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, $CHCl_3$ was distilled prior column chromatography. $PO(OMe)_3$ was dried, distilled and $POCl_3$ distilled using conventional methods prior use. All compounds were fully characterized by NMR spectroscopy and the spectra were recorded on a Bruker Avance-IIIHD 600 (^{1}H at 600.1 MHz, and ^{13}C at 150.9 MHz) or on a Bruker Avance-IIIHD 500 (500.0 MHz for ^{1}H, 125.7 MHz for ^{13}C, 202.3 MHz for ^{31}P and 95.4 MHz for ^{77}Se) spectrometer. ^{1}H and ^{13}C resonances were assigned using H,H-COSY, H,C-HSQC and H,C-HMBC 2D NMR spectra. The samples were measured in DMSO-d_6 or in D_2O and chemical shifts (δ-scale, in ppm) were referenced to residual solvent signal (DMSO (δ (^{1}H) = 2.50 ppm, δ (^{13}C) = 39.70 ppm) or to 1,4-dioxane as external standard in the case of D_2O solutions (δ (^{1}H) = 3.75 ppm, δ (^{13}C) = 69.30 ppm); ^{31}P spectra were referenced to H_3PO_4 as an external standard (δ (^{31}P) = 0 ppm) and ^{77}Se spetra were referenced to Me_2Se as an external standard (δ (^{77}Se) = 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 F_{254} (Merck) and analysed at 254 and/or 365 nm. Column chromatography was performed using Silica gel Geduram 60 F_{254} (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^I) (Method A):

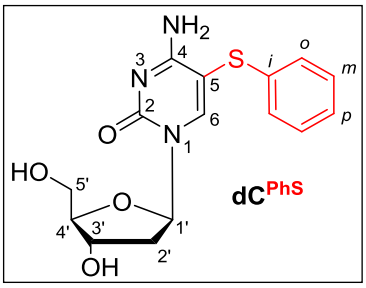
To a U-shaped microwave vial sealable with a Teflon cap were added **dN^I** (0.255 mmol, 1 equiv.), Cu powder (16.2 mg, 0.255 mmol, 1 equiv.), 2,2'-bipyridyl (9.9 mg, 0.063 mmol, 0.25 equiv.) and corresponding diselenide or disulfide (0.140 mmol, 0.55 equiv.). The vessel was capped and then evacuated 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_2 using $CHCl_3$/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_2 by co-evaporation from $CHCl_3$/MeOH, and purified by column chromatography (SiO_2, $CHCl_3$/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_2O=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^I) using copper(I) thiophenolate (Method B):

To a U-shaped microwave vial sealable with a Teflon cap were added **dN^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_2 ($CHCl_3$/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_2 by co-evaporation from $CHCl_3$/MeOH, and purified by column chromatography (SiO_2, $CHCl_3$/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_2O=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 (dC^{PhS})



Method A - starting from $(PhS)_2$ (31 mg), conditions: 110 °C, 6 hours. Yield: 50 mg (58 %). White foam.

Method B - starting from $PhSCu^{I}$ (48.6 mg), conditions: 100 °C, 1.5 hours. Yield: 48 mg (56 %). White foam.

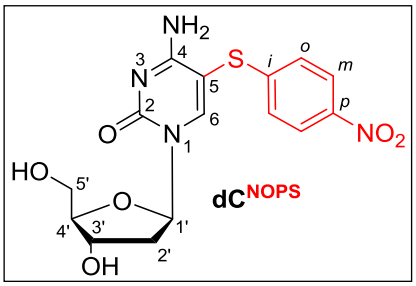
^{1}H NMR (500.0 MHz, DMSO-d_6): 2.06 (dt, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.3, H-2′b); 2.21 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.9, H-2′a); 3.54 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 4.9, $J_{5'b,4'}$ = 3.4, H-5′b); 3.62 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 4.9, $J_{5'a,4'}$ = 3.4, H-5′a); 3.81 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.4, H-4′); 4.22 (m, 1H, H-3′); 5.09 (t, 1H, $J_{OH,5'}$ = 4.9, OH-5′); 5.24 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3′); 6.11 (t, 1H, $J_{1',2'}$ = 6.3, H-1′); 6.93 (bs, 1H, NH$_a$**H$_b$**); 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, N**H$_a$**H$_b$); 8.43 (s, 1H, H-6).

^{13}C NMR (125.7 MHz, DMSO-d_6): 41.47 (CH_2-2′); 61.30 (CH_2-5′); 70.38 (CH-3′); 86.11 (CH-1′); 87.95 (CH-4′); 94.42 (C-5); 126.47 (CH-*p*-Ph); 126.85 (CH-*o*-Ph); 129.67 (CH-*m*-Ph); 136.59 (C-*i*-Ph); 149.66 (CH-6); 154.75 (C-2); 165.41 (C-4).

MS-ESI ($C_{15}H_{17}O_4N_3S$) *m/z* (% int.) calcd: 358.4 $[M + Na]^+$. Found: 358.1 $[M + Na]^+$ (100), 693.3 $[2M + Na]^+$ (60).

HRMS-ESI ($C_{15}H_{17}O_4N_3S$) *m/z* (% int.) calcd: 358.08320 $[M + Na]^+$. Found: 358.08331 $[M + Na]^+$.

5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine (dC^{NOPS})



Method A - starting from $(4\text{-}NO_2\text{-}PhS)_2$ (43.2 mg), conditions: 80 °C, 4.5 hours. Yield: 48.5 mg (50 %). Yellowish microcrystals.

^{1}H NMR (600.1 MHz, DMSO-d_6): 2.09 (dt, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.2, H-2′b); 2.23 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 4.1, H-2′a); 3.52 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.0, $J_{5'b,4'}$ = 3.5, H-5′b); 3.60 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.5, H-5′a); 3.81 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.5, H-4′); 4.21 (dddd, 1H, $J_{3',2'}$ = 6.2, 4.1, $J_{3',OH}$ = 4.4, $J_{3',4'}$ = 3.5, H-3′); 5.03 (t, 1H, $J_{OH,5'}$ = 5.0, OH-5′); 5.22 (d, 1H, $J_{OH,3'}$ = 4.4, OH-3′); 6.10 (t, 1H,

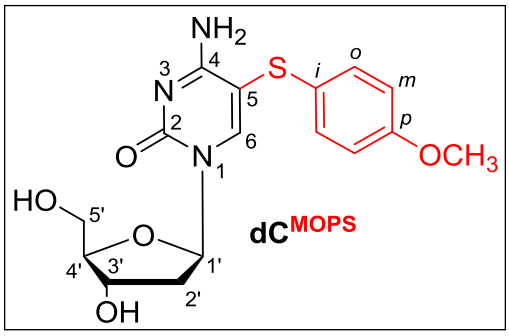
$J_{1',2'}$ = 6.2, H-1′); 7.07 (bs, 1H, NH$_a$**H$_b$**); 7.34 (m, 2H, H-*o*-C$_6$H$_4$NO$_2$); 7.70 (bs, 1H, N**H$_a$**H$_b$); 8.15 (m, 2H, H-*m*-C$_6$H$_4$NO$_2$); 8.46 (s, 1H, H-6).

^{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$_6$N$_4$S) *m/z* (% int.) calcd: 403.4 [M + Na]$^+$. Found: 403.1 [M + Na]$^+$ (100), 783.3 [2M + Na]$^+$ (30).

HRMS-ESI (C$_{15}$H$_{16}$O$_6$N$_4$S) *m/z* (% int.) calcd: 403.06828 [M + Na]$^+$. Found: 403. 06835 [M + Na]$^+$.

5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine (dC^{MOPS})



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

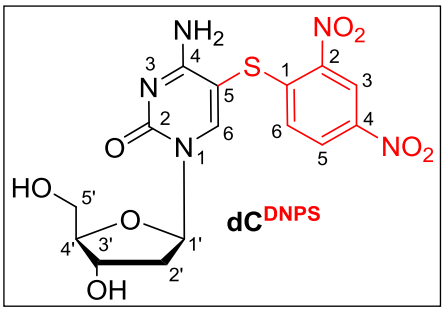
^{1}H NMR (600.1 MHz, DMSO-*d*$_6$): 2.05 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.7, $J_{2'b,3'}$ = 5.9, H-2′b); 2.20 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 3.9, H-2′a); 3.57, 3.64 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 4.8, $J_{5',4'}$ = 3.5, H-5′); 3.72 (s, 3H, CH$_3$O); 3.82 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 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$_a$**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, N**H$_a$**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]$^+$.

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.

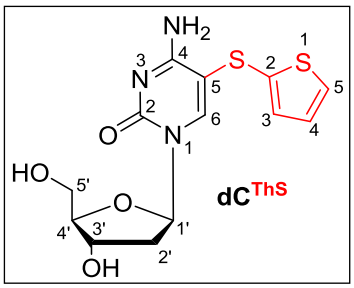
^{1}H NMR (500.0 MHz, DMSO-d_6): 2.12 (dt, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.2, H-2′b); 2.25 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 4.3, H-2′a); 3.53 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.0, $J_{5'b,4'}$ = 3.5, H-5′b); 3.62 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.5, H-5′a); 3.82 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.5, H-4′); 4.22 (dddd, 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$_a$**H$_b$**); 7.47 (d, 1H, $J_{6,5}$ = 9.0, H-6-$C_6H_3(NO_2)_2$); 7.67 (bs, 1H, N**H$_a$**H$_b$); 8.40 (dd, 1H, $J_{5,6}$ = 9.0, $J_{5,3}$ = 2.5, H-5-$C_6H_3(NO_2)_2$); 8.53 (s, 1H, H-6); 8.88 (d, 1H, $J_{3,5}$ = 2.5, H-3-$C_6H_3(NO_2)_2$).

^{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-$C_6H_3(NO_2)_2$); 127.82 (CH-5-$C_6H_3(NO_2)_2$); 128.63 (CH-6-$C_6H_3(NO_2)_2$); 144.68 (C-4-$C_6H_3(NO_2)_2$); 145.13 (C-1-$C_6H_3(NO_2)_2$); 145.32 (C-2-$C_6H_3(NO_2)_2$); 150.50 (CH-6); 154.36 (C-2); 164.18 (C-4).

MS-ESI ($C_{15}H_{15}N_5O_8S$) *m/z* (% int.) calcd: 448.4 $[M + Na]^+$. Found: 448.1 $[M + Na]^+$ (100), 873.1 $[2M + Na]^+$ (55).

HRMS-ESI ($C_{15}H_{15}N_5O_8S$) *m/z* (% int.) calcd: 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, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.2, H-2′b); 2.19 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.9, H-2′a); 3.59 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 4.8, $J_{5'b,4'}$ = 3.5, H-5′b); 3.66 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 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,

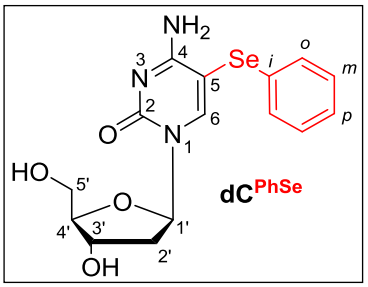
$J_{1',2'}$ = 6.2, H-1′); 7.01 (dd, 1H, $J_{4,5}$ = 5.3, $J_{4,3}$ = 3.6, H-4-thienyl); 7.16 (bs, 1H, $NH_a\mathbf{H_b}$); 7.32 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.3, H-3-thienyl); 7.58 (dd, 1H, $J_{5,4}$ = 5.3, $J_{5,3}$ = 1.3, H-5-thienyl); 7.83 (bs, 1H, $N\mathbf{H_a}H_b$); 8.50 (s, 1H, H-6).

^{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 ($C_{13}H_{15}O_4N_3S_2$) *m/z* (% int.) calcd: 364.4 $[M + Na]^+$. Found: 364.2 $[M + Na]^+$ (100), 705.5 $[2M + Na]^+$ (80).

HRMS-ESI ($C_{13}H_{15}O_4N_3S_2$) *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, $NH_a\mathbf{H_b}$); 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, $N\mathbf{H_a}H_b$); 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); 154.62 (C-2); 165.18 (C-4).

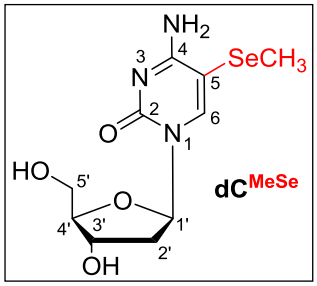
^{77}Se NMR (95.4 MHz, DMSO-d_6): 283.80.

MS-ESI ($C_{15}H_{17}O_4N_3Se$) *m/z* (% int.) calcd: 406.0 $[M + Na]^+$. Found: 406.1 $[M + Na]^+$ (100), 787.2 $[2M + Na]^+$ (10).

HRMS-ESI ($C_{15}H_{17}O_4N_3Se$) *m/z* (% int.) calcd: 406.02765 $[M + Na]^+$. Found: 406. 02783 $[M + Na]^+$.

5-Methylselanyl-2'-deoxycytidine (dC^{MeSe})

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 H_2O. Yield: 15 mg (18 %). White microcrystals.



^{1}H NMR (500.0 MHz, DMSO-d_6): 2.00 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 6.0, H-2′b); 2.11 (s, 3H, CH_3Se); 2.13 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.7, H-2′a); 3.54 (dd, 1H, J_{gem} = 11.8, $J_{5'b,4'}$ = 3.7, H-5′b); 3.61 (dd, 1H, J_{gem} = 11.8, $J_{5'a,4'}$ = 3.7, H-5′a); 3.78 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.7, H-4′); 4.21 (dt, 1H, $J_{3',2'}$ = 6.0, 3.7, $J_{3',4'}$ = 3.7, H-3′); 6.10 (dd, 1H, $J_{1',2'}$ = 7.0, 6.1, H-1′); 6.78, 7.69 (2 × bs, 2 × 1H, NH_2); 8.20 (s, 1H, H-6).

^{13}C NMR (125.7 MHz, DMSO-d_6): 9.41 (CH_3Se); 40.94 (CH_2-2′); 61.24 (CH_2-5′); 70.33 (CH-3′); 85.42 (CH-1′); 87.55 (CH-4′); 92.35 (C-5); 147.26 (CH-6); 154.72 (C-2); 165.18 (C-4).

^{77}Se NMR (95.4 MHz, DMSO-d_6): 89.74 (q, $J_{Se,H}$ = 10.9).

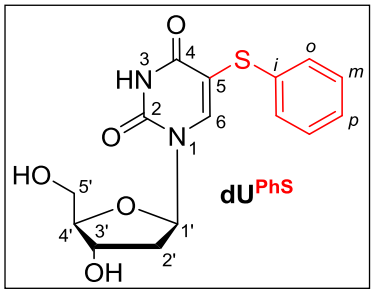
MS-ESI ($C_{10}H_{15}O_4N_3Se$) *m/z* (% int.) calcd: 344.0 $[M + Na]^+$. Found: 344.1 $[M + Na]^+$ (100), 665.3 $[2M + Na]^+$ (50).

HRMS-ESI ($C_{10}H_{15}O_4N_3Se$) *m/z* (% int.) calcd: 344.01200 $[M + Na]^+$. Found: 344.01215 $[M + Na]^+$.

5-Phenylsulfanyl-2'-deoxyuridine (dU^{PhS})[S1]

Method B - starting from $PhSCu^I$ (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.



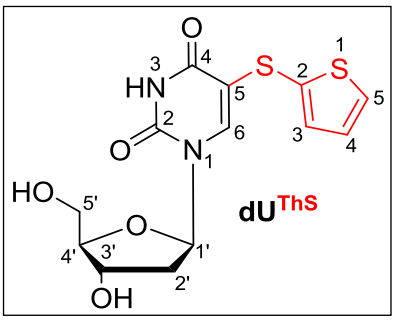
^{1}H NMR (500.0 MHz, DMSO-d_6): 2.16 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 6.5, $J_{2'b,3'}$ = 4.5, H-2′b); 2.20 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.7, $J_{2'a,3'}$ = 5.4, H-2′a); 3.54, 3.60 (2 × bdd, 2 × 1H, J_{gem} = 11.8, $J_{5',4'}$ = 3.4, H-5′); 3.81 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 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, $J_{1',2'}$ = 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).

^{13}C NMR (125.7 MHz, DMSO-d_6): 40.50 (CH_2-2′); 61.03 (CH_2-5′); 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).

MS-ESI ($C_{15}H_{16}O_5N_2S$) *m/z* (% int.) calcd: 359.4 $[M + Na]^+$. Found: 359.0 $[M + Na]^+$ (100), 695.1 $[2M + Na]^+$ (35).

HRMS-ESI ($C_{15}H_{16}O_5N_2S$) *m/z* (% int.) calcd: 359.06721 $[M + Na]^+$. Found: 359.06730 $[M + Na]^+$.

5-[(2-Thienyl)sulfanyl]-2'-deoxyuridine (dU^{ThS})



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

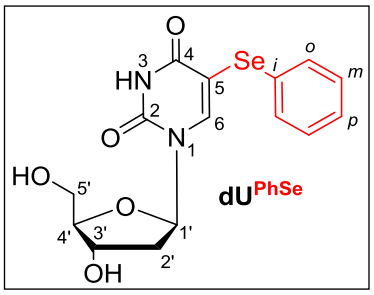
^{1}H NMR (500.0 MHz, DMSO-d_6): 2.08 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 5.9, H-2′b); 2.14 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.6, H-2′a); 3.51 (bdd, 1H, J_{gem} = 11.7, $J_{5'b,4'}$ = 3.6, H-5′b); 3.56 (bdd, 1H, J_{gem} = 11.7, $J_{5'a,4'}$ = 3.6, 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).

^{13}C NMR (125.7 MHz, DMSO-d_6): 40.35 (CH_2-2′); 61.18 (CH_2-5′); 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-6); 150.14 (C-2); 161.18 (C-4).

MS-ESI ($C_{13}H_{14}O_5N_2S_2$) *m/z* (% int.) calcd: 365.4 $[M + Na]^+$. Found: 365.0 $[M + Na]^+$ (100), 707.0 $[2M + Na]^+$ (90).

HRMS-ESI ($C_{13}H_{14}O_5N_2S_2$) *m/z* (% int.) calcd: 365.02363 $[M + Na]^+$. Found: 365.02377 $[M + Na]^+$.

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).

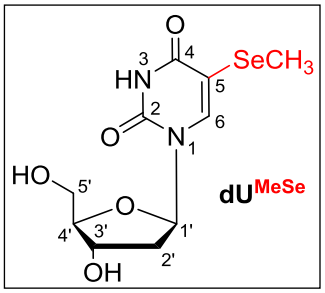
^{13}C NMR (125.7 MHz, DMSO-d_6): 40.33 (CH_2-2′); 61.15 (CH_2-5′); 70.34 (CH-3′); 85.01 (CH-1′); 87.73 (CH-4′); 101.36 (C-5); 126.86 (CH-*p*-Ph); 129.51 (CH-*m*-Ph); 130.20 (CH-*o*-Ph); 131.04 (C-*i*-Ph); 146.07 (CH-6); 150.56 (C-2); 161.67 (C-4).

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

MS-ESI ($C_{15}H_{16}O_5N_2Se$) *m/z* (% int.) calcd: 407.0 $[M + Na]^+$. Found: 407.1 $[M + Na]^+$ (100), 791.0 $[2M + Na]^+$ (40).

HRMS-ESI ($C_{15}H_{16}O_5N_2Se$) *m/z* (% int.) calcd: 407.01166 $[M + Na]^+$. Found: 407.01184 $[M + Na]^+$.

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_2O. 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_3Se); 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, $J_{3',2'}$ = 5.6, 3.6, $J_{3',4'}$ = 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).

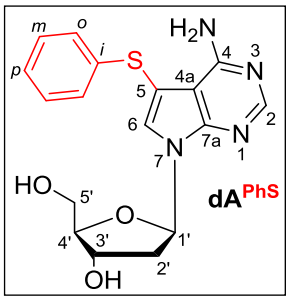
^{13}C NMR (125.7 MHz, DMSO-d_6): 5.42 (CH_3Se); 40.24 (CH_2-2′); 61.26 (CH_2-5′); 70.57 (CH-3′); 84.73 (CH-1′); 87.70 (CH-4′); 104.21 (C-5); 137.75 (CH-6); 150.23 (C-2); 161.87 (C-4).

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

MS-ESI ($C_{10}H_{14}O_5N_2Se$) *m/z* (% int.) calcd: 345.0 $[M + Na]^+$. Found: 345.0 $[M + Na]^+$ (100), 667.0 $[2M + Na]^+$ (35).

HRMS-ESI ($C_{10}H_{14}O_5NaN_2Se$) *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^I (45.7 mg), conditions: 110 °C, 1.5 hours. Yield: 34 mg (40 %). White foam.

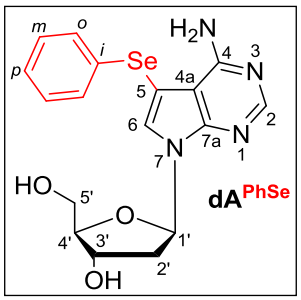
^{1}H NMR (500.0 MHz, DMSO-d_6): 2.23 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.8, H-2′b); 2.54 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.0, $J_{2'a,3'}$ = 5.8, H-2′a); 3.53 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.9, $J_{5'b,4'}$ = 4.2, H-5′b); 3.60 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 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 (dddd, 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 ($C_{17}H_{18}O_3N_4S$) *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 $[M + Na]^+$.

