 13.1, J_{2b,1'} = 6.0, J_{2b,3'} = 2.7, H-2'b$); 2.55 (ddd, 1H, $J_{gem} = 13.1, J_{2a,1'} = 8.1, J_{2a,3'} = 5.7, H-2'a$); 3.52 (ddd, 1H, $J_{gem} = 11.7, J_{5b,OH} = 5.9, J_{5b,4'} = 4.2, H-5'b$); 3.60 (ddd, 1H, $J_{gem} = 11.5, J_{5a,OH} = 5.3, J_{5a,4'} = 4.8, H-5'a$); 3.85 (ddd, 1H, $J_{4',5'} = 4.8, 4.2, J_{4',3'} = 2.4, H-4'$); 4.36 (ddddd, 1H, $J_{5',2'} = 5.7, 2.7, J_{3',OH} = 4.0, J_{3',4'} = 2.4, H-3'$); 5.11 (dd, 1H, $J_{OH,5} = 5.9, 5.3, OH-5'$); 5.30 (d, 1H, $J_{OH,3} = 4.0, OH-3'$); 6.54 (dd, 1H, $J_{1',2'} = 8.1, 5.9, H-1'$); 7.19 (m, 1H, H-p-Ph); 7.22 - 7.30 (m, 4H, H-o,m-Ph); 7.86 (s, 1H, H-6); 8.14 (s, 1H, H-2).

$^{13}$C NMR (125.7 MHz, DMSO-d$_6$): 40.15 (CH-2'); 62.07 (CH-2'); 71.19 (CH-3'); 83.59 (CH-1'); 87.78 (CH-4'); 93.56 (C-5); 103.72 (C-4a); 126.63 (CH-p-Ph); 128.68 (CH-o-Ph); 129.77 (CH-m-Ph); 130.19 (CH-6); 133.28 (C-i-Ph); 150.67 (C-7a); 152.54 (CH-2); 157.77 (C-4).

$^{77}$Se NMR (95.4 MHz, DMSO-d$_6$): 230.26.

MS-ESI (C$_{17}$H$_{18}$O$_3$N$_4$Se) m/z (% int.) calcd: 429.0 [M + Na]$^+$, Found: 429.1 [M + Na]$^+$ (100), 835.3 [2M + Na]$^+$ (35).

HRMS-ESI (C$_{17}$H$_{18}$O$_3$N$_4$Se) m/z (% int.) calcd: 429.04363 [M + Na]$^+$, Found: 429.04370 [M + Na]$^+$.

7-Methylselanyl-7-deaza-2'-deoxyadenosine (dAMeSe)

Method A - starting from (MeSe)$_2$ (1.1 equiv., 50 mg, 25 μl), conditions: 100 °C, 3.5 hours. The compound was separated using HPFC and HPLC (C-18, 0 → 100% MeOH in water). The compound was freeze-dried from H$_2$O. Yield: 11.4 mg (14 %). White microcrystals.

$^1$H NMR (500.0 MHz, DMSO-d$_6$): 2.16 (ddd, 1H, $J_{gem} = 13.1, J_{2b,1'} = 6.0, J_{2b,3'} = 2.7, H-2'b$); 2.19 (s, 3H, CH$_3$Se); 2.50 (ddd, 1H, $J_{gem} = 13.1, J_{2a,1'} = 8.3, J_{2a,3'} = 5.8, H-2'a$); 3.52 (ddd, 1H, $J_{gem} = 11.8, J_{5b,OH} = 5.4, J_{5b,4'} = 4.3, H-5'b$); 3.58 (dt, 1H, $J_{gem} = 11.8, J_{5a,OH} = J_{5a,4'} = 4.6, H-5'a$); 3.83 (ddd, 1H, $J_{4',5'} = 4.6, 4.3, J_{4',3'} = 2.5, H-4'$); 4.34 (ddddd, 1H, $J_{5',2'} = 5.8, 2.7, J_{3',OH} = 13.1, J_{2b,3'} = 2.7, H-2'b$); 2.19 (s, 3H, CH$_3$Se); 2.50 (ddd, 1H, $J_{gem} = 13.1, J_{2a,1'} = 8.3, J_{2a,3'} = 5.8, H-2'a$); 3.52 (ddd, 1H, $J_{gem} = 11.8, J_{5b,OH} = 5.4, J_{5b,4'} = 4.3, H-5'b$); 3.58 (dt, 1H, $J_{gem} = 11.8, J_{5a,OH} = J_{5a,4'} = 4.6, H-5'a$); 3.83 (ddd, 1H, $J_{4',5'} = 4.6, 4.3, J_{4',3'} = 2.5, H-4'$); 4.34 (ddddd, 1H, $J_{5',2'} = 5.8, 2.7, J_{3',OH} = 13.1, J_{2b,3'} = 2.7, H-2'b$); 2.19 (s, 3H, CH$_3$Se); 2.50 (ddd, 1H, $J_{gem} = 13.1, J_{2a,1'} = 8.3, J_{2a,3'} = 5.8, H-2'a$); 3.52 (ddd, 1H, $J_{gem} = 11.8, J_{5b,OH} = 5.4, J_{5b,4'} = 4.3, H-5'b$); 3.58 (dt, 1H, $J_{gem} = 11.8, J_{5a,OH} = J_{5a,4'} = 4.6, H-5'a$); 3.83 (ddd, 1H, $J_{4',5'} = 4.6, 4.3, J_{4',3'} = 2.5, H-4'$); 4.34 (ddddd, 1H, $J_{5',2'} = 5.8, 2.7, J_{3',OH} = 13.1, J_{2b,3'} = 2.7, H-2'b$); 2.19 (s, 3H, CH$_3$Se); 2.50 (ddd, 1H, $J_{gem} = 13.1, J_{2a,1'} = 8.3, J_{2a,3'} = 5.8, H-2'a$); 3.52 (ddd, 1H, $J_{gem} = 11.8, J_{5b,OH} = 5.4, J_{5b,4'} = 4.3, H-5'b$); 3.58 (dt, 1H, $J_{gem} = 11.8, J_{5a,OH} = J_{5a,4'} = 4.6, H-5'a$); 3.83 (ddd, 1H, $J_{4',5'} = 4.6, 4.3, J_{4',3'} = 2.5, H-4'$); 4.34 (ddddd, 1H, $J_{5',2'} = 5.8, 2.7, J_{3',OH} = 13.1, J_{2b,3'} = 2.7, H-2'b$); 2.19 (s, 3H, CH$_3$Se); 2.50 (ddd, 1H, $J_{gem} = 13.1, J_{2a,1'} = 8.3, J_{2a,3'} = 5.8, H-2'a$); 3.52 (ddd, 1H, $J_{gem} = 11.8, J_{5b,OH} = 5.4, J_{5b,4'} = 4.3, H-5'b$); 3.58 (dt, 1H, $J_{gem} = 11.8, J_{5a,OH} = J_{5a,4'} = 4.6, H-5'a$); 3.83 (ddd, 1H, $J_{4',5'} = 4.6, 4.3, J_{4',3'} = 2.5, H-4'$); 4.34 (ddddd, 1H, $J_{5',2'} = 5.8, 2.7, J_{3',OH} =
3.9, \( J_{3',4'} = 2.5 \), H-3'); 5.11 (ddd, 1H, \( J_{OH,5'} = 5.4 \), 4.6, OH-5'); 5.27 (bd, 1H, \( J_{OH,3'} = 4.9 \), OH-3'); 6.49 (dd, 1H, \( J_{1',2'} = 8.3 \), 6.0, H-1'); 7.60 (s, 1H, H-6); 8.11 (s, 1H, H-2).

\( ^{13}C \) NMR (125.7 MHz, DMSO-\( d_6 \)): 12.12 (CH\( _3 \)Se); 40.03 (CH\( _2-2' \)); 62.15 (CH\( _2-5' \)); 71.23 (CH-3'); 83.36 (CH-1'); 87.66 (CH-4'); 96.05 (C-5); 103.72 (C-4a); 127.83 (CH-6); 150.25 (C-7a); 152.29 (CH-2); 157.89 (C-4).

\( ^{77}Se \) NMR (95.4 MHz, DMSO-\( d_6 \)): 33.61 (q, \( J_{Se,H} = 10.8 \)).

MS-ESI (C\(_{12}H_{16}O_3N_4Se\)) \( m/z \) (% int.) calcd: 345.0 [M + H]\(^+\). Found: 345.0 [M + H]\(^+\) (100).

HRMS-ESI (C\(_{12}H_{16}O_3N_4Se\)) \( m/z \) (% int.) calcd: 345.04604 [M + H]\(^+\). Found: 345.04615 [M + H]\(^+\).

7-Phenylsulfanyl-7-deaza-2'-deoxyguanosine (dG\( ^{PhS} \))

Method B - starting from PhSCu\(^1\) (43.7 mg), conditions: 110° C, 1.5 hours. Yield: 33.5 mg (39 %). White foam.

\(^1H\) NMR (500.0 MHz, DMSO-\( d_6 \)): 2.12 (ddd, 1H, \( J_{gem} = 13.0 \), \( J_{2b,1'} = 5.8 \), \( J_{2b,3'} = 2.5 \), H-2'\( b \)); 2.35 (ddd, 1H, \( J_{gem} = 13.0 \), \( J_{2a,1'} = 8.4 \), \( J_{2a,3'} = 5.7 \), H-2'a); 3.48, 3.52 (2 \times dt, 1H, \( J_{gem} = 11.7 \), \( J_{3;OH} = J_{3',4'} = 4.6 \), H-5'); 3.77 (ddd, 1H, \( J_{4',5'} = 4.6 \), \( J_{4',3'} = 2.5 \), H-4'); 4.29 (dq, 1H, \( J_{3',2'} = 5.7 \), 2.5, \( J_{3',4'} = J_{3;OH} = 2.5 \), H-3'); 4.91 (bt, 1H, \( J_{OH,5'} = 4.6 \), OH-5'); 5.22 (bd, 1H, \( J_{OH,3'} = 2.5 \), OH-3'); 6.32 (dd, 1H, \( J_{1',2'} = 8.4 \), 5.8, H-1'); 6.37 (bs, 2H, NH\( _2 \)); 7.08 (m, 1H, H-\( p \)-Ph); 7.10 (m, 2H, H-\( o \)-Ph); 7.22 (m, 2H, H-\( m \)-Ph); 7.25 (s, 1H, H-6); 10.42 (bs, 1H, NH).

\( ^{13}C\) NMR (125.7 MHz, DMSO-\( d_6 \)): 39.97 (CH\( _2-2' \)); 62.04 (CH\( _2-5' \)); 71.11 (CH-3'); 82.59 (CH-1'); 87.38 (CH-4'); 100.39 (C-5); 123.46 (CH-6); 124.97 (CH-\( p \)-Ph); 126.12 (CH-\( o \)-Ph); 128.88 (CH-\( m \)-Ph); 139.56 (C-\( i \)-Ph); 152.02 (C-7a); 153.41 (C-2); 157.96 (C-4).

MS-ESI (C\(_{17}H_{18}O_4N_4S\)) \( m/z \) (% int.) calcd: 397.4 [M + Na]\(^+\). Found: 397.1 [M + Na]\(^+\) (100).

HRMS-ESI (C\(_{17}H_{18}O_4N_4S\)) \( m/z \) (% int.) calcd: 397.09410 [M + Na]\(^+\). Found: 397.09420 [M + Na]\(^+\).

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513
7-Phenylselanyl-7-deaza-2'-deoxyguanosine (dG\textsuperscript{PhSe})

Method A - starting from (PhSe)\textsubscript{2} (39.6 mg), conditions: 80 °C, 1.5 hours. Yield: 43.2 mg (45 %). White foam.

\textsuperscript{1}H NMR (500.0 MHz, DMSO-\textit{d}\textsubscript{6}): 2.09 (ddd, 1H, \textit{J}_{\text{gem}} = 13.0, \textit{J}_{2\text{b},1'\text{a}} = 5.8, \textit{J}_{2\text{b},3'} = 2.5, H-2'\text{b}); 2.33 (ddd, 1H, \textit{J}_{\text{gem}} = 13.0, \textit{J}_{2\text{a},1'\text{a}} = 8.5, \textit{J}_{2\text{a},3'} = 5.6, H-2'\text{a}); 3.45 (ddd, 1H, \textit{J}_{\text{gem}} = 11.5, \textit{J}_{5\text{b},\text{OH}} = 5.3, \textit{J}_{5\text{b},4'} = 4.5, H-5'\text{b}); 3.49 (ddd, 1H, \textit{J}_{\text{gem}} = 11.5, \textit{J}_{5\text{a},\text{OH}} = 5.3, \textit{J}_{5\text{a},4'} = 4.5, H-5'\text{a}); 3.75 (td, 1H, \textit{J}_{4',5'} = 4.5, \textit{J}_{4',3'} = 2.3, H-4'); 4.26 (m, 1H, H-3'); 4.91 (t, 1H, \textit{J}_{\text{OH},5'} = 5.3, OH-5'); 5.23 (d, 1H, \textit{J}_{\text{OH},3'} = 3.7, OH-3'); 6.31 (dd, 1H, \textit{J}_{1',2'} = 8.5, 5.8, H-1'); 6.36 (bs, 2H, NH\textsubscript{2}); 7.13 (s, 1H, H-6); 7.15 (m, 1H, H-\textit{p}-Ph); 7.22 (m, 2H, H-\textit{m}-Ph); 7.30 (m, 2H, H-\textit{o}-Ph); 10.43 (bs, 1H, NH).

\textsuperscript{13}C NMR (125.7 MHz, DMSO-\textit{d}\textsubscript{6}): 39.93 (CH-2'); 62.09 (CH-2'); 71.18 (CH-3'); 82.57 (CH-1'); 87.37 (CH-4'); 98.19 (C-5); 100.87 (C-4a); 123.30 (CH-6); 126.08 (CH-\textit{p}-Ph); 129.27 (CH-\textit{o}-Ph); 129.43 (CH-\textit{m}-Ph); 133.95 (C-\textit{i}-Ph); 151.90 (C-7a); 153.25 (C-2); 158.28 (C-4).

\textsuperscript{77}Se NMR (95.4 MHz, DMSO-\textit{d}\textsubscript{6}): 262.71.

MS-ESI (C\textsubscript{17}H\textsubscript{18}O\textsubscript{4}N\textsubscript{4}Se) \textit{m}/\textit{z} (% int.) calcd: 423.1 [M + H]\textsuperscript{+}. Found: 423.1 [M + H]\textsuperscript{+} (30).

HRMS-ESI (C\textsubscript{17}H\textsubscript{18}O\textsubscript{4}N\textsubscript{4}Se) \textit{m}/\textit{z} (% int.) calcd: 423.05660 [M + H]\textsuperscript{+}. Found: 423.05658 [M + H]\textsuperscript{+}. 