7-Phenylselanyl-7-deaza-2'-deoxyadenosine (dA^{PhSe})



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, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.7, H-2′b); 2.55 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.1, $J_{2'a,3'}$ = 5.7, H-2′a); 3.52 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.9, $J_{5'b,4'}$ = 4.2, H-5′b); 3.60 (ddd, 1H, J_{gem} = 11.5, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 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 (dddd, 1H, $J_{3',2'}$ = 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).

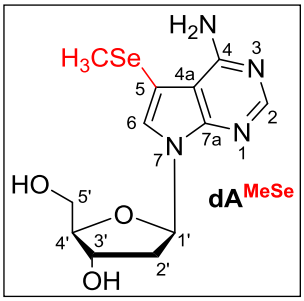
^{13}C NMR (125.7 MHz, DMSO-d_6): 40.15 (CH_2-2′); 62.07 (CH_2-5′); 71.19 (CH-3′); 83.59 (CH-1′); 87.78 (CH-4′); 93.56 (C-5); 103.72 (C-4a); 126.63 (CH-*p*-Ph); 128.68 (CH-*o*-Ph); 129.77 (CH-*m*-Ph); 130.19 (CH-6); 133.28 (C-*i*-Ph); 150.67 (C-7a); 152.54 (CH-2); 157.77 (C-4).

^{77}Se NMR (95.4 MHz, DMSO-d_6): 230.26.

MS-ESI ($C_{17}H_{18}O_3N_4Se$) *m/z* (% int.) calcd: 429.0 $[M + Na]^+$. Found: 429.1 $[M + Na]^+$ (100), 835.3 $[2M + Na]^+$ (35).

HRMS-ESI ($C_{17}H_{18}O_3N_4Se$) *m/z* (% int.) calcd: 429.04363 $[M + Na]^+$. Found: 429.04370 $[M + Na]^+$.

7-Methylselanyl-7-deaza-2'-deoxyadenosine (dA^{MeSe})



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_2O. Yield: 11.4 mg (14 %). White microcrystals.

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.16 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.7, H-2′b); 2.19 (s, 3H, CH_3Se); 2.50 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.3, $J_{2'a,3'}$ = 5.8, H-2′a); 3.52 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.4, $J_{5'b,4'}$ = 4.3, H-5′b); 3.58 (dt, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 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 (dddd, 1H, $J_{3',2'}$ = 5.8, 2.7, $J_{3',OH}$ =

3.9, $J_{3',4'}$ = 2.5, H-3′); 5.11 (bdd, 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).

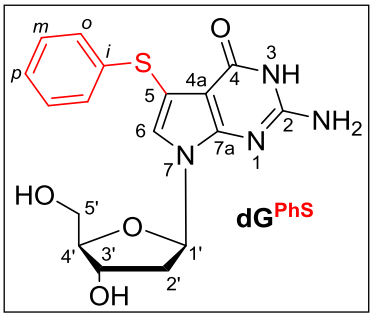
^{13}C NMR (125.7 MHz, DMSO-d_6): 12.12 (CH_3Se); 40.03 (CH_2-2′); 62.15 (CH_2-5′); 71.23 (CH-3′); 83.36 (CH-1′); 87.66 (CH-4′); 96.05 (C-5); 103.72 (C-4a); 127.83 (CH-6); 150.25 (C-7a); 152.29 (CH-2); 157.89 (C-4).

^{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 $PhSCu^I$ (43.7 mg), conditions: 110 °C, 1.5 hours. Yield: 33.5 mg (39 %). White foam.

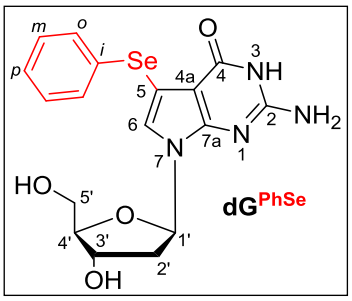
^{1}H NMR (500.0 MHz, DMSO-d_6): 2.12 (ddd, 1H, J_{gem} = 13.0, $J_{2'b,1'}$ = 5.8, $J_{2'b,3'}$ = 2.5, H-2′b); 2.35 (ddd, 1H, J_{gem} = 13.0, $J_{2'a,1'}$ = 8.4, $J_{2'a,3'}$ = 5.7, H-2′a); 3.48, 3.52 (2 × dt, 1H, J_{gem} = 11.7, $J_{5',OH}$ = $J_{5',4'}$ = 4.6, H-5′); 3.77 (ddd, 1H, $J_{4',5'}$ = 4.6, $J_{4',3'}$ = 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); 103.73 (C-4a); 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 $[M + Na]^+$.

7-Phenylselanyl-7-deaza-2'-deoxyguanosine (dG^{PhSe})



Method A - starting from $(PhSe)_2$ (39.6 mg), conditions: 80 °C, 1.5 hours. Yield: 43.2 mg (45 %). White foam.

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.09 (ddd, 1H, J_{gem} = 13.0, $J_{2'b,1'}$ = 5.8, $J_{2'b,3'}$ = 2.5, H-2′b); 2.33 (ddd, 1H, J_{gem} = 13.0, $J_{2'a,1'}$ = 8.5, $J_{2'a,3'}$ = 5.6, H-2′a); 3.45 (ddd, 1H, J_{gem} = 11.5, $J_{5'b,OH}$ = 5.3, $J_{5'b,4'}$ = 4.5, H-5′b); 3.49 (ddd, 1H, J_{gem} = 11.5, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 4.5, H-5′a); 3.75 (td, 1H, $J_{4',5'}$ = 4.5, $J_{4',3'}$ = 2.3, H-4′); 4.26 (m, 1H, H-3′); 4.91 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5′); 5.23 (d, 1H, $J_{OH,3'}$ = 3.7, OH-3′); 6.31 (dd, 1H, $J_{1',2'}$ = 8.5, 5.8, H-1′); 6.36 (bs, 2H, NH_2); 7.13 (s, 1H, H-6); 7.15 (m, 1H, H-*p*-Ph); 7.22 (m, 2H, H-*m*-Ph); 7.30 (m, 2H, H-*o*-Ph); 10.43 (bs, 1H, NH).

^{13}C NMR (125.7 MHz, DMSO-d_6): 39.93 (CH_2-2′); 62.09 (CH_2-5′); 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-*p*-Ph); 129.27 (CH-*o*-Ph); 129.43 (CH-*m*-Ph); 133.95 (C-*i*-Ph); 151.90 (C-7a); 153.25 (C-2); 158.28 (C-4).

^{77}Se NMR (95.4 MHz, DMSO-d_6): 262.71.

MS-ESI ($C_{17}H_{18}O_4N_4Se$) *m/z* (% int.) calcd: 423.1 $[M + H]^+$. Found: 423.1 $[M + H]^+$ (30).

HRMS-ESI ($C_{17}H_{18}O_4N_4Se$) *m/z* (% int.) calcd: 423.05660 $[M + H]^+$. Found: 423.05658 $[M + H]^+$.

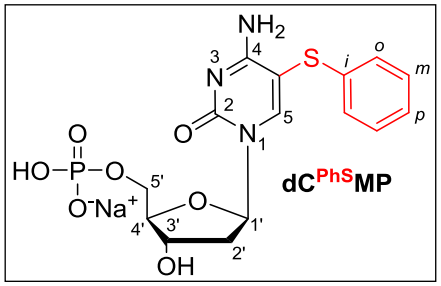
General procedure for copper-mediated sulfanylations and selanylations of dC^{I}MP (Method A):

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^{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_2O 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^{RX} (Method B):

To an argon-purged flask containing under reduced presure dried (2 hours, 80 °C) **dC^{RX}** (0.1-0.2 mmol), dry $PO(OMe)_3$ (0.1 ml for every 10 mg of **dC^{RX}**) was added and the suspension/solution cooled to 0 °C on an ice bath. Freshly distilled $POCl_3$ (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_2O : 7 NH_4OH). 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^{PhS}MP)



Method A starting from $(PhS)_2$ (222.2 mg). Yield: 2.9 mg (5 %). White solid.

Method B: **dC^{PhS}** (100 mg, 0.3 mmol), $POCl_3$ (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_2O): 2.36 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.4, $J_{2'b,3'}$ = 6.4, H-2′b); 2.46 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.6, H-2′a); 3.92 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 5.1, $J_{5'b,4'}$ = 4.8, H-5′b); 3.96 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 5.9, $J_{5'a,4'}$ = 4.8, H-5′a); 4.18 (td, 1H, $J_{4',5'}$ = 4.8, $J_{4',3'}$ = 3.4, H-4′); 4.53 (ddd, 1H, $J_{3',2'}$ = 6.5, 3.6, $J_{3',2'}$ = 3.4, H-3′); 6.28 (dd, 1H, $J_{1',2'}$ = 7.4, 6.2, H-1′); 7.22-7.30 (m, 3H, H-*o*,*p*-Ph); 7.35 (m, 2H, H-*m*-Ph); 8.30 (s, 1H, H-6).

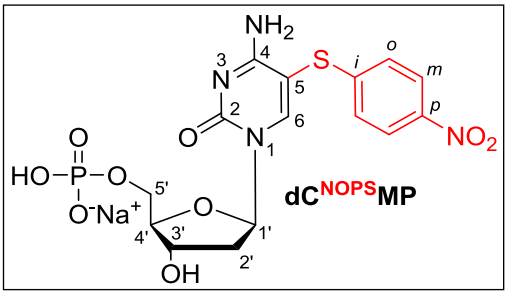
^{13}C NMR (125.7 MHz, D_2O): 41.87 (CH_2-2′); 66.62 (d, $J_{C,P}$ = 4.6, CH_2-5′); 73.81 (CH-3′); 88.74 (d, $J_{C,P}$ = 8.2, CH-4′); 89.14 (CH-1′); 100.62 (C-5); 129.24 (CH-*o*-Ph); 129.32 (CH-*p*-Ph); 132.21 (CH-*m*-Ph); 137.24 (C-*i*-Ph); 151.64 (CH-6); 159.73 (C-2); 168.43 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O): 3.87.

MS-ESI ($C_{15}H_{17}O_7N_3PS^-$) *m/z* (% int.) calcd: 414.3 [M]. Found: 414.0 [M] (100).

HRMS-ESI ($C_{15}H_{17}O_7N_3PS^-$) *m/z* (% int.) calcd: 414.05303 [M]. Found: 414.05266 [M].

5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine 5'-*O*-monophosphate sodium salt (dC^{NOPS}MP)



Method A: The amount of DMF used for the reaction was doubled due to precipitate formation during the reaction. Starting from $(4\text{-}NO_2\text{-}PhS)_2$ (222.2 mg). Yield: 4.8 mg (7 %) of yellowish solid.

Method B: **dC^{NOPhS}** (59 mg, 0.16 mmol), $POCl_3$ (30.9 mg, 18.9 µl, 0.2 mmol, 1.3 equiv.), 80 minutes. Yield: 20 mg (27 %). Yellowish solid.

^{1}H NMR (500.0 MHz, D_2O): 2.37 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.6, H-2′b); 2.48 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.6, H-2′a); 3.93 (ddd, 1H, J_{gem} = 11.4, $J_{H,P}$ = 5.2, $J_{5'b,4'}$ = 4.7, H-5′b); 3.60 (ddd, 1H, J_{gem} = 11.4, $J_{H,P}$ = 5.8, $J_{5'a,4'}$ = 4.7, H-5′a); 4.18 (td, 1H, $J_{4',5'}$ = 4.7, $J_{4',3'}$ = 3.6, H-4′); 4.53 (dt, 1H, $J_{3',2'}$ = 6.6, 3.6, $J_{3',4'}$ = 3.6, H-3′); 6.27 (dd, 1H, $J_{1',2'}$ = 7.3, 6.2, H-1′); 7.34 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.14 (m, 2H, H-*m*-$C_6H_4NO_2$); 8.36 (s, 1H, H-6).

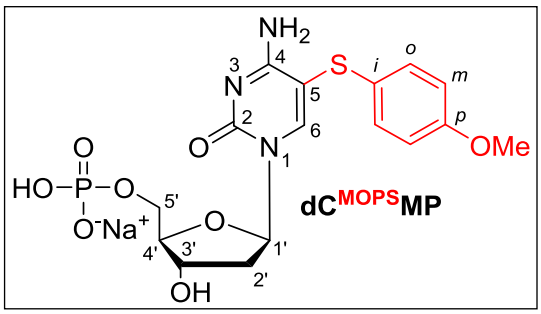
^{13}C NMR (125.7 MHz, D_2O): 42.04 (CH_2-2′); 66.58 (d, $J_{C,P}$ = 4.6, CH_2-5′); 73.76 (CH-3′); 88.82 (d, $J_{C,P}$ = 8.3, CH-4′); 89.35 (CH-1′); 98.15 (C-5); 127.06 (CH-*m*-$C_6H_4NO_2$); 128.45 (CH-*o*-$C_6H_4NO_2$); 147.99 (C-*i*-$C_6H_4NO_2$); 148.22 (C-*p*-$C_6H_4NO_2$); 152.65 (CH-6); 159.60 (C-2); 168.17 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O): 3.79.

MS-ESI ($C_{15}H_{16}O_9N_4PS^-$) *m/z* (% int.) calcd: 459.3 [M]. Found: 459.0 [M] (100).

HRMS-ESI ($C_{15}H_{16}O_9N_4PS^-$) *m/z* (% int.) calcd: 459.03811 [M]. Found: 459.03751 [M].

5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine 5'-*O*-monophosphate sodium salt (dC^{MOPS}MP)



Method B: **dC^{MOPS}** (32 mg, 0.09 mmol), $POCl_3$ (17.4 mg, 10.5 µl, 0.117 mmol, 1.3 equiv.), 90 minutes. Yield: 9 mg (22 %). White solid.

^{1}H NMR (500.0 MHz, D_2O): 2.36 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.5, $J_{2'b,3'}$ = 6.5, H-2′b); 2.44 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.6, H-2′a); 3.80 (s, 3H, CH_3O); 3.91 (dt, 1H, J_{gem} = 11.2, $J_{H,P}$ = $J_{5'b,4'}$ = 5.1, H-5′b); 3.96 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.0, $J_{5'a,4'}$ = 5.1, H-5′a); 4.17 (dt, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.6 H-4′); 4.53 (dt, 1H, $J_{3',2'}$ = 6.5, 3.6, $J_{3',4'}$ = 3.6, H-3′); 6.26 (dd, 1H, $J_{1',2'}$ = 7.5, 6.2, H-1′); 6.96 (m, 2H, H-*m*-C_6H_4OMe); 7.30 (m, 2H, H-*o*-C_6H_4OMe); 8.29 (s, 1H, H-6).

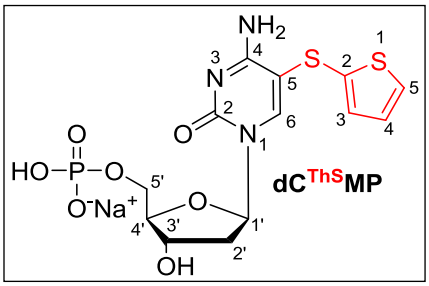
^{13}C NMR (125.7 MHz, D_2O): 41.71 (CH_2-2′); 58.20 (CH_3O); 66.44 (d, $J_{C,P}$ = 4.5, CH_2-5′); 73.86 (CH-3′); 88.81 (d, $J_{C,P}$ = 8.1, CH-4′); 89.03 (CH-1′); 102.29 (C-5); 117.91 (CH-*m*-C_6H_4OMe); 128.00 (C-*i*-C_6H_4OMe); 132.34 (CH-*o*-C_6H_4OMe); 150.94 (CH-6); 159.68 (C-2); 160.98 (C-*p*-C_6H_4OMe); 168.33 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O): 3.96.

MS-ESI ($C_{16}H_{19}N_3O_8PS^-$) *m/z* (% int.) calcd: 444.4 [M]. Found: [M] 444.1 (100).

HRMS-ESI ($C_{16}H_{19}N_3O_8PS^-$) *m/z* (% int.) calcd: 444.06359 [M]. Found: 444.06293 [M].

5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-*O*-monophosphate (dC^{ThS}MP)



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

^{1}H NMR (500.0 MHz, D_2O): 2.31 (dt, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 6.3, H-2′b); 2.44 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.6, H-2′a); 4.03 (dd, 2H, $J_{H,P}$ = 5.6, $J_{5',4'}$ = 4.5, H-5′); 4.20 (td, 1H, $J_{4',5'}$ = 4.5, $J_{4',3'}$ = 3.6, H-4′); 4.53 (dt, 1H, $J_{3',2'}$ = 6.3, 3.6, $J_{3',4'}$ = 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).

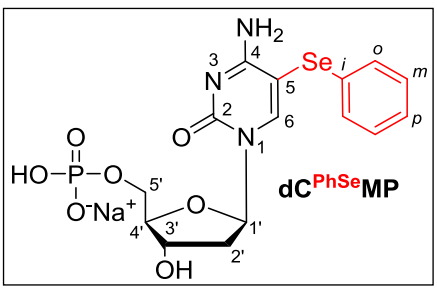
^{13}C NMR (125.7 MHz, D_2O): 42.08 (CH_2-2′); 66.96 (d, $J_{C,P}$ = 4.7, CH_2-5′); 73.74 (CH-3′); 88.65 (d, $J_{C,P}$ = 8.3, CH-4′); 89.26 (CH-1′); 104.66 (C-5); 130.79 (CH-4-thienyl); 132.68 (CH-5-thienyl); 135.25 (CH-3-thienyl); 135.53 (C-2-thienyl); 150.41 (CH-6); 159.38 (C-2); 167.88 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O): 2.72.

MS-ESI ($C_{13}H_{15}O_7N_3PS_2^-$) *m/z* (% int.) calcd: 420.4 [M]. Found: 420.1[M] (100).

HRMS-ESI ($C_{13}H_{15}O_7N_3PS_2^-$) *m/z* (% int.) calcd: 420.00945 [M]. Found: 420.00928 [M].

5-Phenylselanyl-2'-deoxycytidine 5'-*O*-monophosphate sodium salt (dC^{PhSe}MP)



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

^{1}H NMR (500.0 MHz, D_2O): 2.36 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.5, $J_{2'b,3'}$ = 6.5, H-2′b); 2.44 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.6, H-2′a); 3.92 (dt, 1H, J_{gem} = 11.3,

$J_{5'b,4'}$ = $J_{H,P}$ = 5.1, H-5′b); 3.95 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 5.9, $J_{5'a,4'}$ = 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_2O): 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′); 88.96 (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_2O): 4.44.

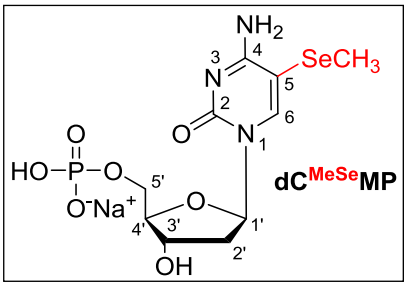
^{77}Se NMR (95.4 MHz, D_2O): 284.52.

MS-ESI ($C_{15}H_{17}O_7N_3PSe^-$) *m/z* (% int.) calcd: 462.0 [M]. Found: 462.1 [M] (100).

HRMS-ESI ($C_{15}H_{17}O_7N_3PSe^-$) *m/z* (% int.) calcd: 461.99748 [M]. Found: 461.99731 [M].

5-Methylselanyl-2'-deoxycytidine 5'-*O*-monphosphate sodium salt (dC^{MeSe}MP)

Method B: **dC^{MeSe}** (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_2O): 2.18 (s, 3H, CH_3Se); 2.33 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.6, $J_{2'b,3'}$ = 6.4, H-2′b); 2.42 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.4, H-2′a); 4.01 (dd, 2H, $J_{H,P}$ = 5.4, $J_{5',4'}$ = 4.3, H-5′); 4.19 (tdd, 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_2O): 11.47 (CH_3Se); 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_2O): 2.09.

^{77}Se NMR (95.4 MHz, D_2O): 85.79 (q, $J_{Se,H}$ = 11.4).

MS-ESI ($C_{10}H_{15}O_7N_3PSe^-$) *m/z* (% int.) calcd: 400.0 [M]. Found: 400.0 [M] (100).

HRMS-ESI ($C_{10}H_{15}O_7N_3PSe^-$) *m/z* (% int.) calcd: 399.98183 [M]. Found: 399.98160 [M].

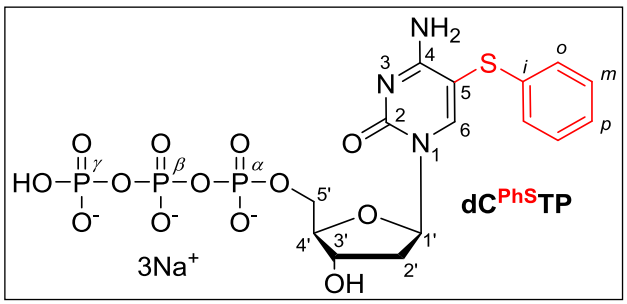
General procedure for copper-mediated sulfanylations or selanylations of dC^{I}TP (Method A):

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'-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^+) of **dC^{I}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 *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_2O 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[X3]) followed by freeze-drying from water afforded the product as a white, fluffy powder.

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

To a U-shaped microwave vial sealable with a Teflon cap were added $PhSCu^{I}$ (55 mg, 0.318 mmol, 1.1 equiv.) and 2,2'-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^+) of **dC^{I}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 *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_2O 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[S4]) followed by freeze-drying from water afforded the product as a white, fluffy powder.