S14
**General procedure for copper-mediated sulfanylations and selanylations of dC\textsuperscript{I}MP (Method A):**

To a U-shaped microwave vial sealable with a Teflon cap were added Cu powder (91.5 mg, 1.440 mmol, 10 equiv.), 2,2’-bipyridyl (74.3 mg, 0.476 mmol, 3.30 equiv.) and corresponding diselenide or disulfide (0.721 mmol, 5 equiv.). The vessel was capped and then evacuated and backfilled with Ar three times. Triethylammonium salt (TEA\(^+\)) of dC\textsuperscript{I}MP (77 mg, 0.144 mmol, 1 equiv.) in DMF (1.1 ml, degassed) was injected via a syringe and the vessel was evacuated and backfilled with Ar and the contents were sonicated. The reaction mixture was heated to 80 °C and vigorously stirred for 60 - 70 minutes. The reaction was quenched by slow addition of cold water (5 ml), the precipitate was filtered off and the filtrate evaporated to dryness in vacuo. The product was purified by reverse phase HPLC [C-18, 0 → 90% MeOH in 0.1M aq. TEAB (triethylammonium bicarbonate); solid residue was dissolved in approx. 5 ml H\textsubscript{2}O and filtered through two syringe filters prior injection]. Several co-evaporations with water, conversion into a sodium form (Dowex 50WX8 in Na\(^+\) cycle followed by Chelex 100 resin in Na\(^+\) cycle\(^{[X2]}\)) followed by freeze-drying from water afforded the product as a white, fluffy powder.

**General procedure for monophosphorylation of S- or Se-linked nucleosides dC\textsuperscript{RX} (Method B):**

To an argon-purged flask containing under reduced pressure dried (2 hours, 80 °C) dC\textsuperscript{RX} (0.1-0.2 mmol), dry PO(OMe)\textsubscript{3} (0.1 ml for every 10 mg of dC\textsuperscript{RX}) was added and the suspension/solution cooled to 0 °C on an ice bath. Freshly distilled POCl\textsubscript{3} (1.3 equiv. – 1.5 equiv.) was slowly added with a Hamilton syringe, the reaction was stirred for 80 – 180 minutes and monitored with TLC (eluent 11 iPrOH : 2 H\textsubscript{2}O : 7 NH\textsubscript{4}OH). The reaction was quenched with 2M TEAB (1 ml) and evaporated to dryness. The product was purified by reverse phase HPLC (C-18, 0 → 90% MeOH in 0.1M aq. TEAB). Several co-evaporations with water, conversion into a sodium form (Dowex 50WX8 in Na\(^+\) cycle) followed by freeze-drying from water afforded the product as a fluffy powder.
5-Phenylsulfanyl-2'-deoxycytidine 5'-O-monophosphate sodium salt (dC\textsuperscript{PhSMP})

Method A starting from (PhS)\textsubscript{2} (222.2 mg). Yield: 2.9 mg (5 %). White solid.

Method B: \(\text{dC}^{\text{PhS}}\) (100 mg, 0.3 mmol), POCl\textsubscript{3} (59.4 mg, 36 µl, 0.39 mmol, 1.3 equiv.), 70 minutes. Yield: 55 mg (43 %). White solid.

\(^1\)H NMR (500.0 MHz, D\textsubscript{2}O): 2.36 (ddd, 1H, \(J_{\text{gem}} = 14.0\), \(J_{2b,1'} = 7.4\), \(J_{2b,3'} = 6.4\), H-2'b); 2.46 (ddd, 1H, \(J_{\text{gem}} = 14.0\), \(J_{2'a,1'} = 6.2\), \(J_{2'a,3'} = 3.6\), H-2'a); 3.92 (ddd, 1H, \(J_{\text{gem}} = 11.3\), \(J_{5'b,4'} = 4.8\), H-5'b); 3.96 (ddd, 1H, \(J_{\text{gem}} = 11.3\), \(J_{5'a,4'} = 4.8\), H-5'a); 4.18 (td, 1H, \(J_{4',5'} = 4.8\), \(J_{4',3'} = 4.8\), H-4').

\(^{13}\)C NMR (125.7 MHz, D\textsubscript{2}O): 41.87 (CH\textsubscript{2}-2'); 66.62 (d, \(J_{C,P} = 4.6\), CH\textsubscript{2}-5'); 73.81 (CH-3'); 88.74 (d, \(J_{C,P} = 8.2\), CH-4'); 89.14 (CH-1'); 100.62 (C-5); 129.24 (CH-o-Ph); 129.32 (CH-p-Ph); 132.21 (CH-m-Ph); 137.24 (C-i-Ph); 151.64 (CH-6); 159.73 (C-2); 168.43 (C-4).

\(^{31}\)P\textsuperscript{[\(\text{1H}\)]} NMR (202.3 MHz, D\textsubscript{2}O): 3.87.

MS-ESI (C\textsubscript{15}H\textsubscript{17}O\textsubscript{7}N\textsubscript{3}PS\textsuperscript{-}) \(m/z\) (% int.) calcd: 414.3 [M]. Found: 414.0 [M] (100).

HRMS-ESI (C\textsubscript{15}H\textsubscript{17}O\textsubscript{7}N\textsubscript{3}PS\textsuperscript{-}) \(m/z\) (% int.) calcd: 414.05303 [M]. Found: 414.05266 [M].

5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine 5'-O-monophosphate sodium salt (dC\textsuperscript{NO\textsubscript{PS}MP})

Method A: The amount of DMF used for the reaction was doubled due to precipitate formation during the reaction. Starting from (4-NO\textsubscript{2}-PhS)\textsubscript{2} (222.2 mg). Yield: 4.8 mg (7 %) of yellowish solid.

Method B: \(\text{dC}^{\text{NOPS}}\) (59 mg, 0.16 mmol), POCl\textsubscript{3} (30.9 mg, 18.9 µl, 0.2 mmol, 1.3 equiv.), 80 minutes. Yield: 20 mg (27 %). Yellowish solid.
1H NMR (500.0 MHz, D2O): 2.37 (ddd, 1H, Jgem = 14.0, J2b,1′ = 7.3, J2b,3′ = 6.6, H-2′b); 2.48 (ddd, 1H, Jgem = 14.0, J2a,1′ = 6.2, J2a,3′ = 3.6, H-2′a); 3.93 (ddd, 1H, Jgem = 11.4, JHP = 5.2, J5b,4′ = 4.7, H-5′b); 3.60 (ddd, 1H, Jgem = 11.4, JHP = 5.8, J5a,4′ = 4.7, H-5′a); 4.18 (td, 1H, J4′,5′ = 4.7, J4′,3′ = 3.6, H-4′); 4.53 (dt, 1H, J3′,2′ = 6.6, 3.6, J3′,4′ = 3.6, H-3′); 6.27 (dd, 1H, J1′,2′ = 7.3, 6.2, H-1′); 7.34 (m, 2H, H-o-C6H4NO2); 8.14 (m, 2H, H-m-C6H4NO2); 8.36 (s, 1H, H-6).

13C NMR (125.7 MHz, D2O): 42.04 (CH2-2′); 66.58 (d, JCP = 4.6, CH2-5′); 88.82 (d, JCP = 8.3, CH-4′); 89.35 (CH-1′); 98.15 (C-5); 127.06 (CH-m-C6H4NO2); 128.45 (CH-o-C6H4NO2); 147.99 (C-i-C6H4NO2); 148.22 (C-p-C6H4NO2); 152.65 (CH-6); 159.60 (C-2); 168.17 (C-4).

31P{1H} NMR (202.3 MHz, D2O): 3.79.

MS-ESI (C15H16O9N4PS) m/z (% int.) calcd: 459.3 [M]. Found: 459.0 [M] (100).

HRMS-ESI (C15H16O9N4PS) m/z (% int.) calcd: 459.03811 [M]. Found: 459.03751 [M].

5-[(4-Methoxyphenyl)sulfanyl]-2′-deoxycytidine 5′-O-monophosphate sodium salt (dCMOPSMP)

Method B: dCMOPS (32 mg, 0.09 mmol), POCl3 (17.4 mg, 10.5 µl, 0.117 mmol, 1.3 equiv.), 90 minutes.

Yield: 9 mg (22 %). White solid.

1H NMR (500.0 MHz, D2O): 2.37 (ddd, 1H, Jgem = 14.0, J2b,1′ = 7.5, J2b,3′ = 6.5, H-2′b); 2.44 (ddd, 1H, Jgem = 14.0, J2a,1′ = 6.2, J2a,3′ = 3.6, H-2′a); 3.80 (s, 3H, CH3O); 3.91 (dt, 1H, Jgem = 11.2, JHP = J5b,4′ = 5.1, H-5′b); 3.96 (ddd, 1H, Jgem = 11.2, JHP = 6.0, J5a,4′ = 5.1, H-5′a); 4.17 (dt, 1H, J4′,5′ = 5.1, J4′,3′ = 3.6 H-4′); 4.53 (dt, 1H, J3′,2′ = 6.5, 3.6, J3′,4′ = 3.6, H-3′); 6.26 (dd, 1H, J1′,2′ = 7.5, 6.2, H-1′); 6.96 (m, 2H, H-m-C6H4OMe); 7.30 (m, 2H, H-o-C6H4OMe); 8.29 (s, 1H, H-6).

13C NMR (125.7 MHz, D2O): 41.71 (CH2-2′); 58.20 (CH3O); 66.44 (d, JCP = 4.5, CH2-5′); 73.86 (CH-3′); 88.81 (d, JCP = 8.1, CH-4′); 89.03 (CH-1′); 102.29 (C-5); 117.91 (CH-m-C6H4OMe); 128.00 (C-i-C6H4OMe); 132.34 (CH-o-C6H4OMe); 150.94 (CH-6); 159.68 (C-2); 160.98 (C-p-C6H4OMe); 168.33 (C-4).

31P{1H} NMR (202.3 MHz, D2O): 3.96.
MS-ESI (C_{16}H_{19}N_{3}O_{8}PS^{-}) m/z (% int.) calcd: 444.4 [M]. Found: [M] 444.1 (100).

HRMS-ESI (C_{16}H_{19}N_{3}O_{8}PS^{-}) m/z (% int.) calcd: 444.06359 [M]. Found: 444.06293 [M].

5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-O-monophosphate (dCThSMP)

Method A - starting from bis(2-thienyl)disulfide (168.2 mg). Yield: 29 mg (45 %). White solid.

$^{1}$H NMR (500.0 MHz, D$_2$O): 2.31 (dt, 1H, $J_{gem}$ = 14.0, $J_{2b,1'}$ = 7.0, $J_{2b,3'}$ = 6.3, H-2b); 2.44 (ddd, 1H, $J_{gem}$ = 14.0, $J_{2a,1'}$ = 6.2, $J_{2a,3'}$ = 3.6, H-2a); 4.03 (dd, 2H, $J_{H,P}$ = 5.6, $J_{5',4'}$ = 4.5, H-5'); 4.20 (td, 1H, $J_{4',3'}$ = 4.5, $J_{4',5'}$ = 3.6, H-4'); 4.53 (dt, 1H, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 3.6, H-3'); 6.21 (dd, 1H, $J_{1',2'}$ = 7.0, 6.2, H-1'); 7.01 (dd, 1H, $J_{4,5}$ = 5.3, $J_{4,3}$ = 3.6, H-4-thienyl); 7.29 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.4, H-3-thienyl); 7.45 (dd, 1H, $J_{5,4}$ = 5.3, $J_{5,3}$ = 1.4, H-5-thienyl); 8.32 (s, 1H, H-6).

$^{13}$C NMR (125.7 MHz, D$_2$O): 42.08 (CH$_2$-2'); 66.96 (d, $J_{C,P}$ = 4.7, CH$_2$-5'); 73.74 (CH-3'); 88.65 (d, $J_{C,P}$ = 8.3, CH-4'); 89.26 (CH-1'); 104.66 (C-5); 130.79 (CH-4-thienyl); 132.68 (CH-5-thienyl); 135.25 (CH-3-thienyl); 135.53 (C-2-thienyl); 150.41 (CH-6); 159.38 (C-2); 167.88 (C-4).

$^{31}$P{$^{1}$H} NMR (202.3 MHz, D$_2$O): 2.72.

MS-ESI (C$_{13}$H$_{15}$O$_{7}$N$_{3}$PS$_{2}$) m/z (% int.) calcd: 420.4 [M]. Found: 420.1[M] (100).

HRMS-ESI (C$_{13}$H$_{15}$O$_{7}$N$_{3}$PS$_{2}$) m/z (% int.) calcd: 420.00945 [M]. Found: 420.00928 [M].

5-Phenylselanyl-2'-deoxycytidine 5'-O-monophosphate sodium salt (dCPhSeMP)

Method A - starting from (PhSe)$_2$ (225 mg). Yield: 15.5 mg (21 %). White solid.

$^{1}$H NMR (500.0 MHz, D$_2$O): 2.36 (ddd, 1H, $J_{gem}$ = 14.0, $J_{2b,1'}$ = 7.5, $J_{2b,3'}$ = 6.5, H-2b); 2.44 (ddd, 1H, $J_{gem}$ = 14.0, $J_{2a,1'}$ = 6.2, $J_{2a,3'}$ = 3.6, H-2a); 3.92 (dt, 1H, $J_{gem}$ = 11.3,
$J_{5b,4'} = J_{H,P} = 5.1$, H-5'b; 3.95 (ddd, 1H, $J_{gem} = 11.3$, $J_{H,P} = 5.9$, $J_{5b,4'} = 5.1$, H-5’a); 4.17 (td, 1H, $J_{4',5'} = 5.1$, $J_{4',3'} = 3.6$, H-4’); 4.52 (dt, 1H, $J_{3',2'} = 6.5$, 3.6, $J_{3',2'} = 3.6$, H-3’); 6.27 (dd, 1H, $J_{1',2'} = 7.5$, 6.2, H-1’); 7.26 – 7.37 (m, 3H, H-m,p-Ph); 7.41 (m, 2H, H-o-Ph); 8.32 (s, 1H, H-6).

$^{13}$C NMR (125.7 MHz, D$_2$O): 41.67 (CH$_2$-2’); 66.52 (d, $J_{C,P} = 4.5$, CH$_2$-5’); 73.85 (CH-3’); 88.73 (d, $J_{C,P} = 8.2$, CH-4’); 89.96 (CH-1’); 97.23 (C-5); 130.03 (CH-p-Ph); 132.24 (CH-o-Ph); 132.44 (CH-m-Ph); 132.57 (C-i-Ph); 152.03 (CH-6); 159.91 (C-2); 168.53 (C-4).

$^{31}$P{$^1$H} NMR (202.3 MHz, D$_2$O): 4.44.

$^{77}$Se NMR (95.4 MHz, D$_2$O): 284.52.