5-Phenylsulfanyl-2'-deoxycytidine 5'-*O*-triphosphate sodium salt (dC^{PhS}TP)



Method B - starting from PhSCu^{I} (55 mg, 0.318 mmol, 1.1 equiv.), conditions: 80 °C, 1 hour. Yield: 12 mg (7 %).

^{1}H NMR (500.0 MHz, D_2O): 2.37 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.3, H-2′b); 2.47 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.5, H-2′a); 4.11 – 4.29 (m, 3H, H-4′,5′); 4.62 (dt, 1H, $J_{3',2'}$ = 6.3, 3.5, $J_{3',2'}$ = 3.5, H-3′); 6.29 (dd, 1H, $J_{1',2'}$ = 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).

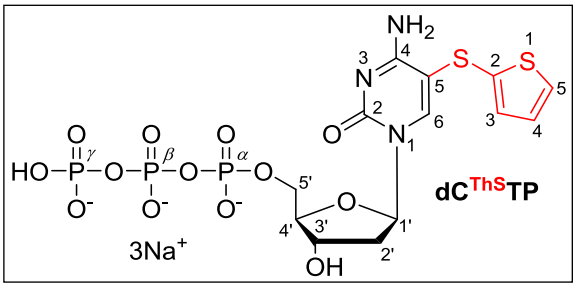
^{13}C NMR (125.7 MHz, D_2O): 42.07 (CH_2-2′); 68.09 (d, $J_{C,P}$ = 5.6, CH_2-5′); 73.44 (CH-3′); 88.36 (d, $J_{C,P}$ = 8.8, CH-4′); 89.26 (CH-1′); 100.99 (C-5); 129.42 (CH-*o*-Ph); 129.65 (CH-*p*-Ph); 132.20 (CH-*m*-Ph); 137.26 (C-*i*-Ph); 151.45 (CH-6); 159.71 (C-2); 168.42 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -22.14 (t, J = 19.7, P$_\beta$); -10.74 (d, J = 19.7, P$_\alpha$); -9.25 (bd, J = 19.7, P$_\gamma$).

MS-ESI ($C_{15}H_{18}O_{13}N_3NaP_3S^-$) *m/z* (% int.) calcd: 596.3 [M-2H+Na]. Found: 596.0 [M-2H+Na] (25), 494.0 [M-PO_3Na] (100).

HRMS-ESI ($C_{15}H_{18}O_{13}N_3NaP_3S^-$) *m/z* (% 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^{ThS}TP)



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

^{1}H NMR (500.0 MHz, D_2O): 2.34 (dt, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.7, H-2′b); 2.44 (ddd, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.7, H-2′a); 4.15 – 4.32 (m, 3H, H-4′,5′); 4.63 (dt, 1H, $J_{3',2'}$ = 6.7, 3.7, $J_{3',4'}$ = 3.7, H-3′); 6.23 (dd, 1H, $J_{1',2'}$ = 6.7, 6.3, H-1′); 7.05 (dd, 1H, $J_{4,5}$ = 5.4, $J_{4,3}$ = 3.6, H-4-thienyl); 7.34 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.3, H-3-thienyl); 7.49 (dd, 1H, $J_{5,4}$ = 5.4, $J_{5,3}$ = 1.3, H-5-thienyl); 8.35 (s, 1H, H-6).

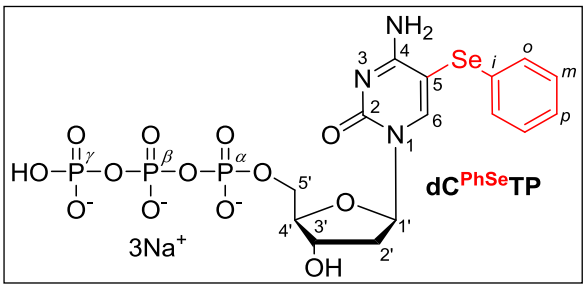
^{13}C NMR (125.7 MHz, D_2O): 42.06 (CH_2-2′); 67.95 (d, $J_{C,P}$ = 5.6, CH_2-5′); 73.17 (CH-3′); 88.46 (d, $J_{C,P}$ = 8.7, CH-4′); 89.15 (CH-1′); 104.86 (br, C-5); 130.81 (CH-4-thienyl); 132.74 (CH-5-thienyl); 135.38 (CH-3-thienyl); 135.67 (C-2-thienyl); 150.40 (CH-6); 159.50 (C-2); 167.97 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -22.26 (t, J = 19.8, P$_\beta$); -11.35 (d, J = 19.8, P$_\alpha$); -7.59 (bd, J = 19.8, P$_\gamma$).

MS-ESI ($C_{13}H_{16}O_{13}N_3NaP_3S_2^-$) *m/z* (% int.) calcd: 601.9 [M-2H+Na]. Found: 601.9 [M-2H+Na] (10), 499.9 [M-PO_3Na] (100).

HRMS-ESI ($C_{13}H_{15}O_{13}N_3Na_2P_3S_2^-$) *m/z* (% int.) calcd: 601.92406 [M-2H+Na]. Found: 601.92290 [M-2H+Na].

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



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

^{1}H NMR (500.0 MHz, D_2O): 2.37 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.5, H-2′b); 2.46 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.9, 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, H-3′); 6.27 (dd, 1H, $J_{1',2'}$ = 7.1, 6.3, 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}C NMR (125.7 MHz, D_2O): 41.85 (CH_2-2′); 66.98 (d, $J_{C,P}$ = 5.3, CH_2-5′); 73.11 (CH-3′); 88.30 (d, $J_{C,P}$ = 8.4, 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}P{^{1}H} NMR (202.3 MHz, D_2O): -21.25 (t, J = 19.2, P$_\beta$); -11.00 (d, J = 19.2, P$_\alpha$); -5.52 (d, J = 19.2, P$_\gamma$).

^{77}Se NMR (95.4 MHz, D_2O): 286.49.

MS-ESI ($C_{15}H_{19}O_{13}N_3P_3Se^-$) *m/z* (% int.) calcd: 621.2 [M-H]. Found: 621.9 [M-H] (100).

HRMS-ESI ($C_{15}H_{19}O_{13}N_3P_3Se^-$) *m/z* (% int.) calcd: 621.93014 [M-H]. Found: 621.93038 [M-H].

Biochemistry

Table S1: List of ON sequences used in this study

Oligo	Sequence
Primer$^{248\text{-}sh}$	5'-CATGGGCGGCATGGG-3'
temp^{oligo1C}	5'-CCCGCCCATGCCGCCCATG-3'
temp^{Prb4baseII}	5'-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3'
primer^{LT25TH}	5'-CAAGGACAAAATACCTGTATTCCTT-3'
primer^{L20}	5'-GACATCATGAGAGACATCGC-3'
temp$^{FVL\text{-}A}$	5'-GACATCATGAGAGACATCGCCTCTGGGCTAATAGGACTACTT CTAATCTGTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGGT ATTTTGTCCTTG-3'

Primer extension - 19-mer template

PEX reactions with **dC^{PhS}TP**, **dC^{PhSe}TP** or **dC^{ThS}TP** 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^{RX}TP** (20 µM), 5'-FAM labelled primer$^{248\text{-}sh}$ (0.5 µM) and 19-mer template (temp^{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^{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^{RX}TP** (200 µM), 5'-FAM labelled primer (primer$^{248\text{-}sh}$, 0.5 µM) and 31-mer template (temp^{Prb4baseII}, 0.75 µM) in buffer (2 µl) supplied by the manufacturer.

Polymerase chain reaction

PCR reactions with **dC^{PhS}TP**, **dC^{PhSe}TP** or **dC^{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 **dC^{ThS}TP** or 1.75 U for **dC^{PhS}TP**, **dC^{PhSe}TP**), dGTP, dATP and TTP (300 µM each), either dCTP or **dC^{RX}TP** (300 µM), primers (primer^{LT25TH} and primer^{L20}, 1 µM each) and 98-mer template (temp^{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.

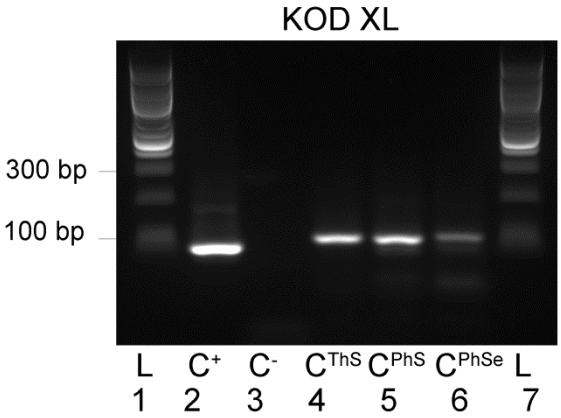


Figure S1: PCR experiments using KOD XL DNA polymerase. Lanes 1,7 , L: ladder ; lane 2, C^+: products of PEX with natural dNTPs; lane 3, C^-: products of PEX with dTTP, dATP, dGTP; lanes 4-6, C^{RX}: products of PCR with dTTP, dATP, dGTP and functionalized **dC^{RX}TP**.

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 **C$^+$**, **C$^-$**, **C^{ThS}**, **C^{PhS}**, **C^{PhSe}**) of MilliQ water. The final concentration of prepared modified DNAs was quantified with NanoDrop instrument: 41.1 ng/µl (**C$^+$**), 45.2 ng/µl (**C^{ThS}**), 42.0 ng/µl (**C^{PhS}**), 28.3 ng/µl (**C^{PhSe}**). Samples were than separated by use of a 2 % agarose gel with GelRed as an intercalator. Visualization was performed by fluorescence imaging.

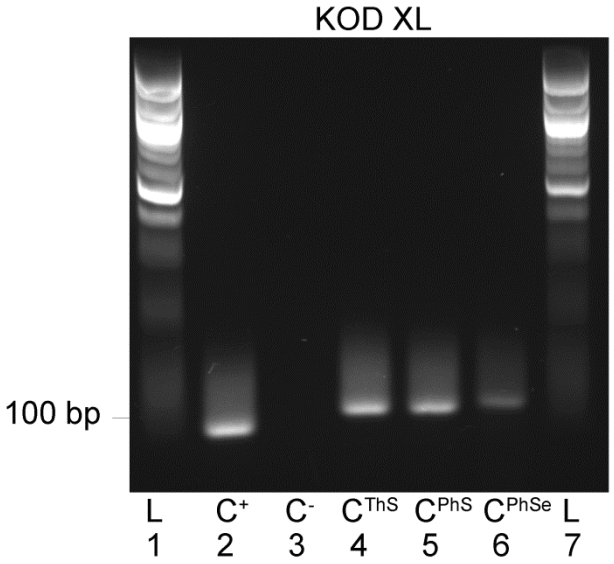


Figure S2: PCR experiments using KOD XL DNA polymerase. Lanes 1,7 , L: ladder ; lane 2, C^{+}: products of PEX with natural dNTPs; lane 3, C^{-}: products of PEX with dTTP, dATP, dGTP; lanes 4-6, C^{RX}: products of PCR with dTTP, dATP, dGTP and functionalized **dC^{RX}TP**.

Optimization of polymerase chain reaction for dC^{PhSe}TP

PCR reactions with 98-mer template (temp^{FVL-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 **dC^{PhSe}TP** 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 **C^{PhSe1}, C^{PhSe2}, C^{PhSe3}, C^{PhSe4}, C^{PhSe5}**) 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 (**C^{PhSe1}**).

b) To the reaction mixture (total volume 20 µl) was added additive: $MgSO_4$ (100 mM, 0.5 µl). Concentration of PCR product was 44.1 ng/µl (**C^{PhSe2}**).

c) Concentration of **dC^{PhSe}TP** in reaction mixture (20 ul) was increased to 450 µM. Concentration of PCR product was 42.6 ng/µl (**C^{PhSe3}**).

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

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

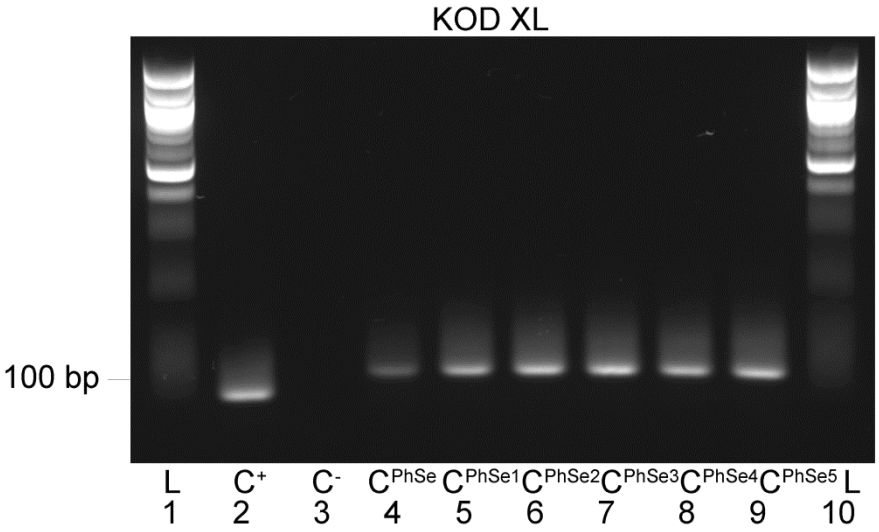


Figure S3: PCR experiments using KOD XL DNA polymerase. Lanes 1,10 , L: ladder ; lane 2, C^{+}: products of PEX with natural dNTPs; lane 3, C^{-}: products of PEX with dTTP, dATP, dGTP; lane 4, C^{PhSe}: products of PCR without additives (dTTP, dATP, dGTP, **dC^{PhSe}TP**); lane 5, C^{PhSe1}: products 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)

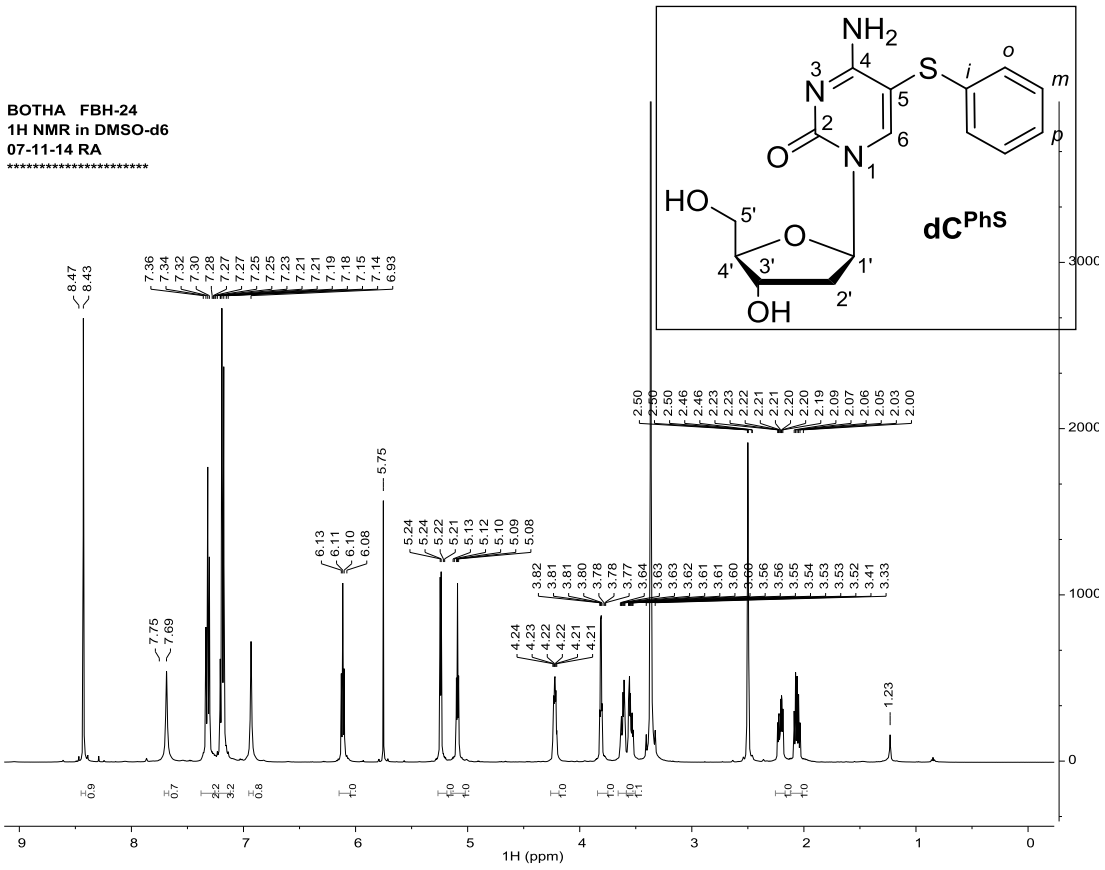
Preparation of ONs for MALDI-TOF Analysis - 31-mer template

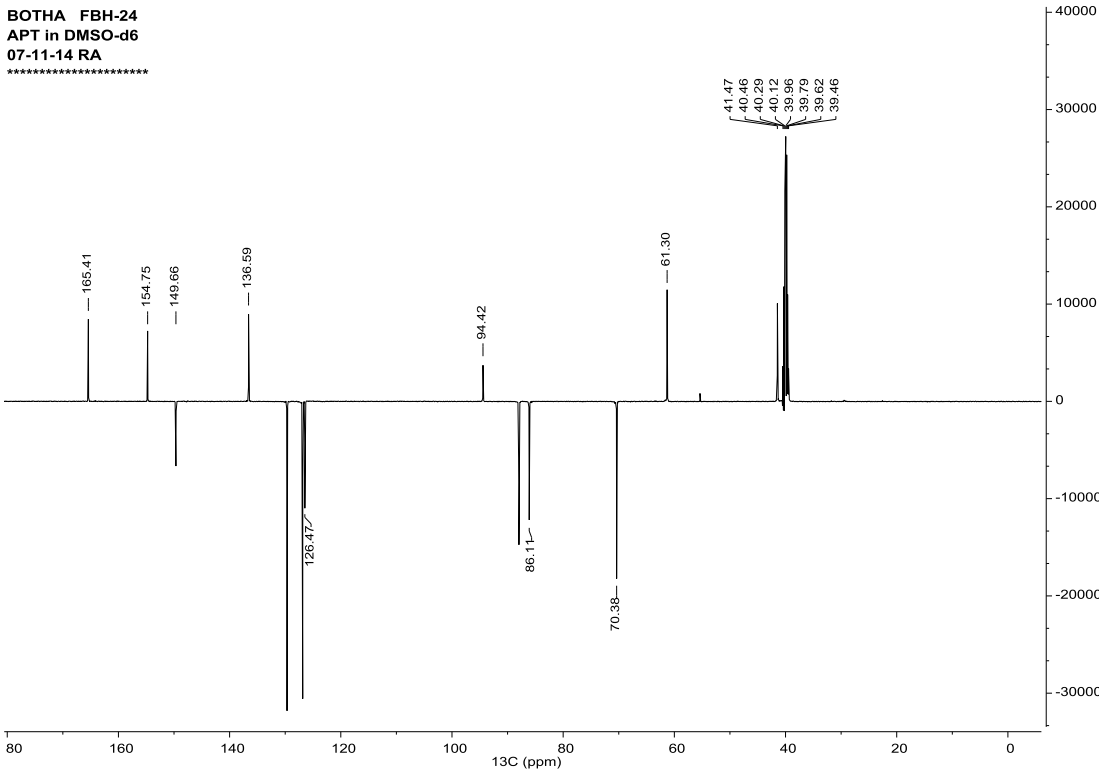
The reaction mixture (60 µl) contained primer (primer$^{248\text{-}sh}$, 3.3 µM), a biotinylated or 31-mer template (5'-biotinylated temp^{Prb4baseII}, 3.3 µM), KOD XL DNA polymerase (0.25 U), dGTP, dATP and TTP (220 µM each), and **dC^{RX}TP** (220 µM), in enzymes reaction buffer supplied by the manufacturer (6 µl). The reaction mixture was shaken (300 rmp) for 40 min at 60 °C in a thermal cycler. Streptavidin magnetic particles stock solution (Roche, 60 µL) was washed with binding buffer (3 × 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.