MS-ESI (C$_{15}$H$_{17}$O$_7$N$_3$PSe) m/z (% int.) calcd: 462.0 [M]. Found: 462.1 [M] (100).

HRMS-ESI (C$_{15}$H$_{17}$O$_7$N$_3$PSe) m/z (% int.) calcd: 461.99748 [M]. Found: 461.99731 [M].

5-Methylselanyl-2'-deoxycytidine 5'-O-monophosphate sodium salt (dCMeSeMP)

Method B: dCMeSe (63 mg, 0.2 mmol), POCl$_3$ (46 mg, 27.4 µl, 0.3 mmol, 1.5 equiv.), 90 minutes. Yield: 42 mg (48%). White solid.

$^1$H NMR (500.0 MHz, D$_2$O): 2.18 (s, 3H, CH$_3$Se); 2.33 (ddd, 1H, $J_{gem} = 14.0$, $J_{2b,1'} = 7.6$, $J_{2b,3'} = 6.4$, H-2’b); 2.42 (dd, 1H, $J_{gem} = 14.0$, $J_{2a,1'} = 6.2$, $J_{2a,3'} = 3.4$, H-2’a); 4.01 (dd, 2H, $J_{H,P} = 5.4$, $J_{5',4'} = 4.3$, H-5’); 4.19 (t, 1H, $J_{4',5'} = 4.3$, $J_{4',3'} = 3.4$, $J_{H,P} = 1.1$, H-4’); 4.54 (dt, 1H, $J_{3',2'} = 6.4$, 3.4, $J_{3',4'} = 3.4$, H-3’); 6.27 (dd, 1H, $J_{1',2'} = 7.6$, 6.2, H-1’); 8.20 (s, 1H, H-6).

$^{13}$C NMR (125.7 MHz, D$_2$O): 41.47 (CH$_3$Se); 41.92 (CH$_2$-2’); 66.91 (d, $J_{C,P} = 4.7$, CH$_2$-5’); 73.85 (CH-3’); 88.59 (d, $J_{C,P} = 8.5$, CH-4’); 88.86 (CH-1’); 98.43 (C-5); 150.32 (CH-6); 160.01 (C-2); 168.77 (C-4).

$^{31}$P{$^1$H} NMR (202.3 MHz, D$_2$O): 2.09.

$^{77}$Se NMR (95.4 MHz, D$_2$O): 85.79 (q, $J_{Se,H} = 11.4$).

MS-ESI (C$_{10}$H$_{15}$O$_7$N$_3$PSe) m/z (% int.) calcd: 400.0 [M]. Found: 400.0 [M] (100).

HRMS-ESI (C$_{10}$H$_{15}$O$_7$N$_3$PSe) m/z (% int.) calcd: 399.98183 [M]. Found: 399.98160 [M].
General procedure for copper-mediated sulfanylations or selanylations of dC\textsuperscript{4}TP (Method A):

To a U-shaped microwave vial sealable with a Teflon cap were added Cu powder (18.5 mg, 0.291 mmol, 1.3 equiv.), 2,2\textsuperscript{'}-bipyridyl (8.8 mg, 0.056 mmol, 0.25 equiv.) and corresponding diselenide or disulfide (0.134 mmol, 0.6 equiv.). The vessel was capped and then evacuated and backfilled with Ar three times. Triethylammonium salt (TEA\textsuperscript{+}) of dC\textsuperscript{4}TP (200 mg, 0.223 mmol, 1 equiv.) or a different iodinated dNTP in DMF (2.0 ml, degassed) was injected via a syringe and the vessel was evacuated and backfilled with Ar and the contents were sonicated. The reaction mixture was heated to 80 °C and vigorously stirred for 60 - 70 minutes. The reaction was quenched by slow addition of cold water (5 ml), the precipitate was filtered off and the filtrate evaporated to dryness \textit{in vacuo} at 34-36 °C. The product was purified by reverse phase HPLC [C-18, 0 → 50% MeOH in 0.1M aq. TEAB (triethylammonium bicarbonate); solid residue was dissolved in approx. 5 ml H\textsubscript{2}O and filtered through two syringe filters prior injection]. Several co-evaporations with water, conversion into a sodium form (Dowex 50WX8 in Na\textsuperscript{+} cycle followed by Chelex 100 resin in Na\textsuperscript{+} cycle\textsuperscript{[X3]}]) followed by freeze-drying from water afforded the product as a white, fluffy powder.

General procedure for sulfanylation of dC\textsuperscript{4}TP using copper(I) thiophenolate (Method B):

To a U-shaped microwave vial sealable with a Teflon cap were added PhSCu\textsuperscript{1} (55 mg, 0.318 mmol, 1.1 equiv.) and 2,2\textsuperscript{'}-bipyridyl (98.9 mg, 0.633 mmol, 2.2 equiv.). The vessel was capped, evacuated and backfilled with Ar three times and the solids were dissolved in DMF (2.6 ml, degassed). Triethylammonium salt (TEA\textsuperscript{+}) of dC\textsuperscript{4}TP (259 mg, 0.289 mmol, 1 equiv.) or a different iodinated dNTP in DMF (2.6 ml, degassed) was injected via a syringe and the vessel was evacuated and backfilled with Ar and the contents were sonicated. The reaction mixture was heated to 80 °C and vigorously stirred for 60 - 70 minutes. The reaction was quenched by slow addition of cold water (5 ml), the precipitate was filtered off and the filtrate evaporated to dryness \textit{in vacuo} at 34-36 °C. The product was purified by reverse phase HPLC [C-18, 0 → 50% MeOH in 0.1M aq. TEAB (triethylammonium bicarbonate); solid residue was dissolved in approx. 5 ml H\textsubscript{2}O and filtered through two syringe filters prior injection]. Several co-evaporations with water, conversion into a sodium form (Dowex 50WX8 in Na\textsuperscript{+} cycle followed by Chelex 100 resin in Na\textsuperscript{+} cycle\textsuperscript{[X3]})) followed by freeze-drying from water afforded the product as a white, fluffy powder.
5-Phenylsulfanyl-2'-deoxycytidine 5'-O-triphosphate sodium salt (dC<sup>PhS</sup>TP)

Method B - starting from PhSCu<sup>1</sup> (55 mg, 0.318 mmol, 1.1 equiv.), conditions: 80 °C, 1 hour. Yield: 12 mg (7 %).

<sup>1</sup>H NMR (500.0 MHz, D<sub>2</sub>O): 2.37 (ddd, 1H, <i>J</i><sub>gem</sub> = 14.0, <i>J</i><sub>2b,1′</sub> = 7.3, <i>J</i><sub>2b,3′</sub> = 6.3, H-2′b); 2.47 (ddd, 1H, <i>J</i><sub>gem</sub> = 14.0, <i>J</i><sub>2a,1′</sub> = 6.2, <i>J</i><sub>2a,3′</sub> = 3.5, H-2′a); 4.11 – 4.29 (m, 3H, H-4′,5′); 4.62 (dt, 1H, <i>J</i><sub>3′,2′</sub> = 6.3, 3.5, <i>J</i><sub>3′,4′</sub> = 3.5, H-3′); 6.29 (dd, 1H, <i>J</i><sub>1′,2′</sub> = 7.3, 6.2, H-1′); 7.25 – 7.31 (m, 3H, H-o,p-Ph); 7.36 (m, 2H, H-m-Ph); 8.32 (s, 1H, H-6).