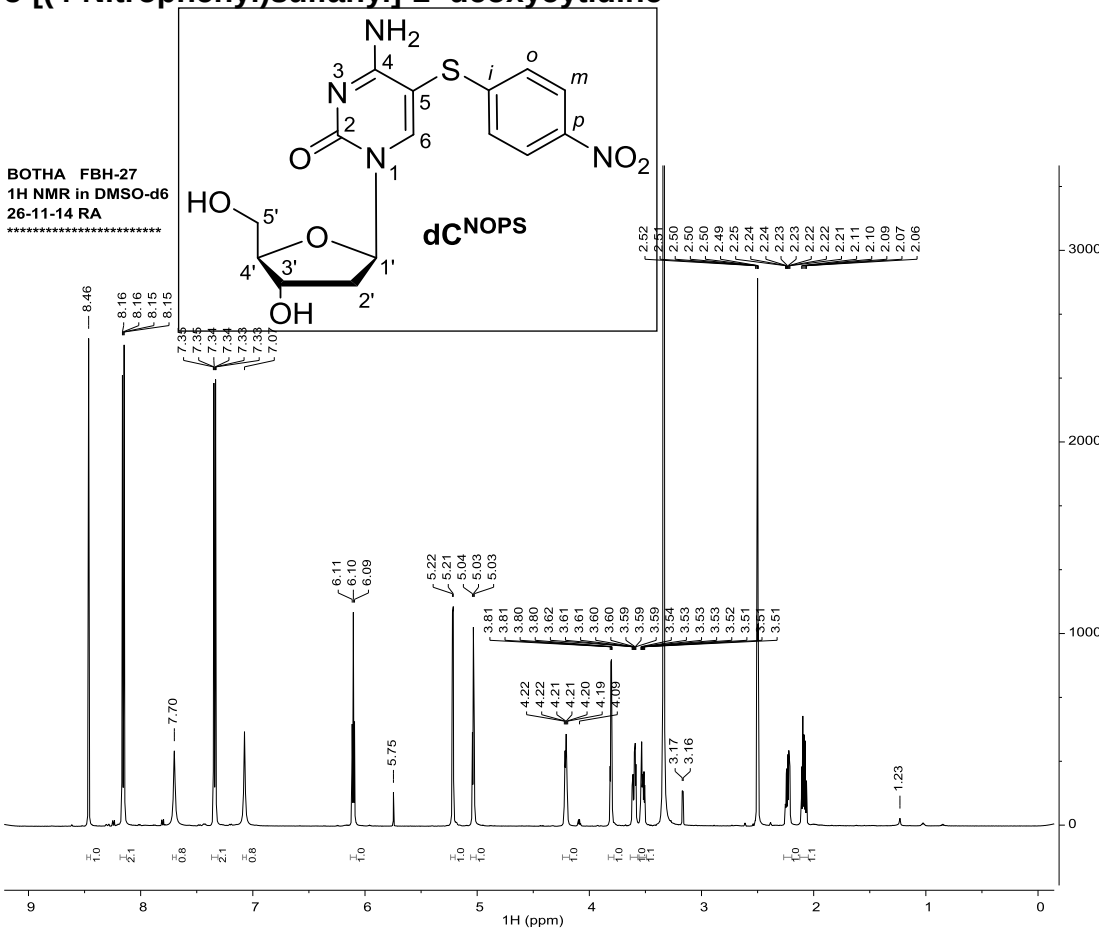
Copies of NMR spectra

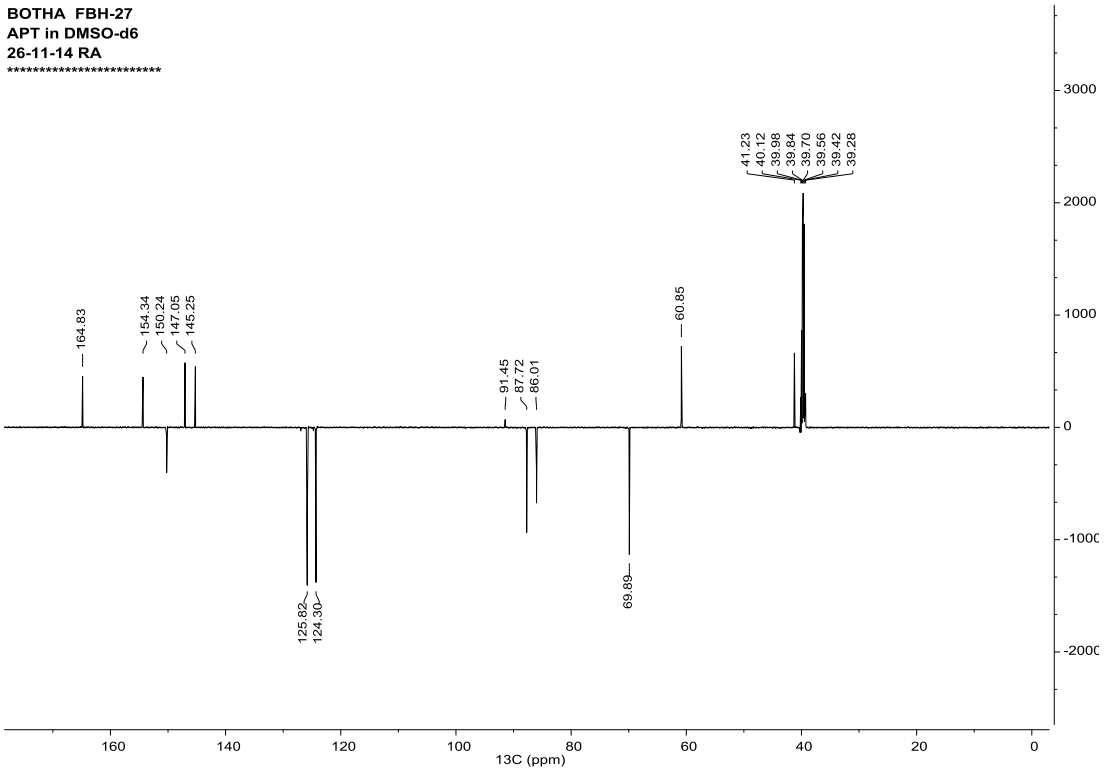
5-Phenylsulfanyl-2'-deoxycytidine



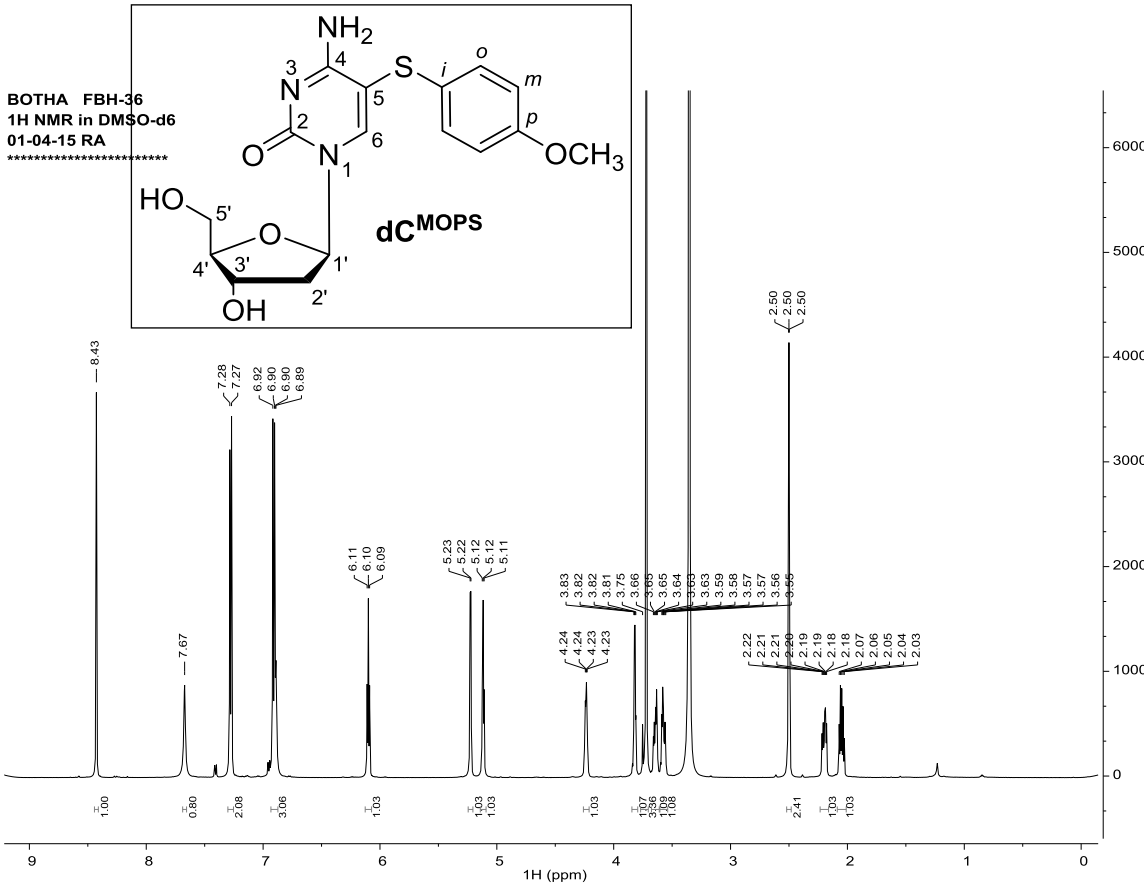


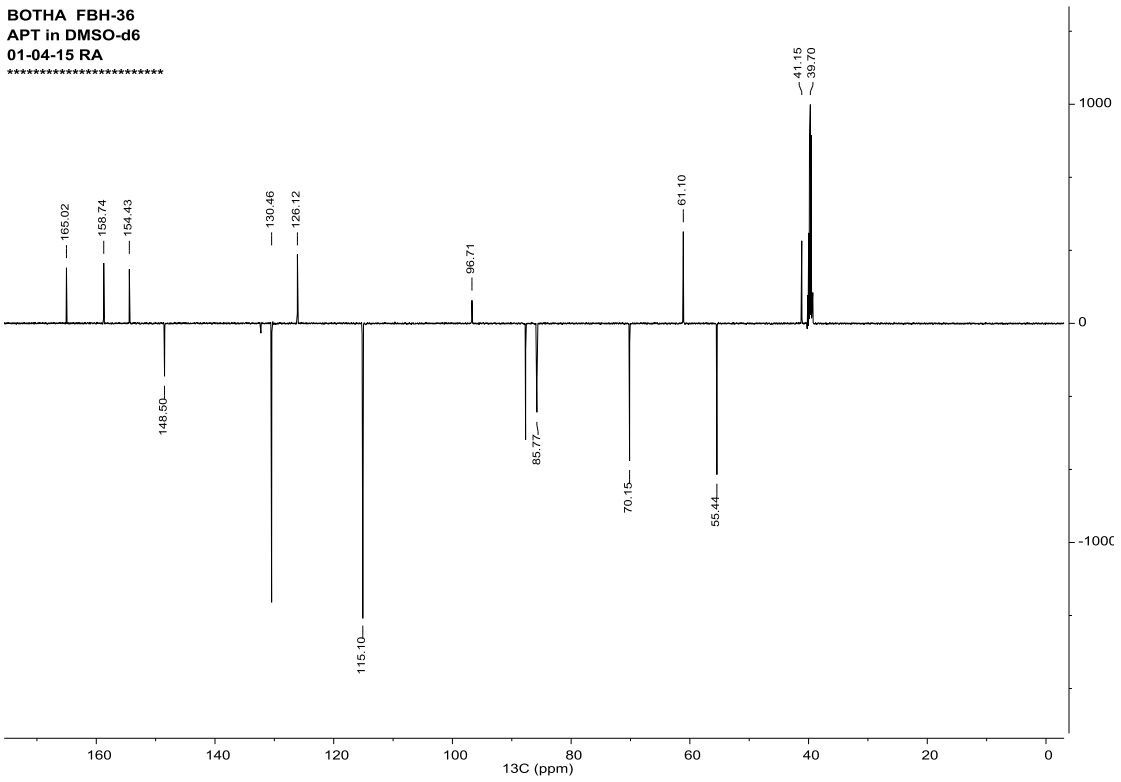
5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine



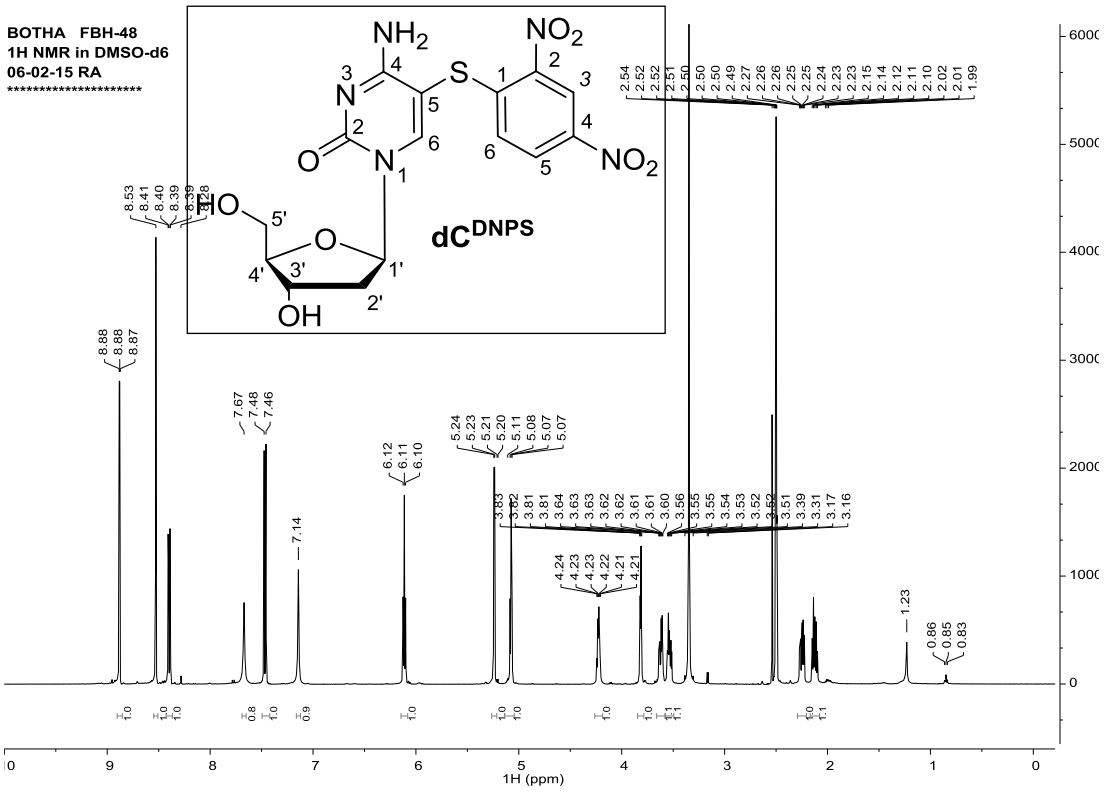


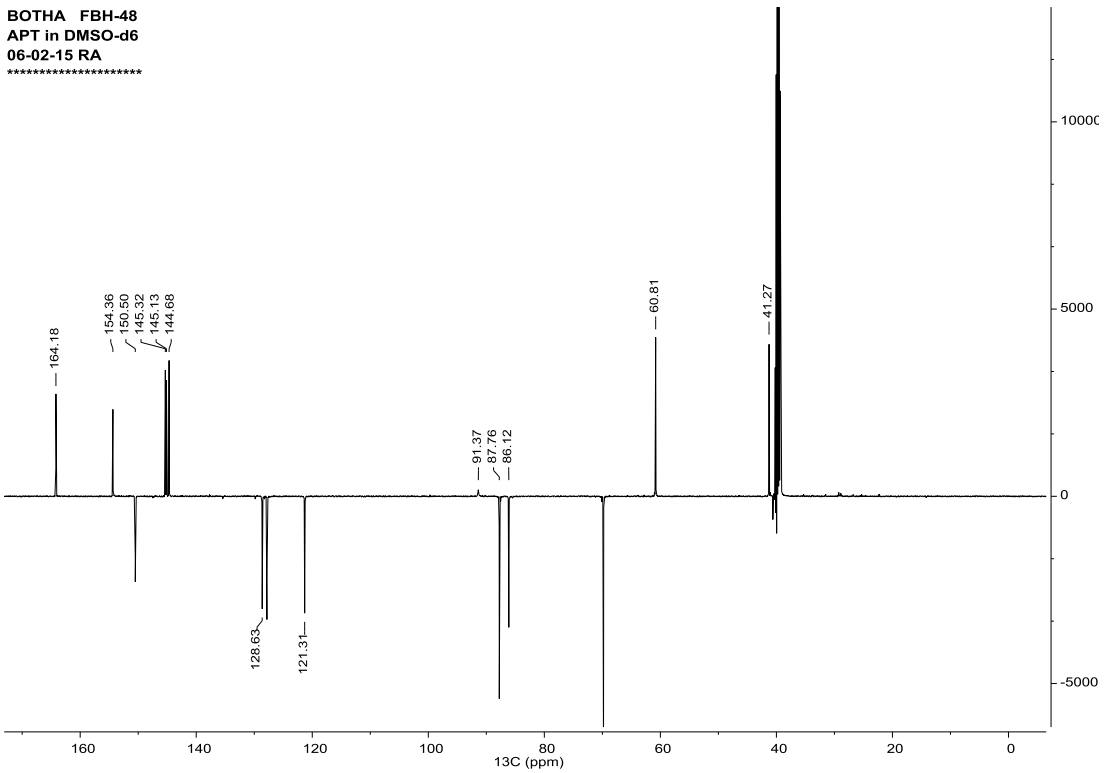
5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine



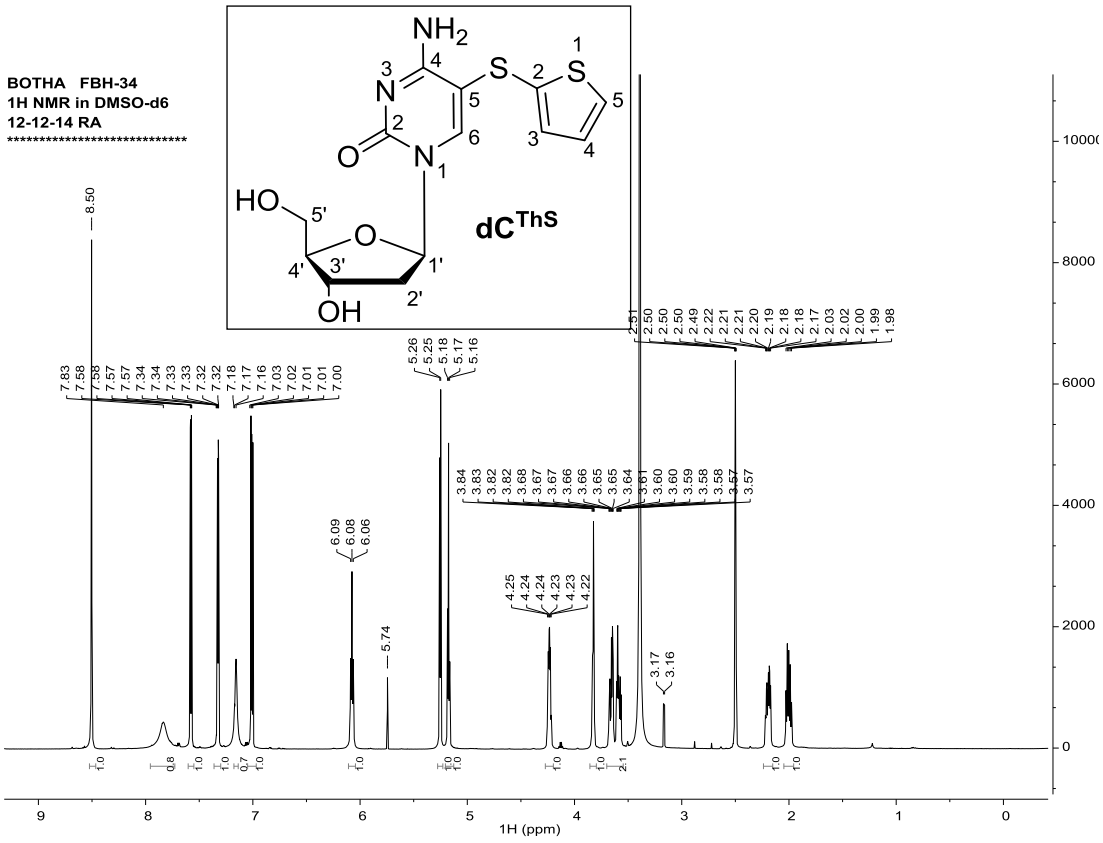


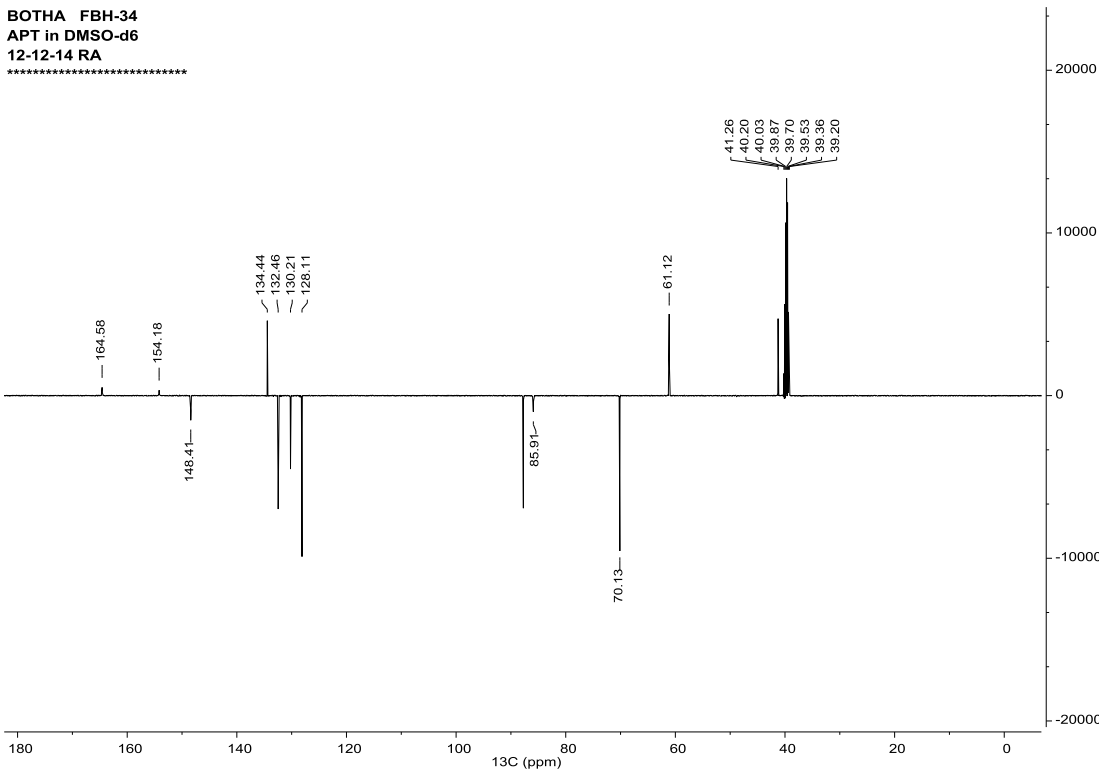
5-[(2,4-Dinitrophenyl)sulfanyl]-2'-deoxycytidine