<sup>13</sup>C NMR (125.7 MHz, D<sub>2</sub>O): 42.07 (CH<sub>-2′</sub>); 68.09 (d, <i>J</i><sub>C,P</sub> = 5.6, CH<sub>-5′</sub>); 73.44 (CH<sub>-3′</sub>); 88.36 (d, <i>J</i><sub>C,P</sub> = 8.8, CH-4′); 89.26 (CH-1′); 129.65 (CH<sub>-m-Ph</sub>); 132.20 (CH<sub>-i-Ph</sub>); 137.26 (C<sub>-i-Ph</sub>); 139.42 (CH<sub>-o-Ph</sub>); 141.45 (CH-6); 151.45 (CH-5); 159.71 (C<sub>-2</sub>); 168.42 (C<sub>-4</sub>).

<sup>31</sup>P{<sup>1</sup>H} NMR (202.3 MHz, D<sub>2</sub>O): -22.14 (t, <i>J</i> = 19.7, P<sub>β</sub>); -10.74 (d, <i>J</i> = 19.7, P<sub>α</sub>); -9.25 (bd, <i>J</i> = 19.7, P<sub>γ</sub>).

MS-ESI (C<sub>15</sub>H<sub>18</sub>O<sub>13</sub>N<sub>3</sub>NaP<sub>3</sub>S<sub>-</sub>) <i>m/z</i> (% int.) calcd: 596.3 [M-2H+Na]. Found: 596.0 [M-2H+Na] (25), 494.0 [M-PO<sub>3</sub>Na] (100).

HRMS-ESI (C<sub>15</sub>H<sub>18</sub>O<sub>13</sub>N<sub>3</sub>NaP<sub>3</sub>S<sub>-</sub>) <i>m/z</i> (% int.) calcd: 595.96764 [M-2H+Na]. Found: 595.96777 [M-2H+Na].

5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-O-triphosphate sodium salt (dC<sup>ThS</sup>TP)

Method A - reaction conditions: 2-thienyl disulfide (30.9 mg), 85 °C, 70 minutes. Yield: 34.5 mg (24 %).

<sup>1</sup>H NMR (500.0 MHz, D<sub>2</sub>O): 2.34 (dt, 1H, <i>J</i><sub>gem</sub> = 14.1, <i>J</i><sub>2b,1′</sub> = <i>J</i><sub>2b,3′</sub> = 6.7, H-2′b); 2.44 (ddd, 1H, <i>J</i><sub>gem</sub> = 14.1, <i>J</i><sub>2a,1′</sub> = 6.3, <i>J</i><sub>2a,3′</sub> = 3.7, H-2′a); 4.15 – 4.29 (m, 3H, H-4′,5′); 4.63 (dt, 1H, <i>J</i><sub>3′,2′</sub> = 6.7, 3.7, <i>J</i><sub>3′,4′</sub> = 3.7, H-3′); 6.23 (dd, 1H, <i>J</i><sub>1′,2′</sub> = 6.7, 6.3, H-1′); 7.05 (dd, 1H, <i>J</i><sub>4′,5′</sub> = 5.4, <i>J</i><sub>3′,4′</sub> = 3.6, H-4-thienyl); 7.34 (dd, 1H, <i>J</i><sub>3′,4′</sub> = 3.6, <i>J</i><sub>3′,5′</sub> = 1.3, H-3-thienyl); 7.49 (dd, 1H, <i>J</i><sub>5′,4′</sub> = 5.4, <i>J</i><sub>5′,3′</sub> = 1.3, H-5-thienyl); 8.35 (s, 1H, H-6).
\(^{13}\text{C}\) NMR (125.7 MHz, D\(_2\)O): 42.06 (CH\(_2\)-2'); 67.95 (d, \(J_{\text{C,P}} = 5.6, \text{CH}_2\)-5'); 73.17 (CH-3'); 88.46 (d, \(J_{\text{C,P}} = 8.7, \text{CH}\)-4'); 89.15 (CH-1'); 104.86 (br, C-5); 130.81 (CH-4-thienyl); 132.74 (CH-5-thienyl); 135.38 (CH-3-thienyl); 135.67 (C-2-thienyl); 150.40 (CH-6); 159.50 (C-2); 167.97 (C-4).

\(^{31}\text{P}\{^{1}\text{H}\}\) NMR (202.3 MHz, D\(_2\)O): -22.26 (t, \(J = 19.8, \text{P}\beta\)); -11.35 (d, \(J = 19.8, \text{P}\alpha\)); -7.59 (bd, \(J = 19.8, \text{P}\gamma\)).

MS-ESI (C\(_{13}\)H\(_{16}\)O\(_3\)NaP\(_3\)S\(_2\)\(^{-}\)) \text{m/z} (%) int. calcd: 601.9 \([\text{M}-2\text{H}+\text{Na}]\). Found: 601.9 \([\text{M}-2\text{H}+\text{Na}]\) (10), 499.9 \([\text{M}-\text{PO}_3\text{Na}]\) (100).

HRMS-ESI (C\(_{13}\)H\(_{15}\)O\(_3\)Na\(_2\)P\(_3\)S\(_2\)\(^{-}\)) \text{m/z} (%) int. calcd: 601.92406 \([\text{M}-2\text{H}+\text{Na}]\). Found: 601.92290 \([\text{M}-2\text{H}+\text{Na}]\).

\(^{77}\text{Se}\) NMR (95.4 MHz, D\(_2\)O): 286.49.

MS-ESI (C\(_{15}\)H\(_{19}\)O\(_{13}\)N\(_3\)P\(_3\)Se\(^{-}\)) \text{m/z} (%) int. calcd: 621.2 \([\text{M-H}]\). Found: 621.9 \([\text{M-H}]\) (100).

HRMS-ESI (C\(_{15}\)H\(_{19}\)O\(_{13}\)N\(_3\)P\(_3\)Se\(^{-}\)) \text{m/z} (%) int. calcd: 621.93014 \([\text{M-H}]\). Found: 621.93038 \([\text{M-H}]\).

5-Phenylselanyl-2'-deoxycytidine 5'-O-triphosphate sodium salt (dC\(^{\text{PhSe}}\)TP)

Method A - starting from (PhSe)$_2$ (41.9 mg), conditions: 80 °C, 1 hour. Yield: 46.8 mg (30.5%).

\(^{1}\text{H}\) NMR (500.0 MHz, D\(_2\)O): 2.37 (ddd, 1H, \(J_{\text{gem}} = 14.0, J_{2b,1'} = 7.1, J_{2b,3'} = 6.5, \text{H-2'b}\)); 2.46 (ddd, 1H, \(J_{\text{gem}} = 14.0, J_{2a,1'} = 6.3, J_{2a,3'} = 3.9, \text{H-2'a}\)); 4.16 – 4.28 (m, 3H, H-4',5'); 4.63 (dt, 1H, \(J_{3',2'} = 6.5, 3.9, J_{3',4'} = 3.9, \text{H-3'}\)); 6.27 (dd, 1H, \(J_{1',2'} = 7.1, 6.3, \text{H-1'}\)); 7.26-7.37 (m, 3H, H-\(m,p\)-Ph); 7.44 (m, 2H, H-\(o\)-Ph); 8.32 (s, 1H, H-6).

\(^{13}\text{C}\) NMR (125.7 MHz, D\(_2\)O): 41.85 (CH\(_2\)-2'); 66.89 (d, \(J_{\text{C,P}} = 5.3, \text{CH}_2\)-5'); 73.11 (CH-3'); 88.30 (d, \(J_{\text{C,P}} = 8.4, \text{CH}\)-4'); 89.06 (CH-1'); 97.52 (C-5); 130.15 (CH-\(p\)-Ph); 132.43 (CH-\(m\)-Ph); 132.55 (C-\(i\)-Ph); 132.69 (CH-\(o\)-Ph); 151.83 (CH-6); 159.87 (C-2); 168.48 (C-4).