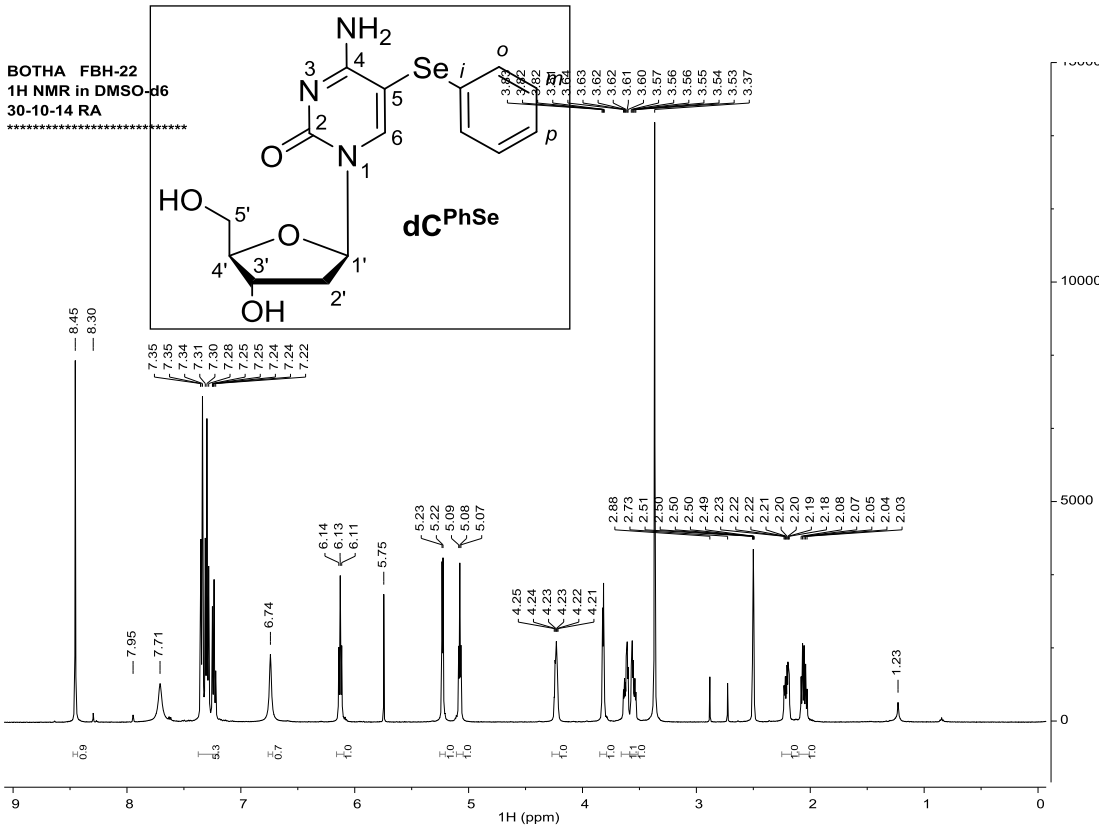


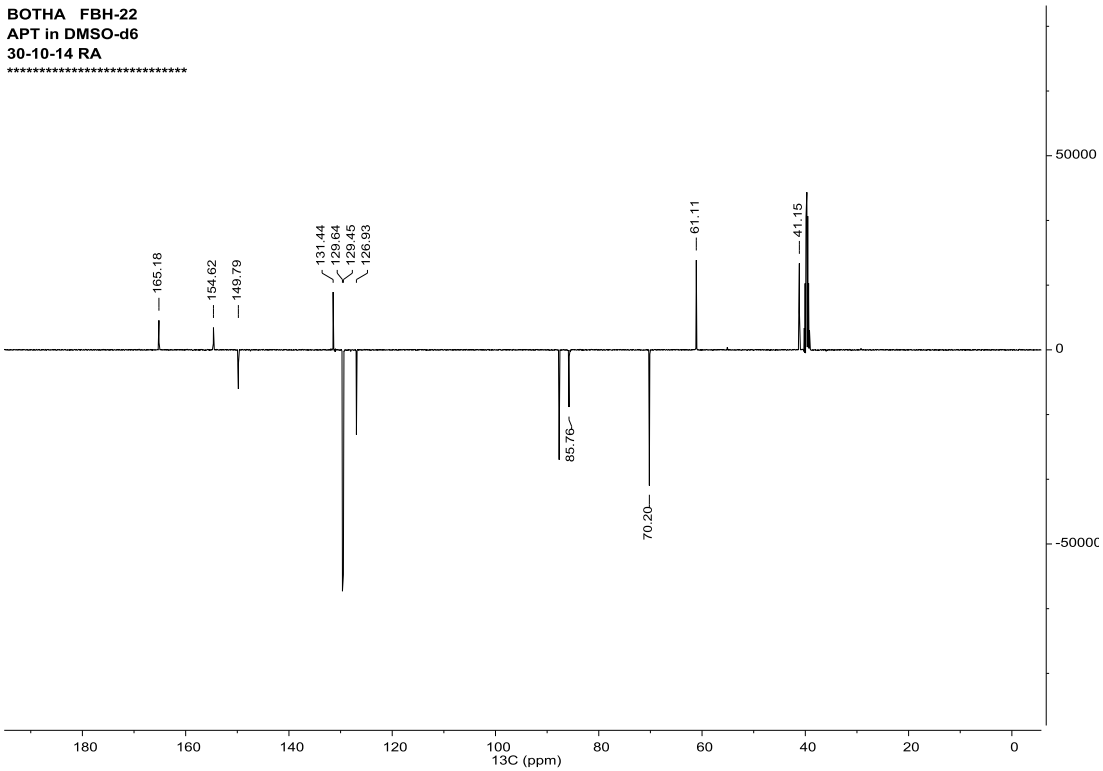
5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine

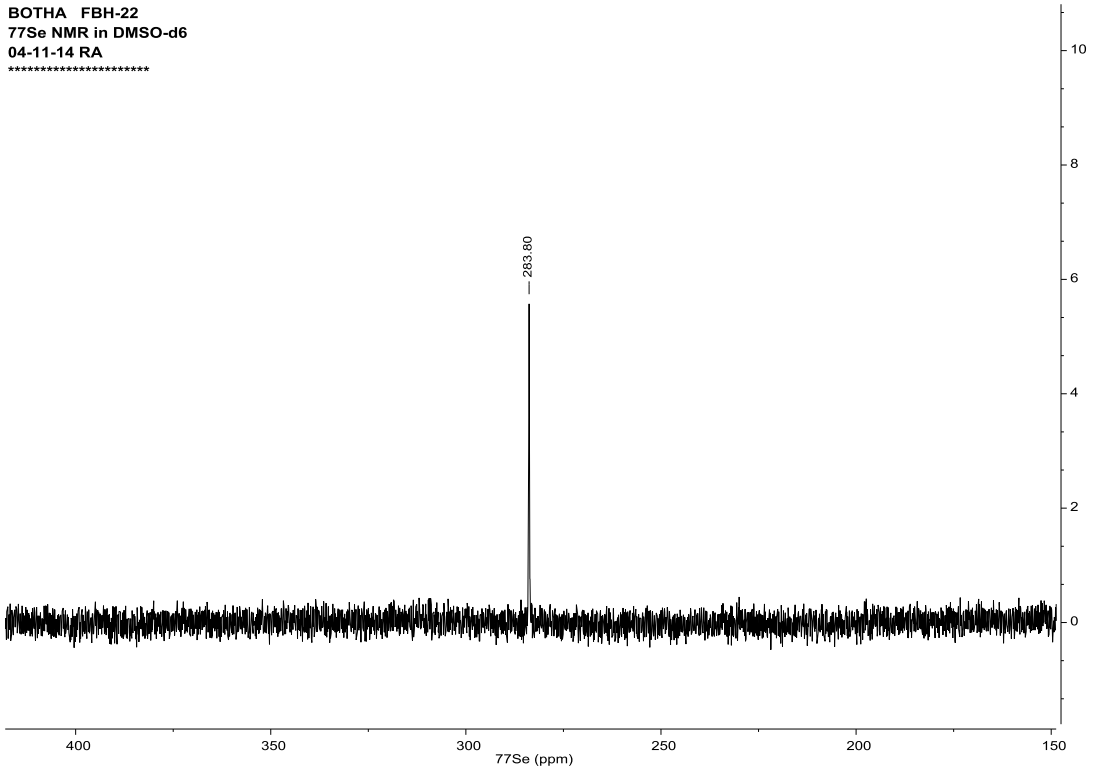




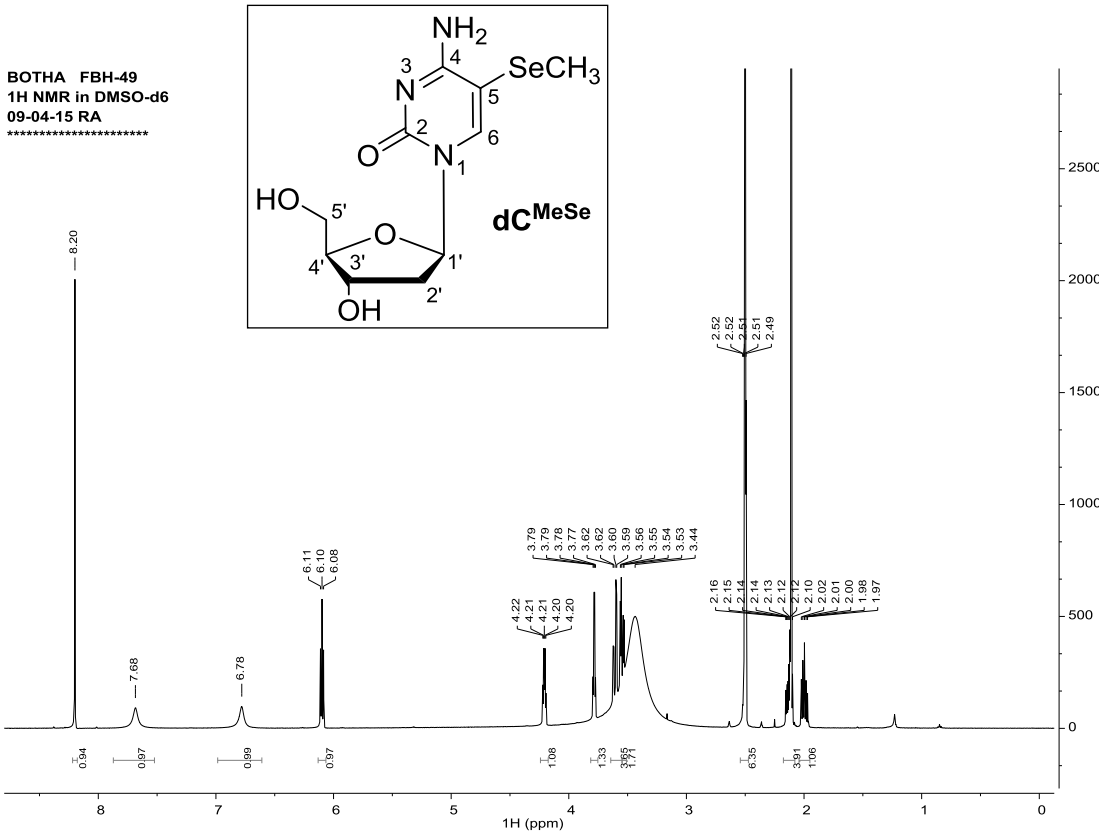
5-Phenylselanyl-2'-deoxycytidine

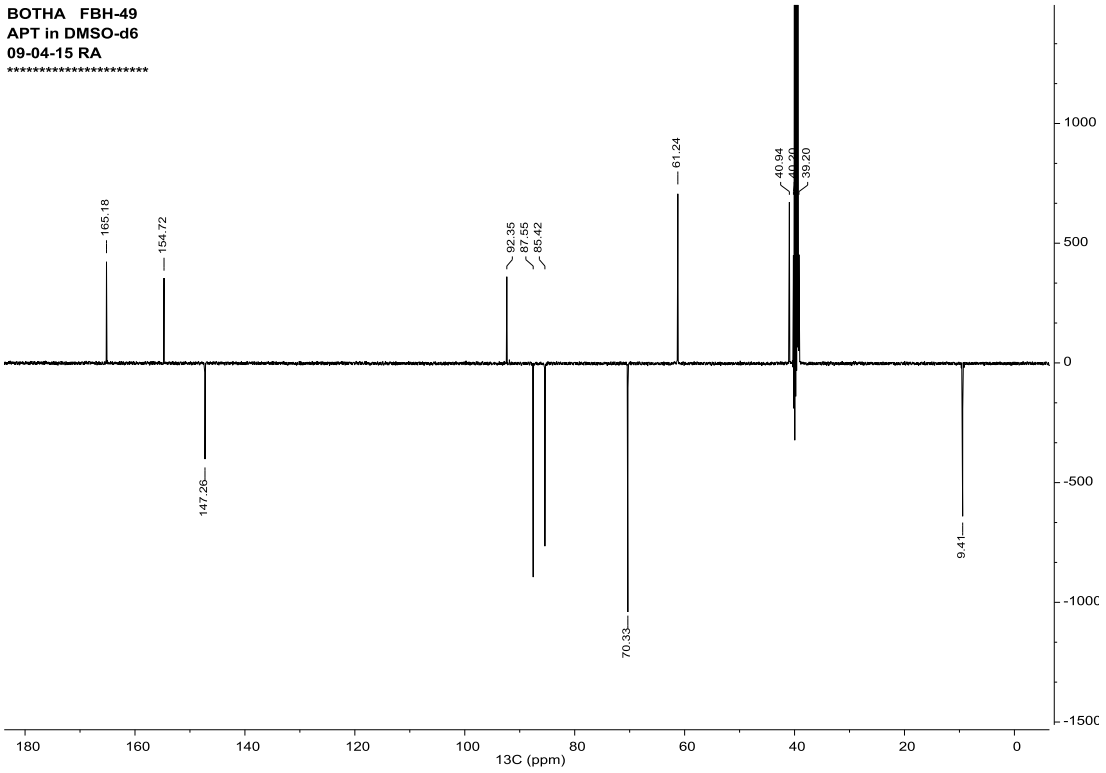


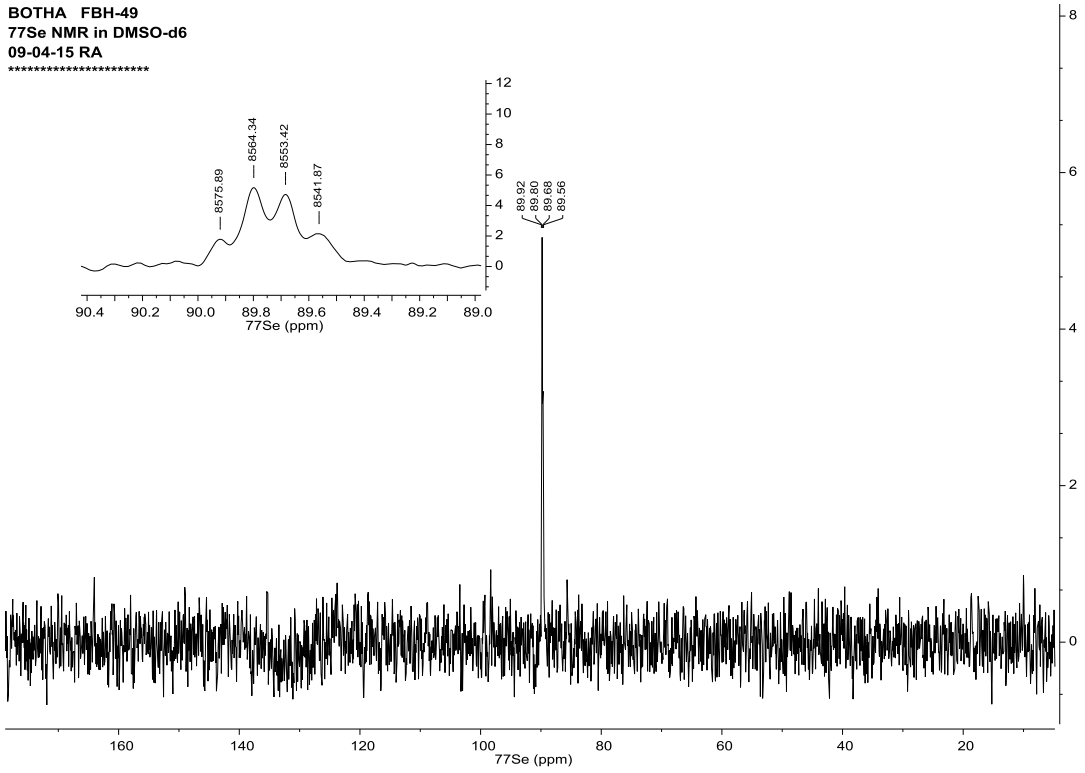



BOTHA FBH-22
77Se NMR in DMSO-d6
04-11-14 RA
****
283.80
10
8
6
4
2
0
400
350
300
250
200
150
77Se (ppm)