\(^{31}\text{P}\{^{1}\text{H}\}\) NMR (202.3 MHz, D\(_2\)O): -21.25 (t, \(J = 19.2, \text{P}\beta\)); -11.00 (d, \(J = 19.2, \text{P}\alpha\)); -5.52 (d, \(J = 19.2, \text{P}\gamma\)).

\(^{77}\text{Se}\) NMR (95.4 MHz, D\(_2\)O): 286.49.

MS-ESI (C\(_{15}\)H\(_{19}\)O\(_{13}\)N\(_3\)P\(_3\)Se\(^{-}\)) \text{m/z} (%) int. calcd: 621.2 \([\text{M-H}]\). Found: 621.9 \([\text{M-H}]\) (100).

HRMS-ESI (C\(_{15}\)H\(_{19}\)O\(_{13}\)N\(_3\)P\(_3\)Se\(^{-}\)) \text{m/z} (%) int. calcd: 621.93014 \([\text{M-H}]\). Found: 621.93038 \([\text{M-H}]\).
Biochemistry

Table S1: List of ON sequences used in this study

<table>
<thead>
<tr>
<th>Oligo</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer248-sh</td>
<td>5’-CATGGGCGGCATGGG-3’</td>
</tr>
<tr>
<td>tempoligo1C</td>
<td>5’-CCCGCCATGCCGCCCATG-3’</td>
</tr>
<tr>
<td>tempPrb4baseII</td>
<td>5’-CTAGCATGAGCTGCCCATGCCGCCCATG-3’</td>
</tr>
<tr>
<td>primerLT25TH</td>
<td>5’-CAAGGACAAAAATACCTGTATTCCCTT-3’</td>
</tr>
<tr>
<td>primerL20</td>
<td>5’-GACATCATGAGAGACATCG-3’</td>
</tr>
<tr>
<td>tempFVL-A</td>
<td>5’-GACATCATGAGAGACATCGCCTCTGGAAGCAATAGGACTACTTTATTITGTCCCTTG-3’</td>
</tr>
</tbody>
</table>

Primer extension - 19-mer template

PEX reactions with dCPhsTP, dCPhSeTP or dCThsTP as substrates were performed in presence of a DNA polymerase (KOD XL, Vent(exo−) or Pwo). The reaction mixture (20 μl) contained KOD XL DNA polymerase (0.05 U), Vent (exo−) DNA Polymerase (0.16 U) or Pwo polymerase (0.2 U), dGTP (20 μM), either dCTP or dC\textsuperscript{R}XTP (20 μM), 5’-FAM labelled primer\textsuperscript{248-sh} (0.5 μM) and 19-mer template (temp\textsuperscript{oligo1C}, 0.75 μM) in buffer (2 μl) supplied by the manufacturer. Reaction mixtures were incubated for 30 min at 60°C. Before gel loading samples were denatured by addition 20 ul of stop solution (80% [v/v] formamide, 20 mM EDTA, 0.025%, [w/v] bromophenol blue, 0.025 % [w/v] xylene cyanol, MilliQ water) and heated for 5 min at 95°C. Reaction mixtures were separated using 12.5 % denaturing PAGE. Visualization was performed by fluorescence imaging.

Primer extension - 31-mer template

PEX reactions with 31-template (temp\textsuperscript{Prb4baseII}) were performed in the same way as above using KOD XL polymerase (0.25 U), Vent (exo−) polymerase (0.2 U) or Pwo polymerase (0.2 U), dGTP, dATP and TTP (200 μM each), dCTP or dC\textsuperscript{R}XTP (200 μM), 5’-FAM labelled primer (primer\textsuperscript{248-sh}, 0.5 μM) and 31-mer template (temp\textsuperscript{Prb4baseII}, 0.75 μM) in buffer (2 μl) supplied by the manufacturer.
Polymerase chain reaction

PCR reactions with \( \text{dC}^{\text{PhS TP}}, \text{dC}^{\text{PhSe TP}} \) or \( \text{dC}^{\text{ThS TP}} \) as substrates were performed using KOD XL polymerase. The PCR reaction mixture (20 μl) contained KOD XL (0.25 U for positive control and \( \text{dC}^{\text{ThS TP}} \) or 1.75 U for \( \text{dC}^{\text{PhS TP}}, \text{dC}^{\text{PhSe TP}} \)), dGTP, dATP and TTP (300 μM each), either dCTP or \( \text{dC}^{\text{RX TP}} \) (300 μM), primers (primer\( ^{\text{LT25TH}} \) and primer\( ^{\text{L20}} \), 1 μM each) and 98-mer template (temp\( ^{\text{FVL-A}} \), 0.025 μM) in reaction buffer (2 μl) supplied by the manufacturer. Thirty PCR cycles were run under the following conditions: denaturation for 1 min at 95°C, annealing for 1 min at 53°C, extension for 1 min at 72°C, followed by final extension step of 2 min at 75°C.

a) Reaction mixtures were than separated without purification by use of a 2 % agarose gel with GelRed as an intercalator. Visualization was performed by fluorescence imaging.

Figure S1: PCR experiments using KOD XL DNA polymerase. Lanes 1,7 , L: ladder ; lane 2, C\(^+\): products of PEX with natural dNTPs; lane 3, C\(^-\): products of PEX with dTTP, dATP, dGTP; lanes 4-6, C\(^{RX}\): products of PCR with dTTP, dATP, dGTP and functionalized \( \text{dC}^{\text{RX TP}} \).

b) Reaction mixtures were purified using QIAquick system according to protocol. In the last step, PCR products were eluted from spin column by either 30 μl (in the case of \( \text{C}^{+}, \text{C}^{-}, \text{C}^{\text{ThS}}, \text{C}^{\text{PhS}}, \text{C}^{\text{PhSe}} \)) of MilliQ water. The final concentration of prepared modified DNAs was quantified with NanoDrop instrument: 41.1 ng/μl (\( \text{C}^{+} \)), 45.2 ng/μl (\( \text{C}^{\text{ThS}} \)), 42.0 ng/μl (\( \text{C}^{\text{PhS}} \)), 28.3 ng/μl (\( \text{C}^{\text{PhSe}} \)). Samples were than separated by use of a 2 % agarose gel with GelRed as an intercalator. Visualization was performed by fluorescence imaging.
**Figure S2:** PCR experiments using KOD XL DNA polymerase. Lanes 1, 7, L: ladder; lane 2, C+: products of PEX with natural dNTPs; lane 3, C⁻: products of PEX with dTTP, dATP, dGTP; lanes 4-6, CRX: products of PCR with dTTP, dATP, dGTP and functionalized dCRXTP.