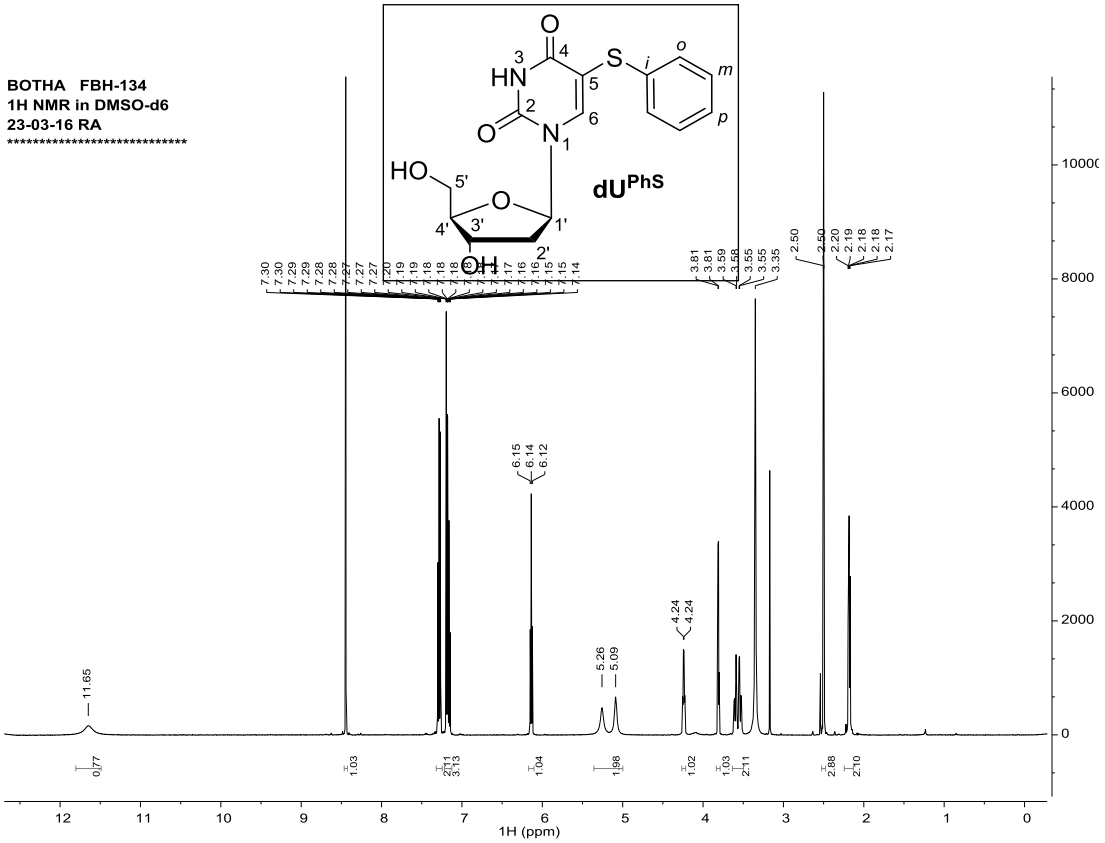
5-Methylselanyl-2'-deoxycytidine

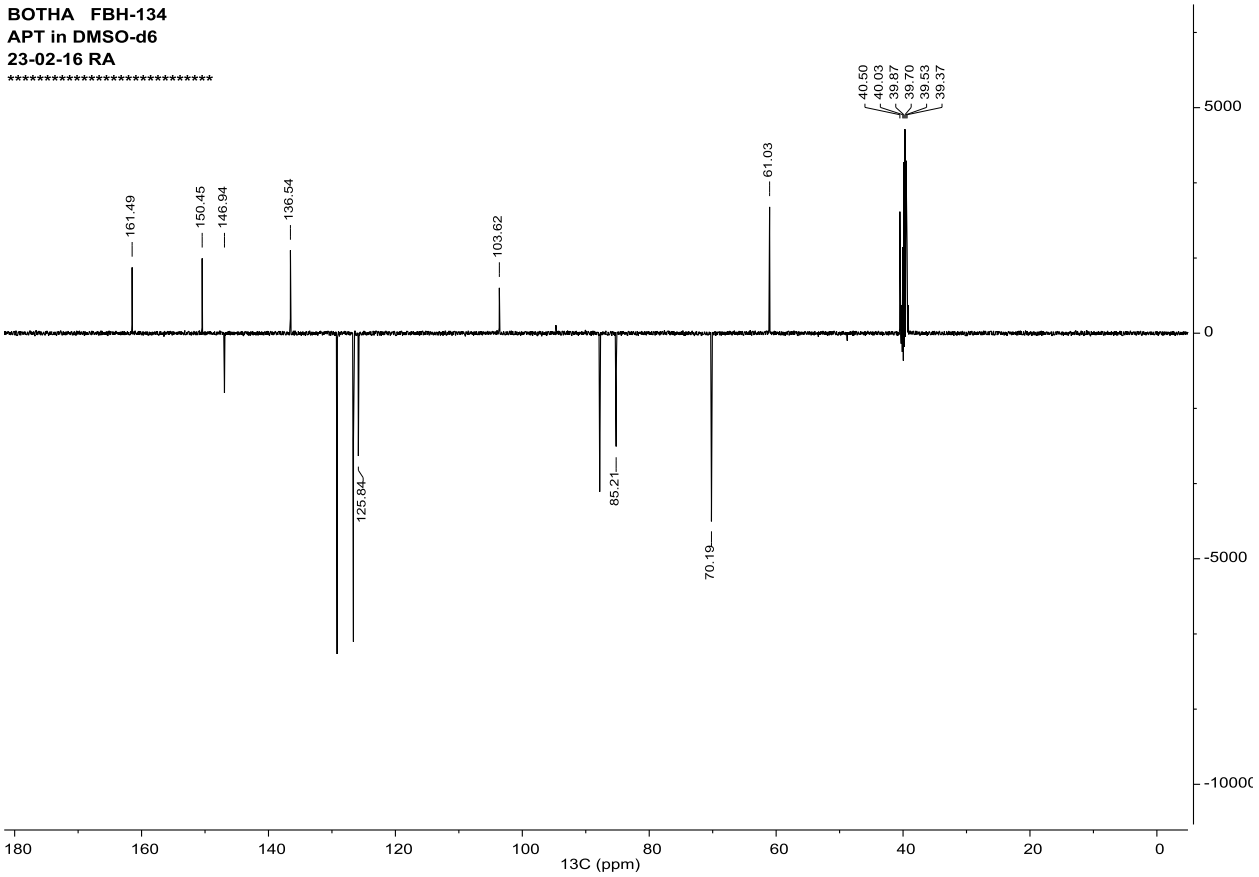




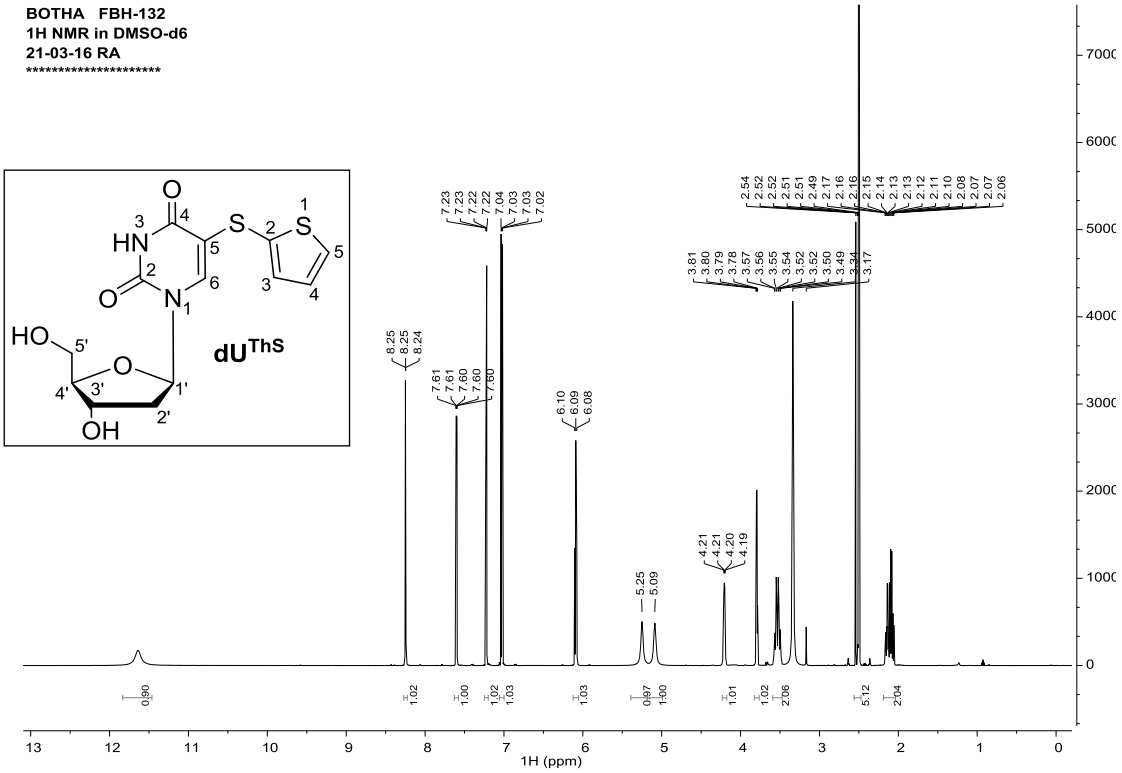

BOTHA FBH-49
77Se NMR in DMSO-d6
09-04-15 RA
****
8575.89
8564.34
8553.42
8541.87
89.92
89.80
89.68
89.56
77Se (ppm)
77Se (ppm)