**Optimization of polymerase chain reaction for dCPhSeTP**

PCR reactions with 98-mer template (tempFVL-A) were performed in 20 µl in the same way as in previous experiment only the additives were added in the reaction mixture or higher concentration of dCPhSeTP was used. Reaction mixtures were purified again using QIAquick system according to protocol. In the last step, PCR products were eluted from spin column by either 30 µl (in the case of CPhSe1, CPhSe2, CPhSe3, CPhSe4, CPhSe5) of MilliQ water. The final concentration of prepared modified DNAs was quantified with NanoDrop instrument.

a) To the reaction mixture (total volume 20 µl) were added additives: DMSO (100 %, 0.5 µl), formamide (5 %, 0.5 µl), betaine (0.75 M, 0.5 µl), TMAC (tetramethylammonium chloride, 50 mM, 0.5 µl). Concentration of PCR product was 38.6 ng/µl (CPhSe1).

b) To the reaction mixture (total volume 20 µl) was added additive: MgSO₄ (100 mM, 0.5 µl). Concentration of PCR product was 44.1 ng/µl (CPhSe2).

c) Concentration of dCPhSeTP in reaction mixture (20 µl) was increased to 450 µM. Concentration of PCR product was 42.6 ng/µl (CPhSe3).
d) Concentration of $dC^{PhSe}TP$ in reaction mixture (20 μl) was increased to 600 μM. Concentration of PCR product was 34.5 ng/μl ($C^{PhSe4}$).

e) Concentration of $dC^{PhSe}TP$ in reaction mixture (20 μl) was increased to 900 μM. Concentration of PCR product was 36.2 ng/μl ($C^{PhSe5}$).

Samples were then separated by use of a 2% agarose gel with GelRed as an intercalator. Visualization was performed by fluorescence imaging.

![KOD XL](image)

**Figure S3:** PCR experiments using KOD XL DNA polymerase. Lanes 1,10, L: ladder; lane 2, C+: products of PEX with natural dNTPs; lane 3, C: products of PEX with dTTP, dATP, dGTP; lane 4, $C^{PhSe}$: products of PCR without additives (dTTP, dATP, dGTP, $dC^{PhSe}TP$); lane 5, $C^{PhSe1}$: products of PCR with using additives - DMSO, TMAC, betain, formamide; lane 6, $C^{PhSe2}$: products of PCR with using $MgSO_4$ as additive; lane 7, $C^{PhSe3}$: products of PCR with increasing concentration of $dC^{PhSe}TP$ (1.5x); lane 8, $C^{PhSe4}$: products of PCR with increasing concentration of $dC^{PhSe}TP$ (2x); lane 9, $C^{PhSe5}$: products of PCR with increasing concentration of $dC^{PhSe}TP$ (3x)

**Preparation of ONs for MALDI-TOF Analysis - 31-mer template**

The reaction mixture (60 μl) contained primer (primer$_{24k-31}$, 3.3 μM), a biotinylated or 31-mer template (5’-biotinylated temp$_{31}$, 3.3 μM), KOD XL DNA polymerase (0.25 U), dGTP, dATP and TTP (220 μM each), and $dC^{RX}TP$ (220 μM), in enzymes reaction buffer supplied by the manufacturer (6 μl). The reaction mixture was shaken (300 rpm) for 40 min at 60 °C in a thermal cycler. Streptavidin magnetic particles stock solution (Roche, 60 μL) was washed with binding buffer (3 x 200 μL, 10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.5). The PEX
solution (prepared as described above) and binding buffer (100 μl) were added. Suspension was shaken (1200 rpm) for 30 min at 15 °C. The magnetic beads were collected on a magnet (DynaMag-2, Invitrogen) and washed with wash buffer (3 × 200 μl, 10 mM Tris, 1 mM EDTA, 500 mM NaCl, pH 7.5) and water (5 × 200 μl). Then water (50 μL) was added and the sample was denatured for 2 min at 55 °C and 900 rpm. The beads were collected on a magnet and the solution was transferred into a clean vial. The product was evaporated to dryness, then dissolved in the mixture of water/acetonitrile (1:1, 5 ul) and analyzed by MALDI-TOF mass spectrometry.
Copies of NMR spectra

5-Phenylsulfanyl-2'-deoxycytidine

BOTHAM FBH-24
1H NMR in DMSO-d6
07-11-14 RA

BOTHAM FBH-24
APT in DMSO-d6
07-11-14 RA
5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine
5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine
5-[(2,4-Dinitrophenyl)sulanyl]-2'-deoxycytidine
5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine
5-Phenylselanyl-2'-deoxycytidine

[Chemical structure diagram]

S33
BOTHAN FBH-22
77Se NMR in DMSO-d6
04-11-14 RA

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5-Methylselanyl-2'-deoxycytidine
BOTH A FBH-49
77Se NMR in DMSO-d6
09-04-15 RA

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90.4 90.2 90.0 89.8 89.6 89.4 89.2 89.0
77Se (ppm)

12
10
8
6
4
2
0

-160 -140 -120 -100 -80 -60 -40 -20
77Se (ppm)
5-Phenylsulfanyl-2'-deoxyuridine
5-[(2-Thienyl)sulfanyl]-2'-deoxyuridine
5-Phenylselanyl-2'-deoxyuridine
5-Methylselanyl-2'-deoxyuridine
7-Phenylsulfanyl-7-deaza-2'-deoxyadenosine
7-Phenylselanyl-7-deaza-2'-deoxyadenosine

BOTH A FBH-127
1H NMR in DMSO-d6
14-03-16 RA

BOTH A FBH-127
APT in DMSO-d6
14-03-16 RA
BOTH A FBH-127
77Se NMR in DMSO-d6
14-03-16 RA

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400 350 300 250 200 150 100 50
---

77Se (ppm)

---

0 5 10 15
---

S45
7-Methylselanyl-7-deaza-2'-deoxyadenosine
7-Phenylsulfanyl-7-deaza-2’-deoxyguanosine

BOTHKA FBH-135
1H NMR in DMSO-d6
30-03-16 RA

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BOTHKA FBH-135
APT in DMSO-d6
30-03-16 RA

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S48
7-Phenylselanyl-7-deaza-2'-deoxyguanosine
5-Phenylsulfanyl-2'-deoxycytidine 5'-O-monophosphate sodium salt
S52
5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine 5’-O-monophosphate sodium salt
5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine 5'-O-monophosphate sodium salt
BOTHA FBH-67
31P(1H) NMR in D2O
14-05-15 RA

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5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-O-monophosphate
BOTH A FBH-52
31P(1H) in D2O
03-03-15 RA

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5-Phenylselanyl-2'-deoxycytidine 5'-O-monophosphate sodium salt
5-Methylselanyl-2'-deoxycytidine 5’-O-monophosphate sodium salt
5-Phenylsulfanyl-2'-deoxycytidine 5'-O-triphosphate sodium salt

![Chemical Structure](attachment:image.png)

**BOTH FAH-131**
1H NMR in D2O
21-04-16 RA

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**BOTH FAH-131**
APT in D2O
21-04-16 RA

---
5-Phenylselanyl-2'-deoxycytidine 5'-O-triphosphate sodium salt

BOTHAS FBBH-35
1H NMR in D2O
27-03-15 RA

BOTHAS FBBH-35
APT in D2O
27-03-15 RA
5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-O-triphosphate sodium salt
MALDI-TOF experiments of PEX products for Prb4Basell

Figure S4: MALDI spectrum of ON4ThS (dCThS TP). M (calc.) = 10073.2; M (found) = 10075.1 [M+2H]^+, 9948.2 [M-T]^+

Figure S5: MALDI spectrum of ON4PhS (dCPhS TP). M (calc.) = 10049.3; M (found) = 10050.8 [M+H]^+, 9924.0 [M-T]^+
Figure S6: MALDI spectrum of ON$_4$$^\text{PhSe}$ ($\text{dC}^\text{PhSeTP}$). $M$ (calc.) = 10241.1; $M$ (found) = 10240.2 [M]$^+$, 10113.3 [M-T]$^+$, 9856.6 [template]$^+$
References