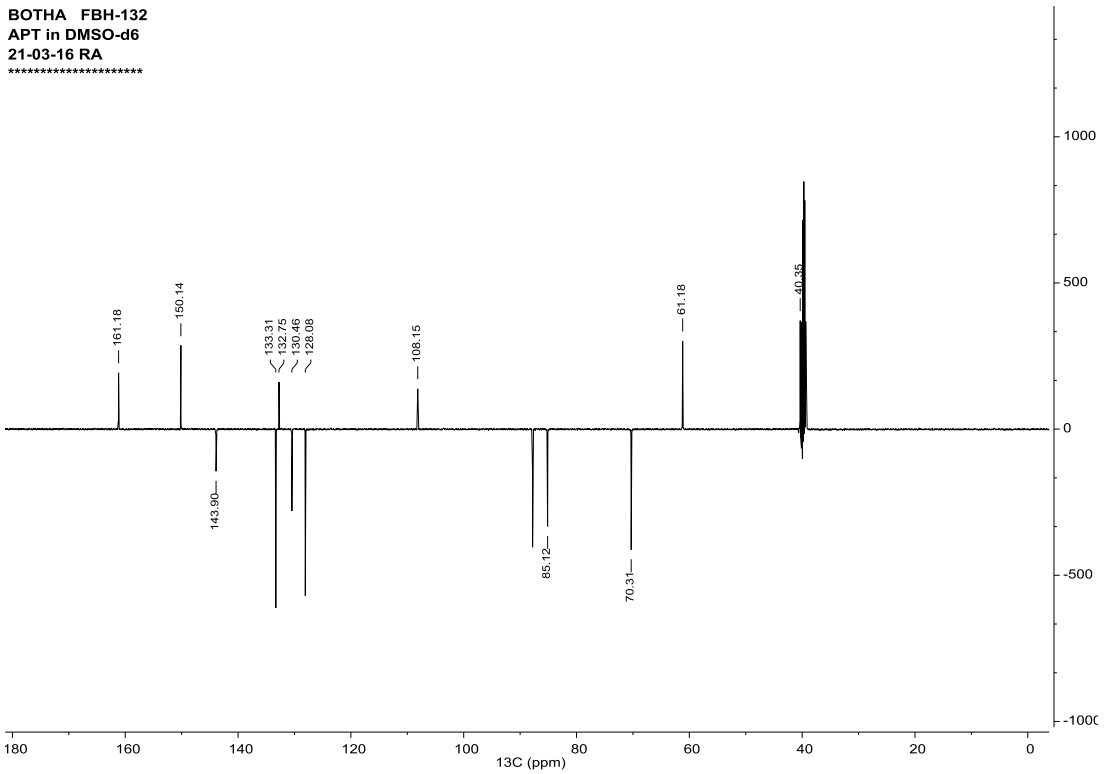
5-Phenylsulfanyl-2'-deoxyuridine



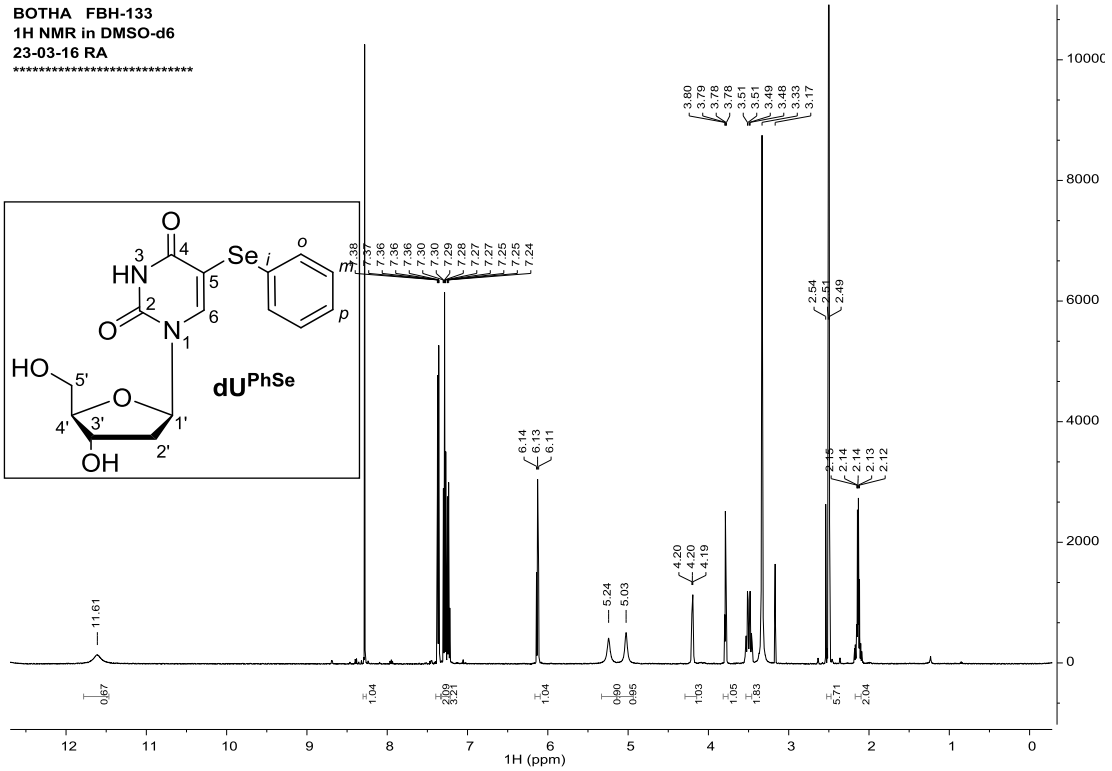


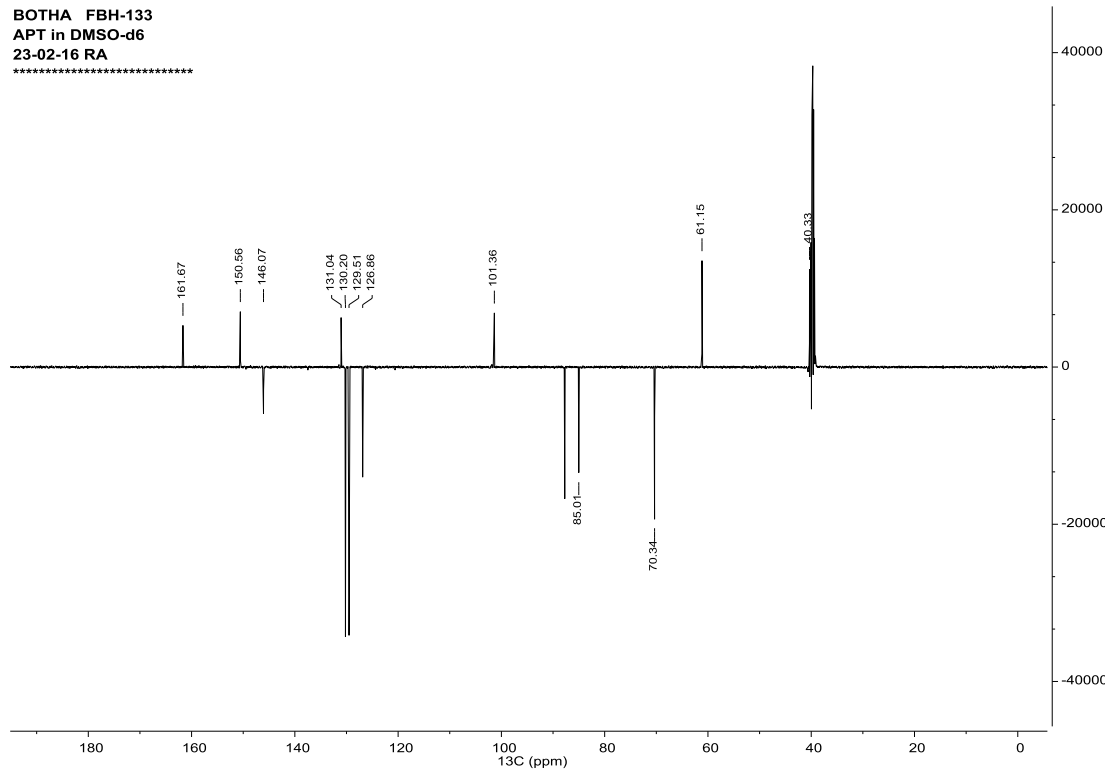
5-[(2-Thienyl)sulfanyl]-2'-deoxyuridine

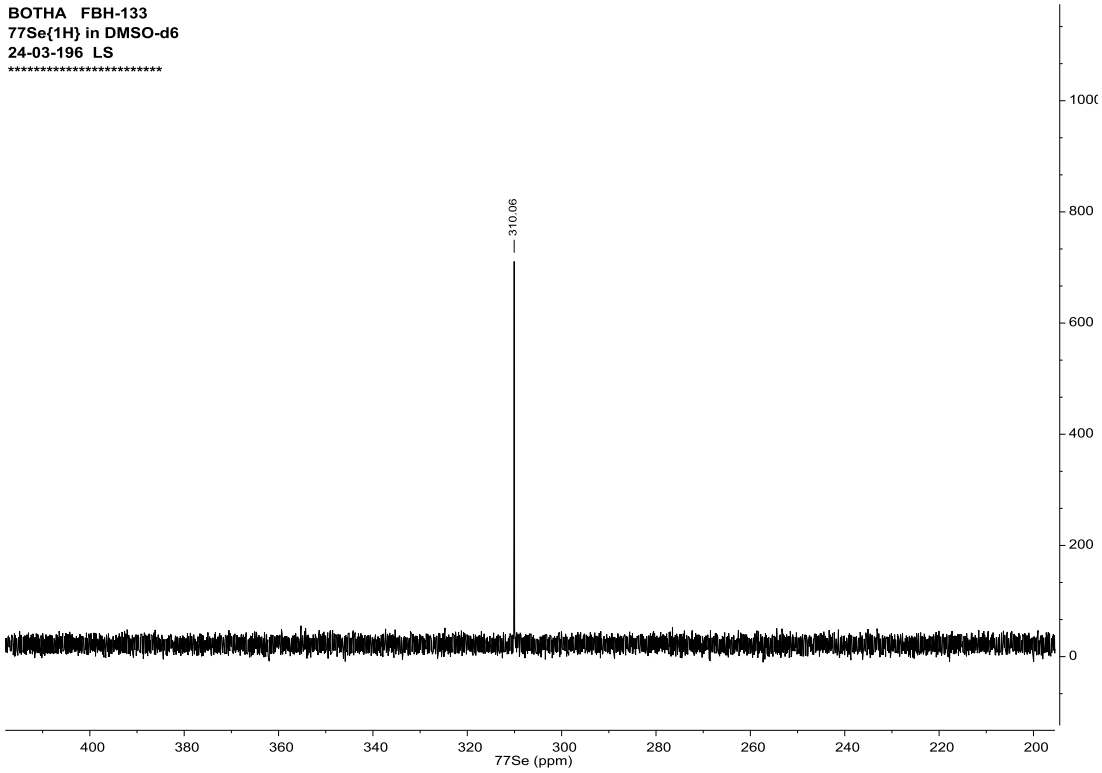




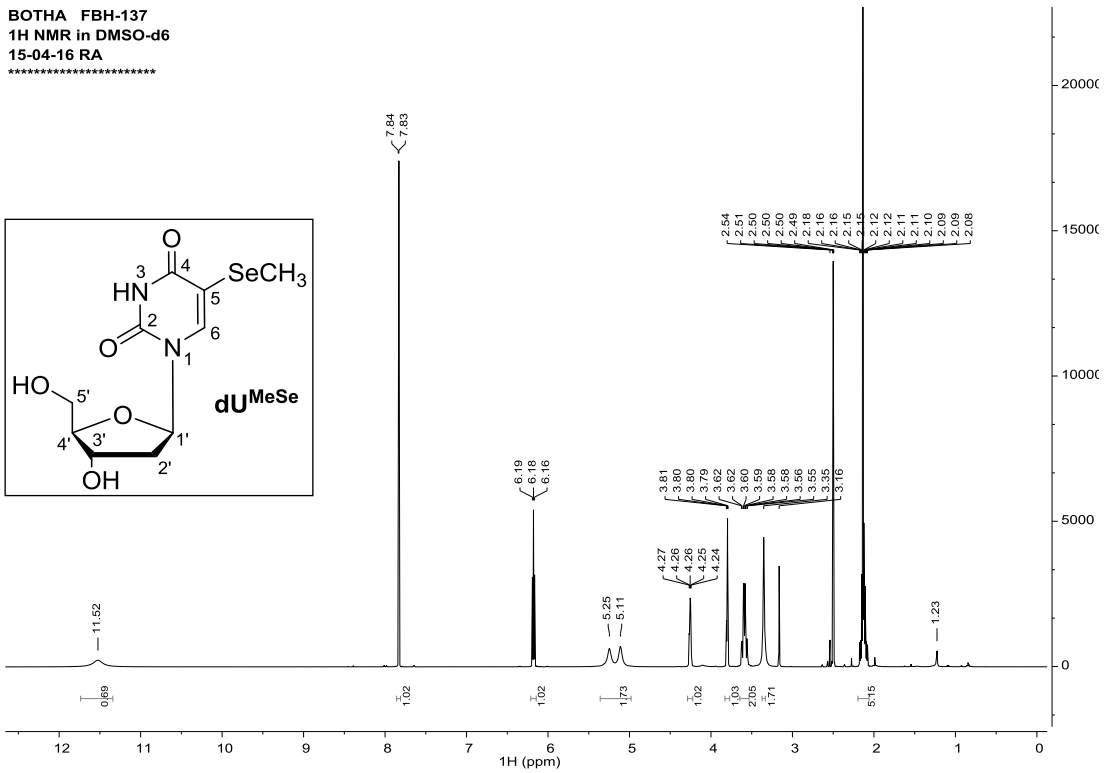
5-Phenylselanyl-2'-deoxyuridine

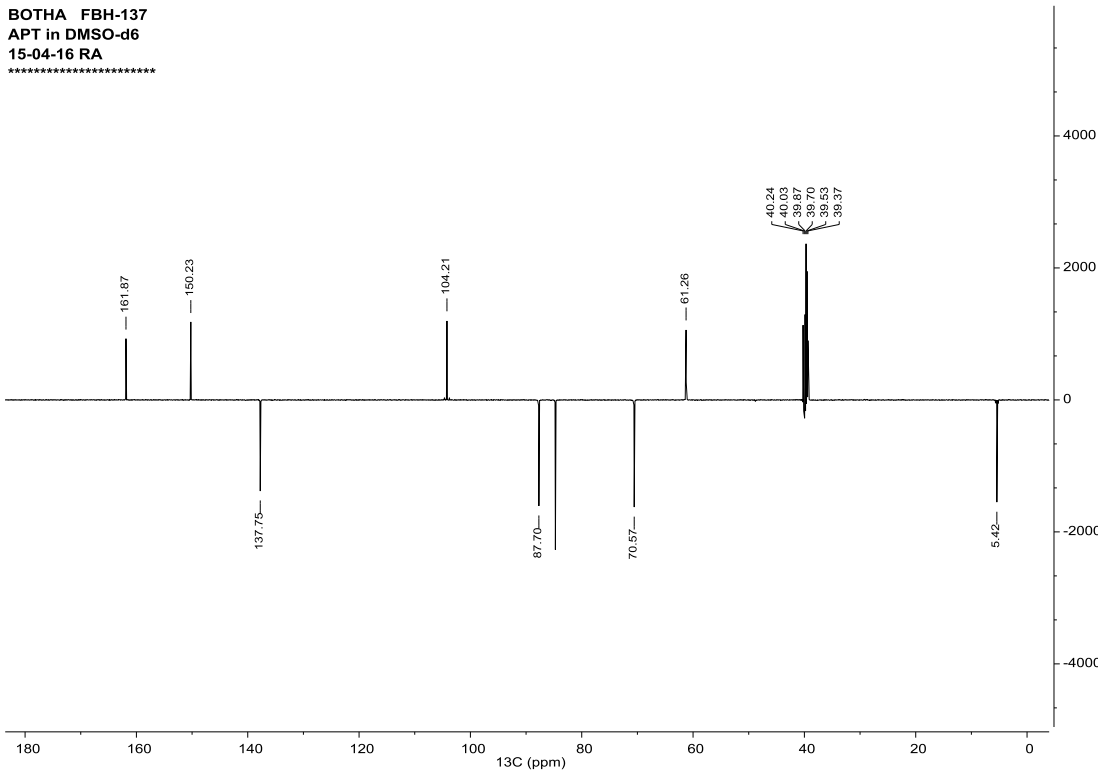


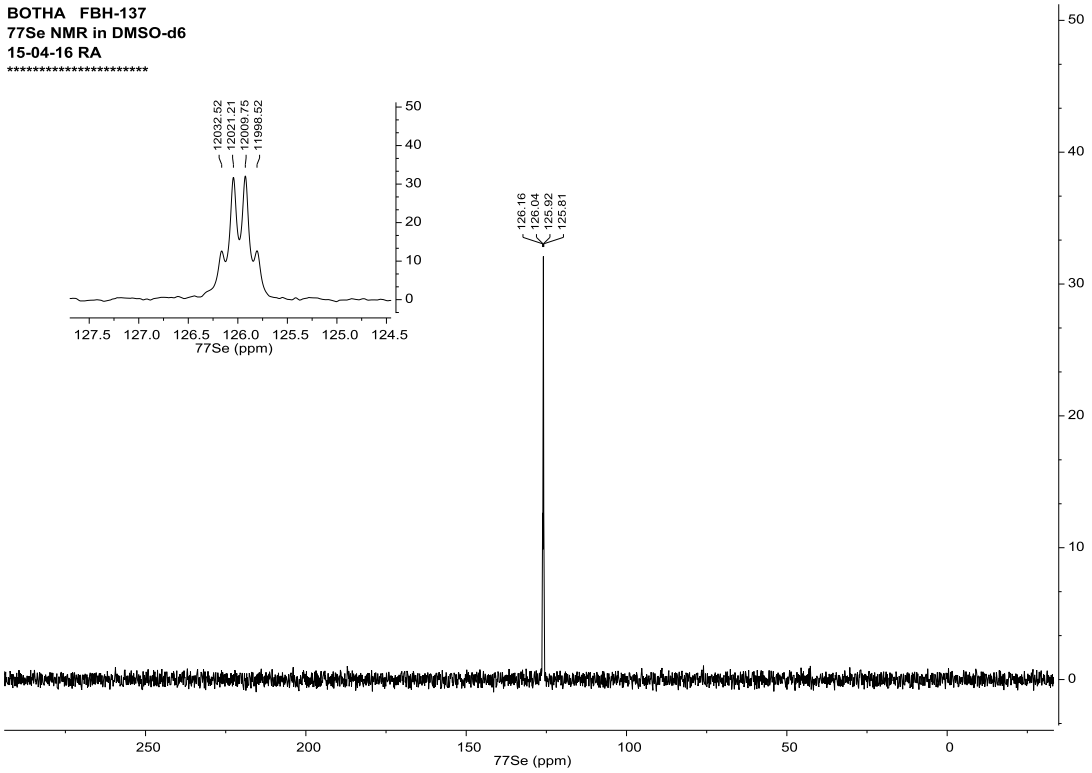



BOTHA FBH-133
77Se{1H} in DMSO-d6
24-03-196 LS
****
310.06
1000
800
600
400
200
0
400
380
360
340
320
300
280
260
240
220
200
77Se (ppm)

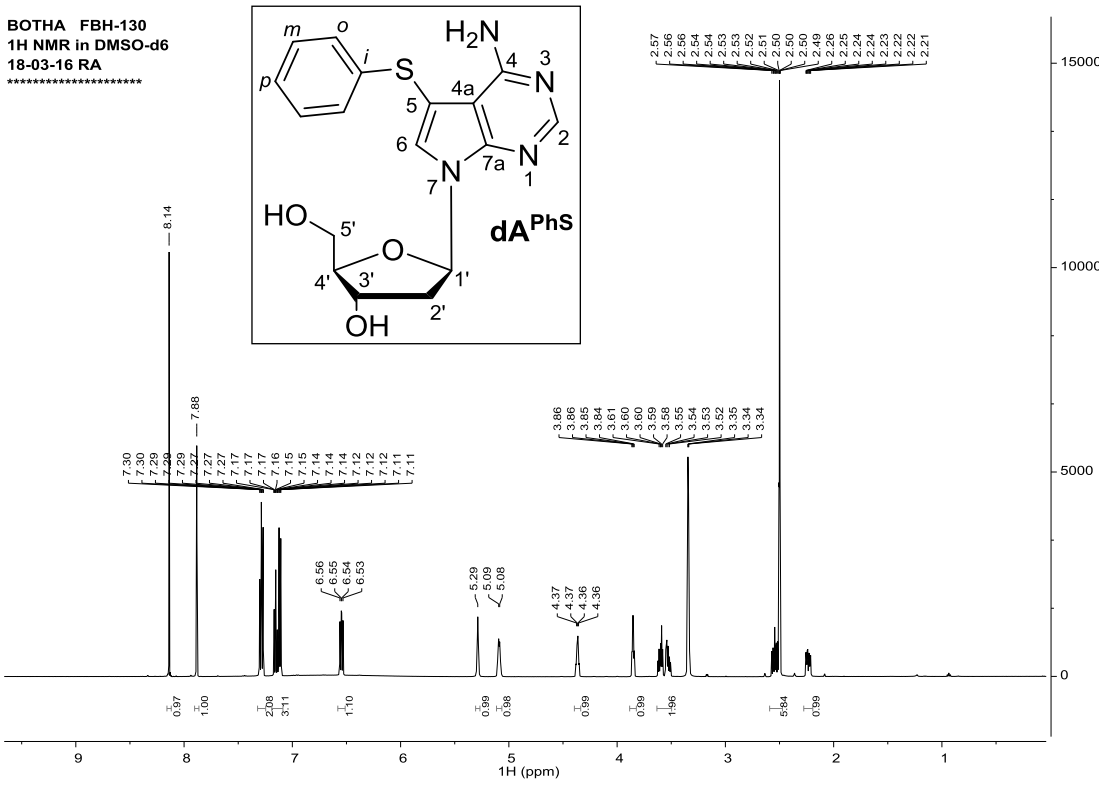
5-Methylselanyl-2'-deoxyuridine

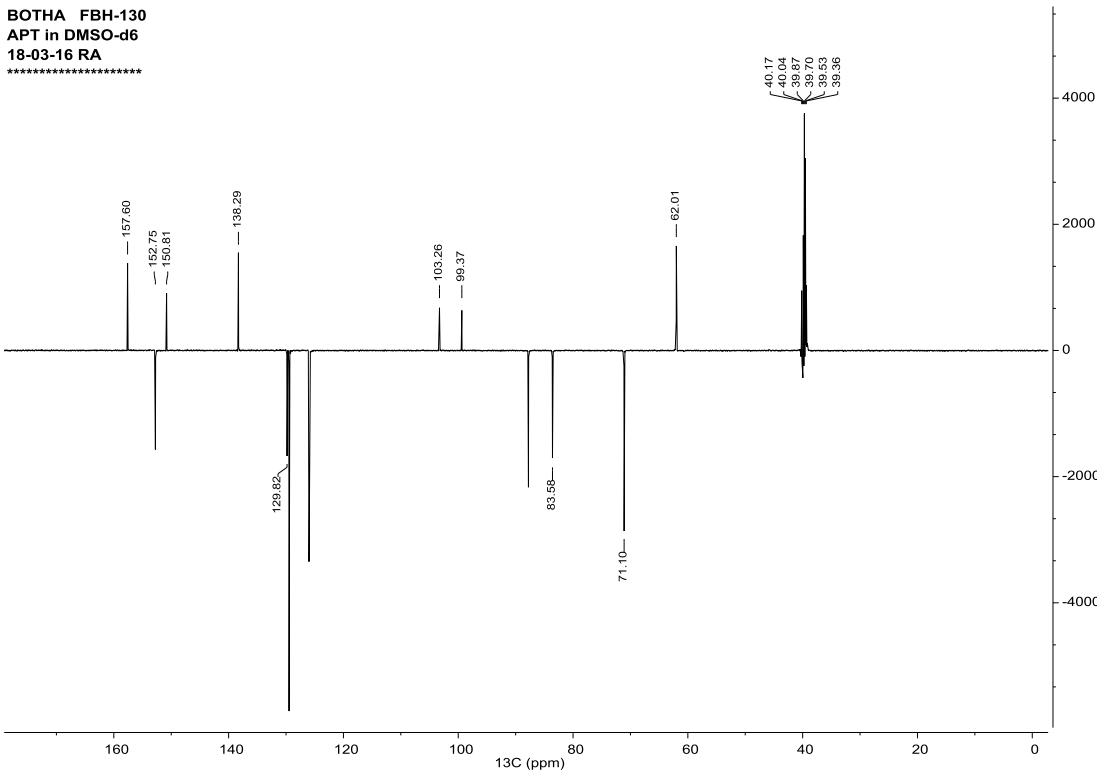




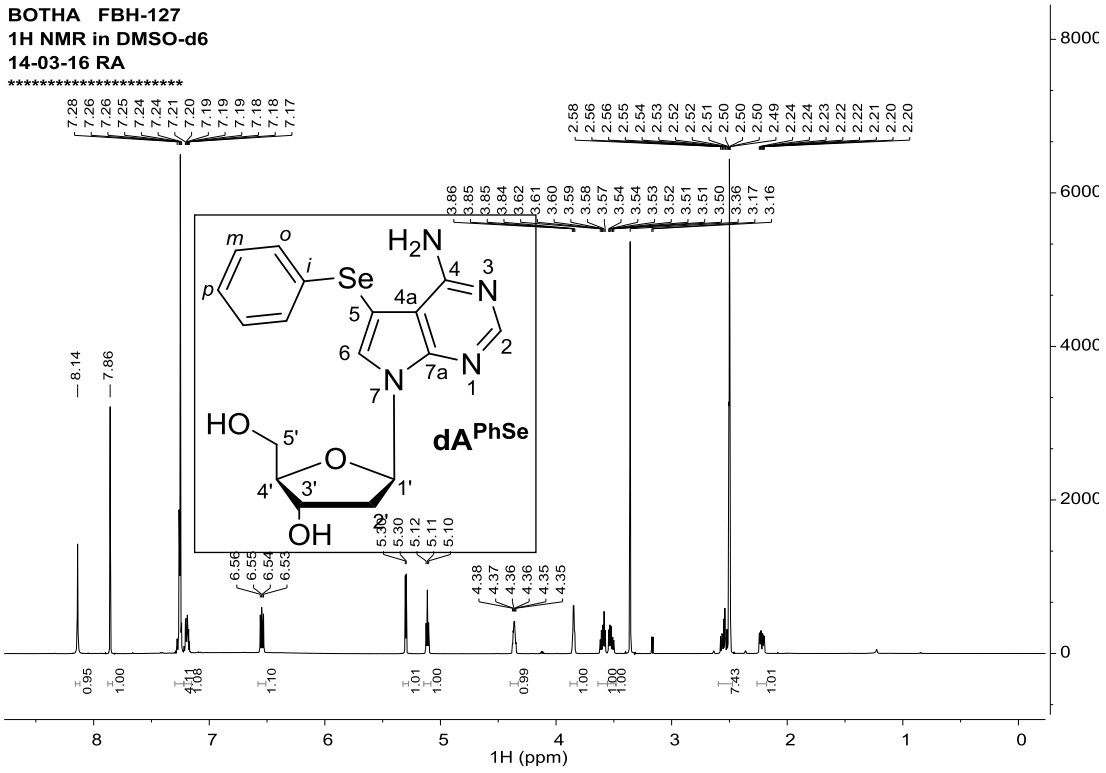

BOTHA FBH-137
77Se NMR in DMSO-d6
15-04-16 RA
****
12032.52
12021.21
12009.75
11998.52
126.16
126.04
125.92
125.81
77Se (ppm)
77Se (ppm)

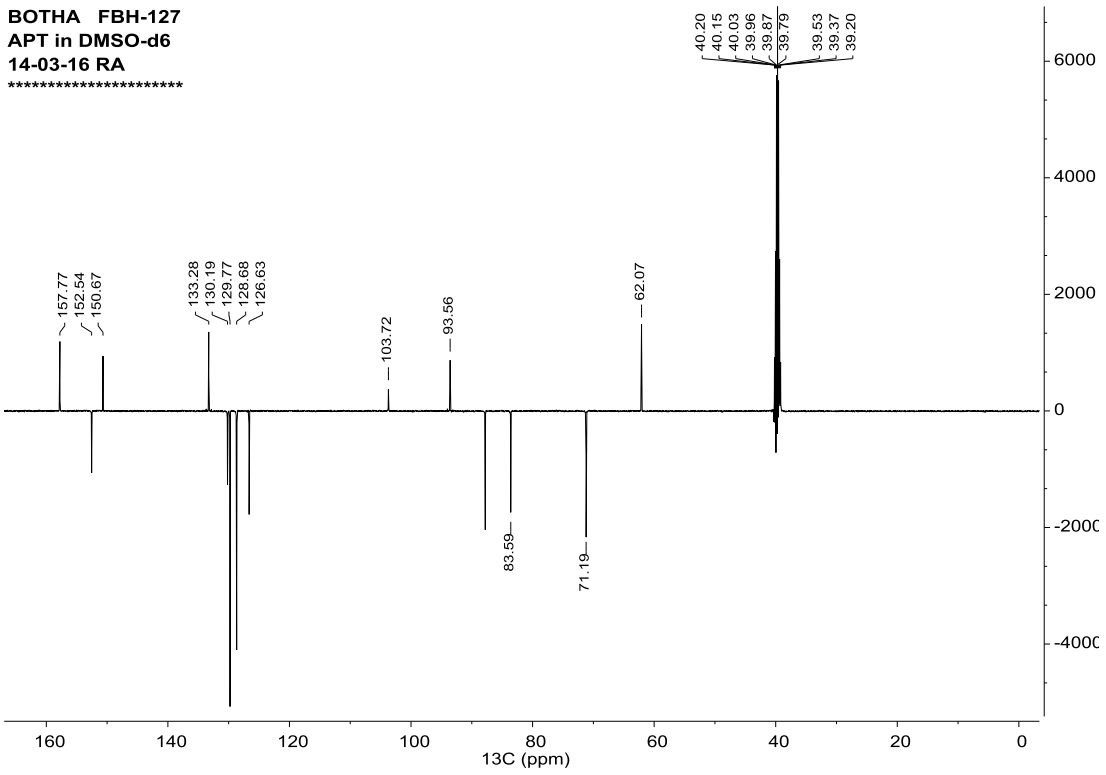
7-Phenylsulfanyl-7-deaza-2'-deoxyadenosine

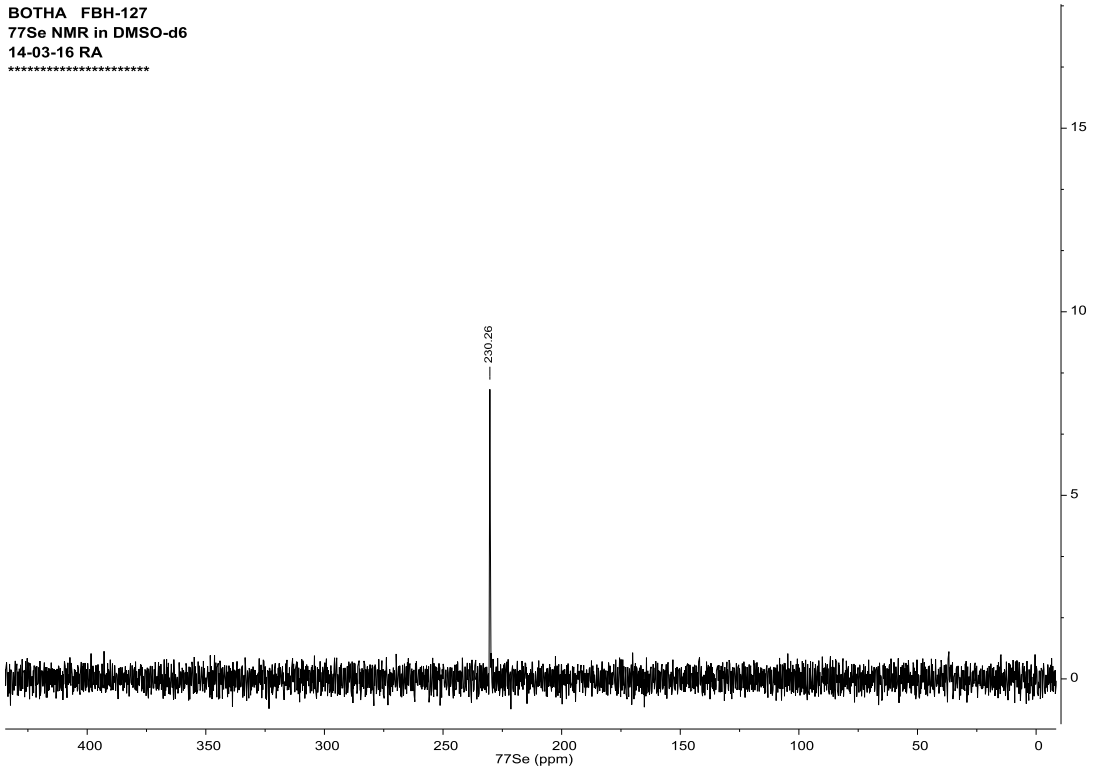




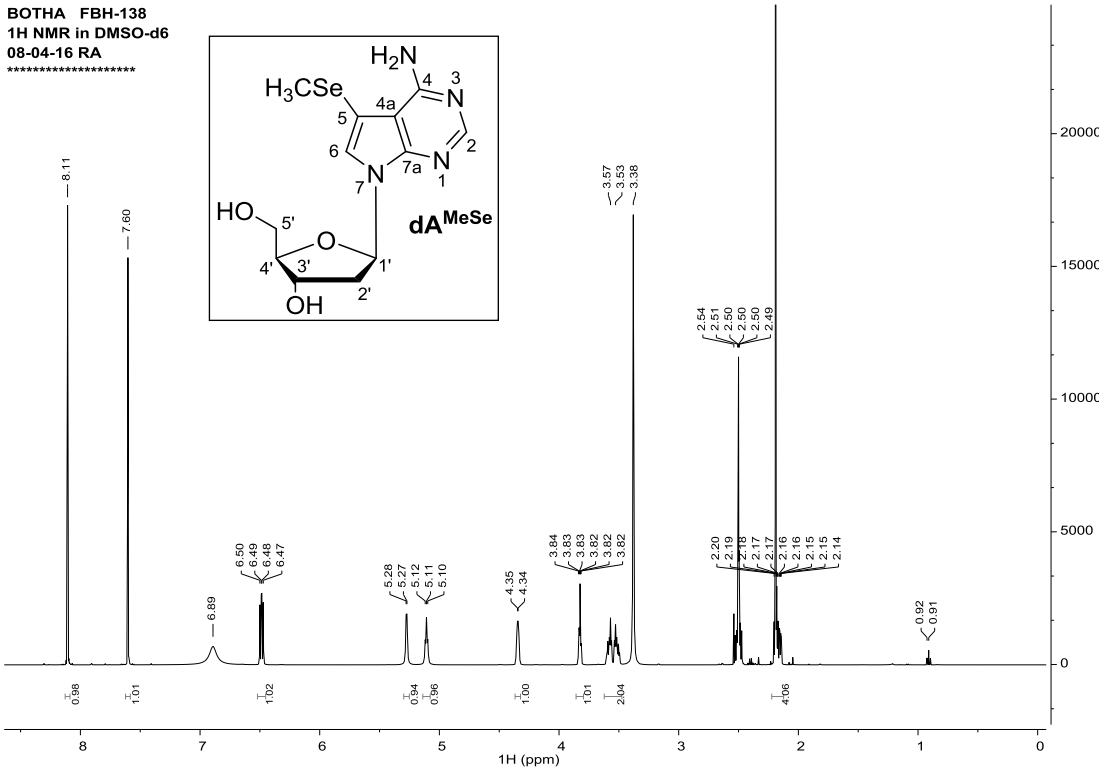
7-Phenylselanyl-7-deaza-2'-deoxyadenosine

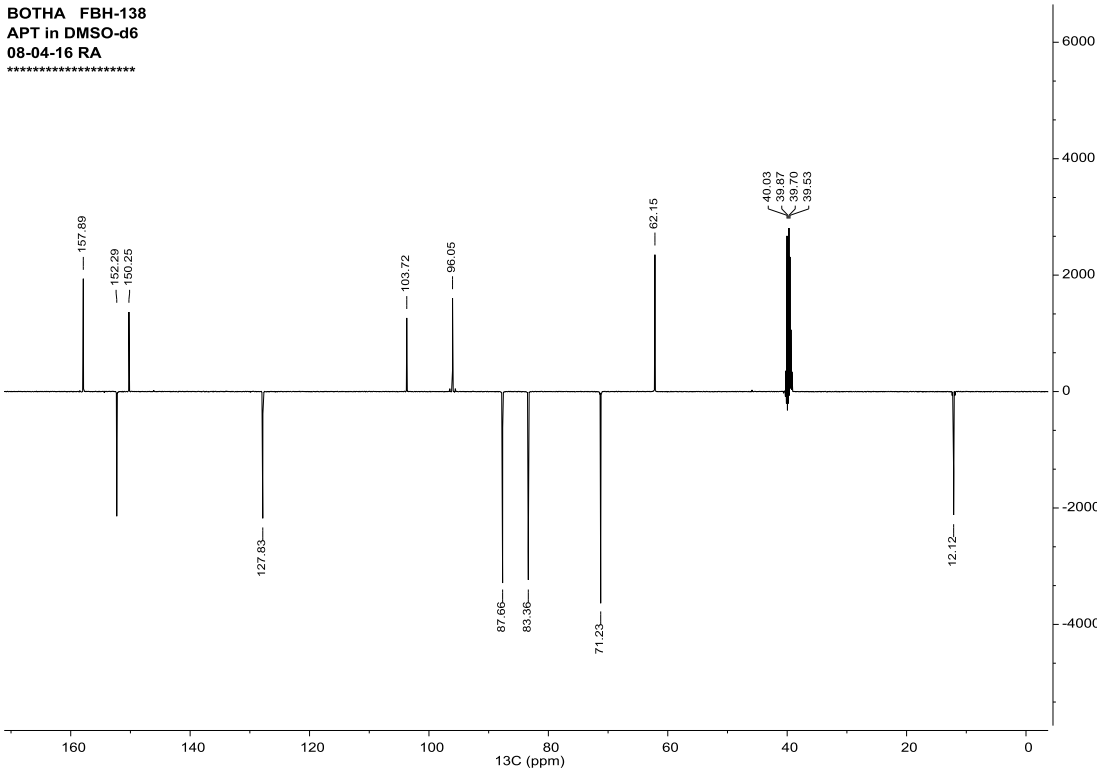


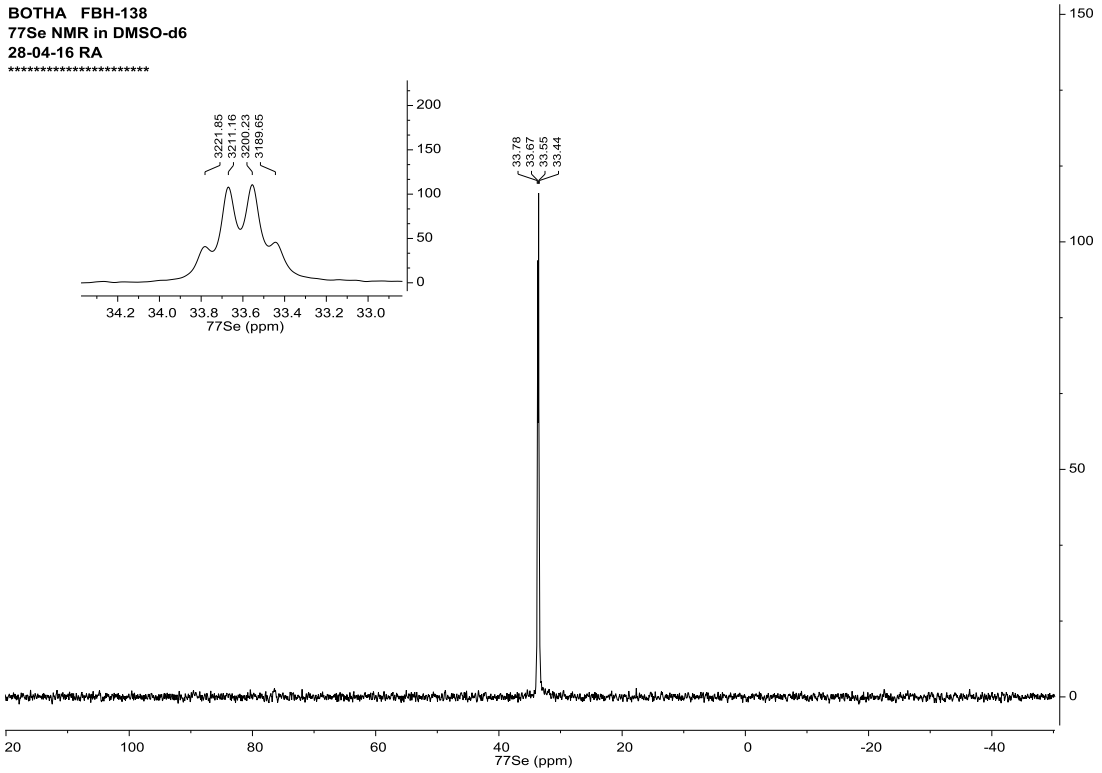



BOTHA FBH-127
77Se NMR in DMSO-d6
14-03-16 RA
****
230.26
15
10
5
0
400
350
300
250
200
150
100
50
0
77Se (ppm)

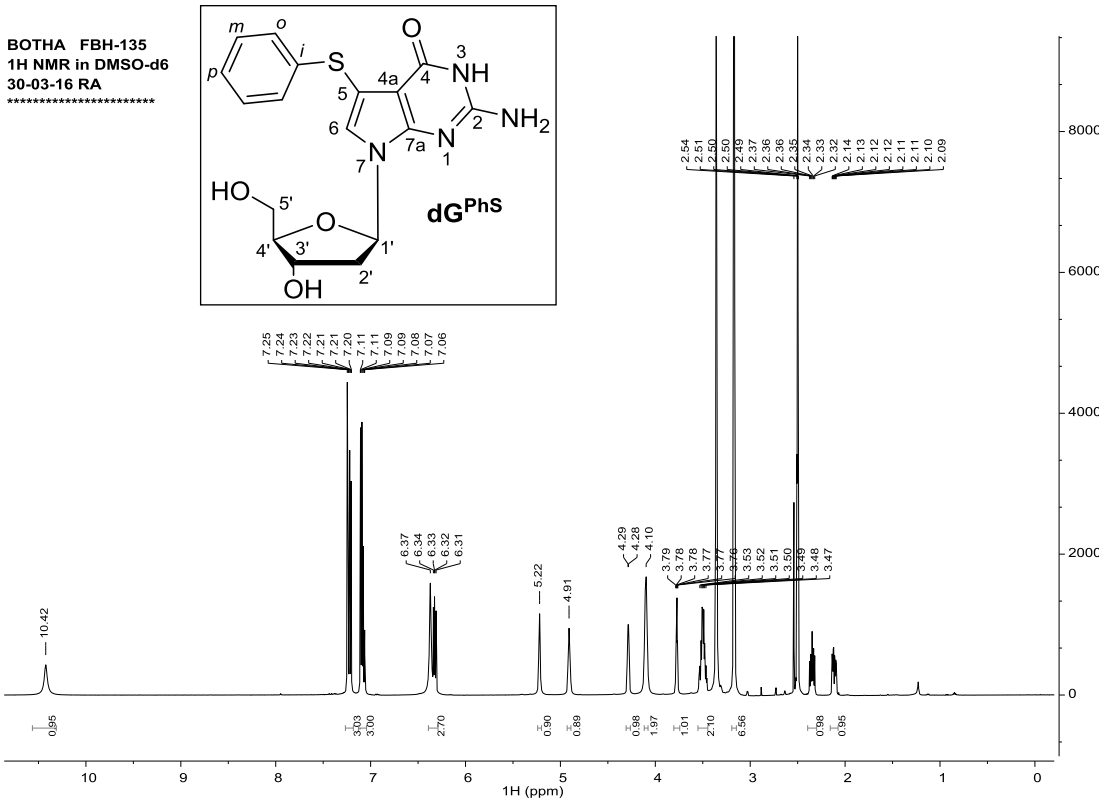
7-Methylselanyl-7-deaza-2'-deoxyadenosine

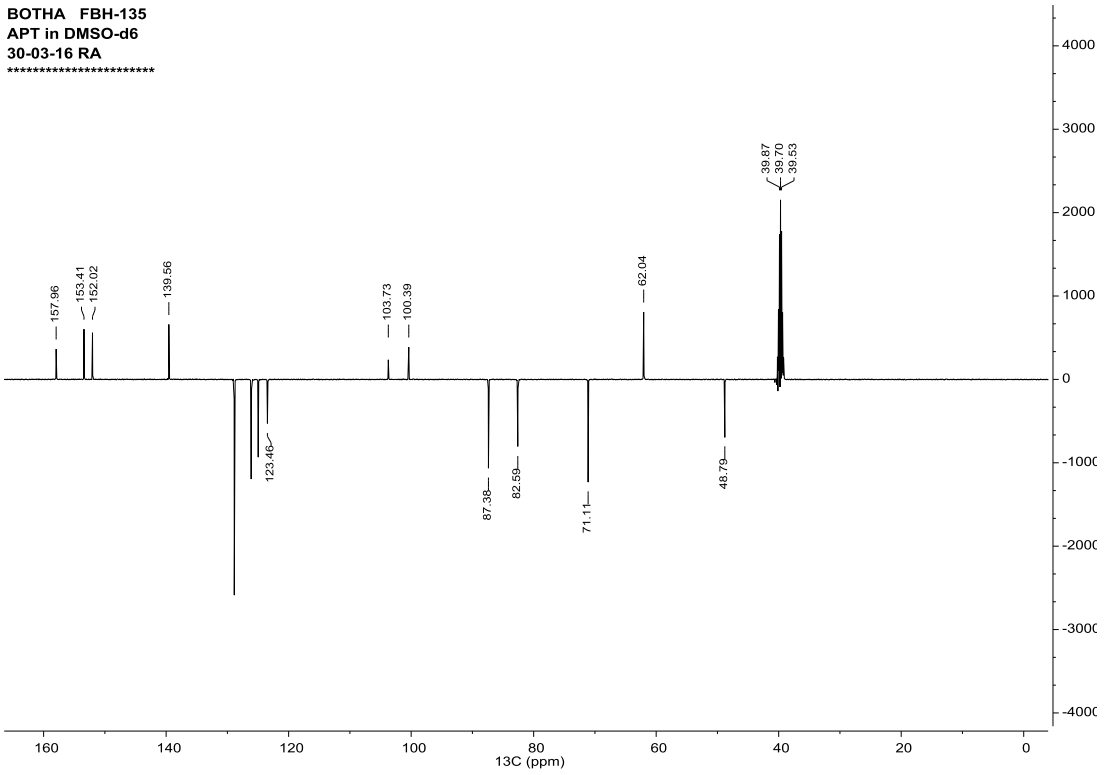




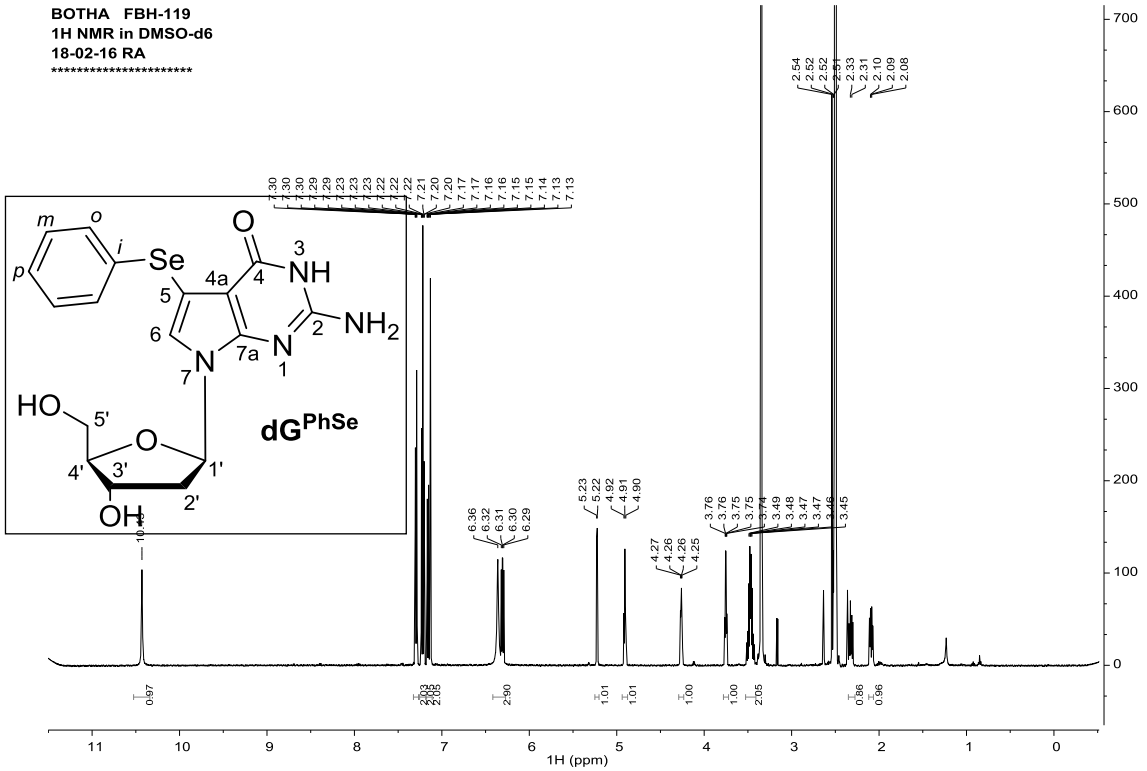

BOTHA FBH-138
77Se NMR in DMSO-d6
28-04-16 RA
****
3221.85
3211.16
3200.23
3189.65
33.78
33.67
33.55
33.44
77Se (ppm)
77Se (ppm)

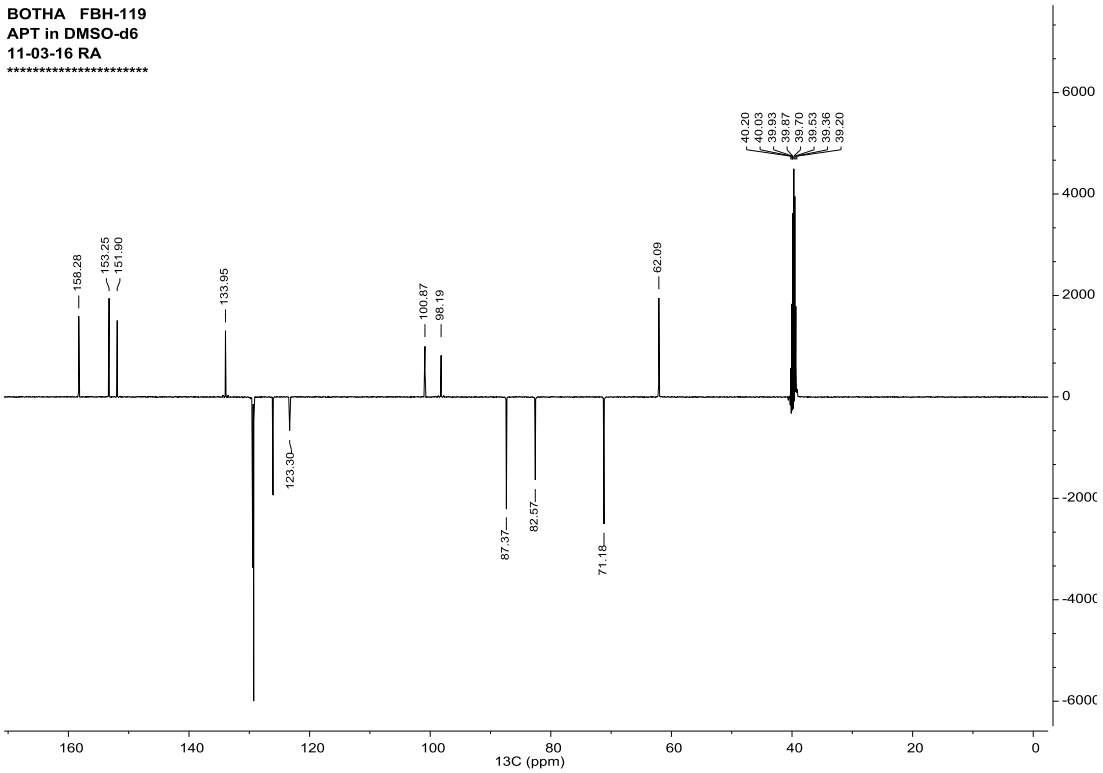
7-Phenylsulfanyl-7-deaza-2'-deoxyguanosine

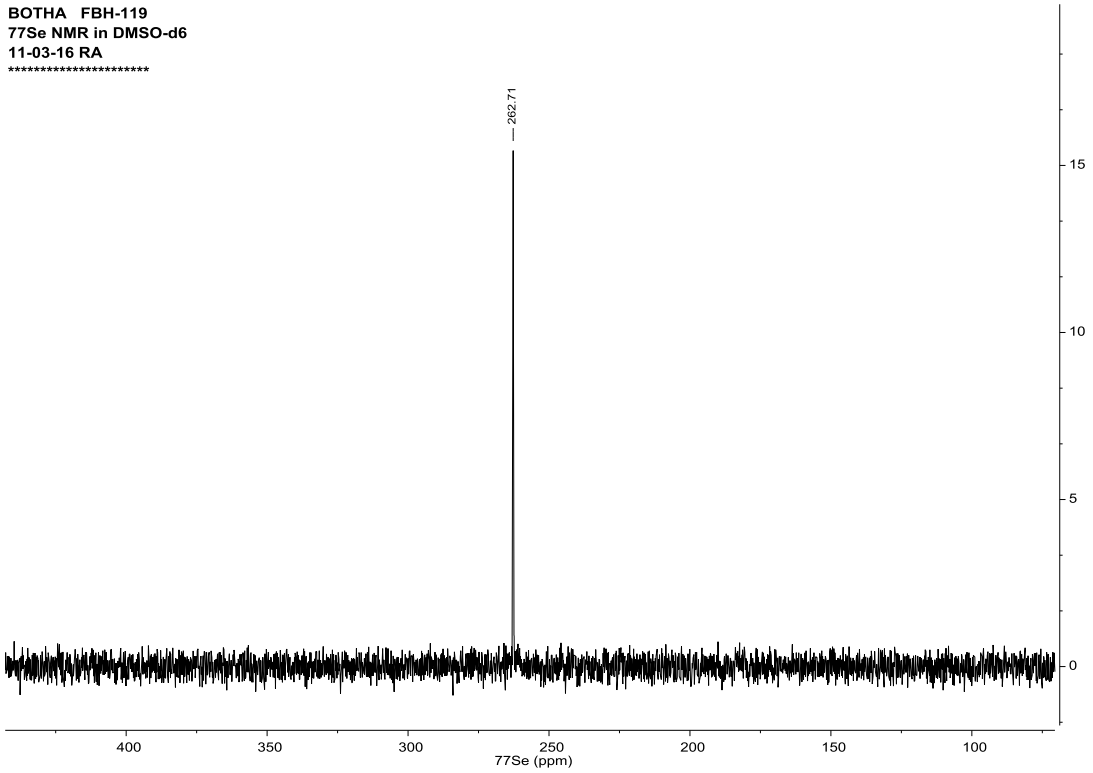




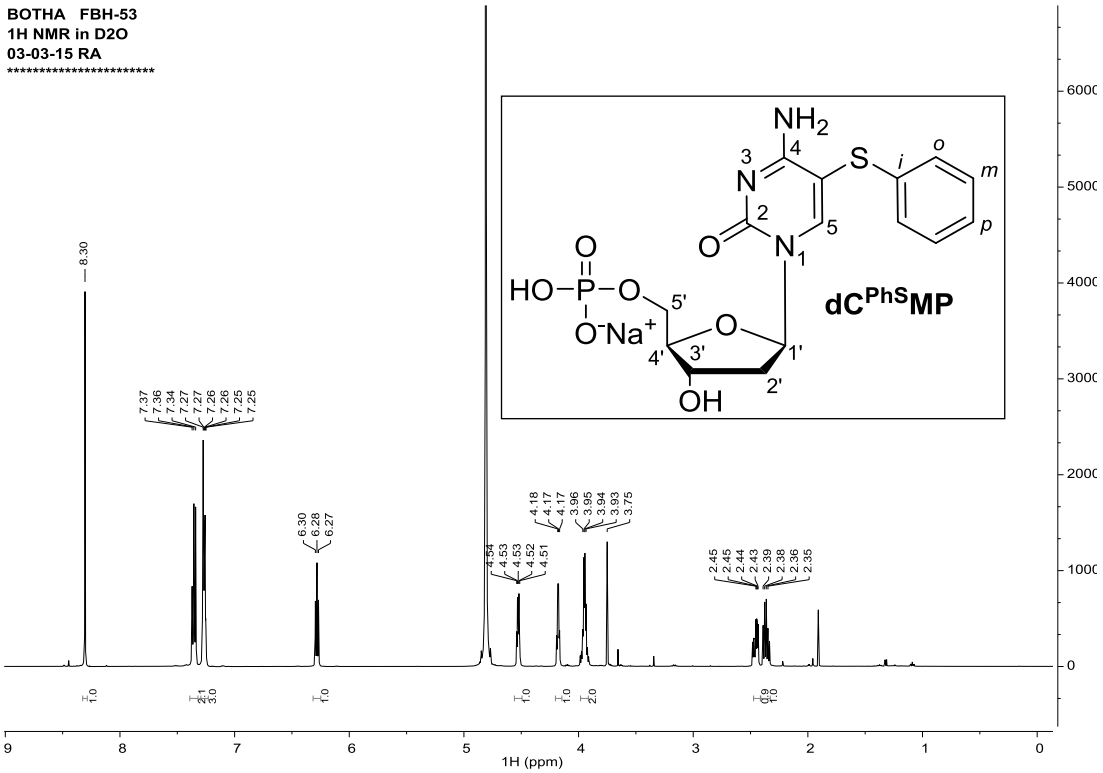
7-Phenylselanyl-7-deaza-2'-deoxyguanosine

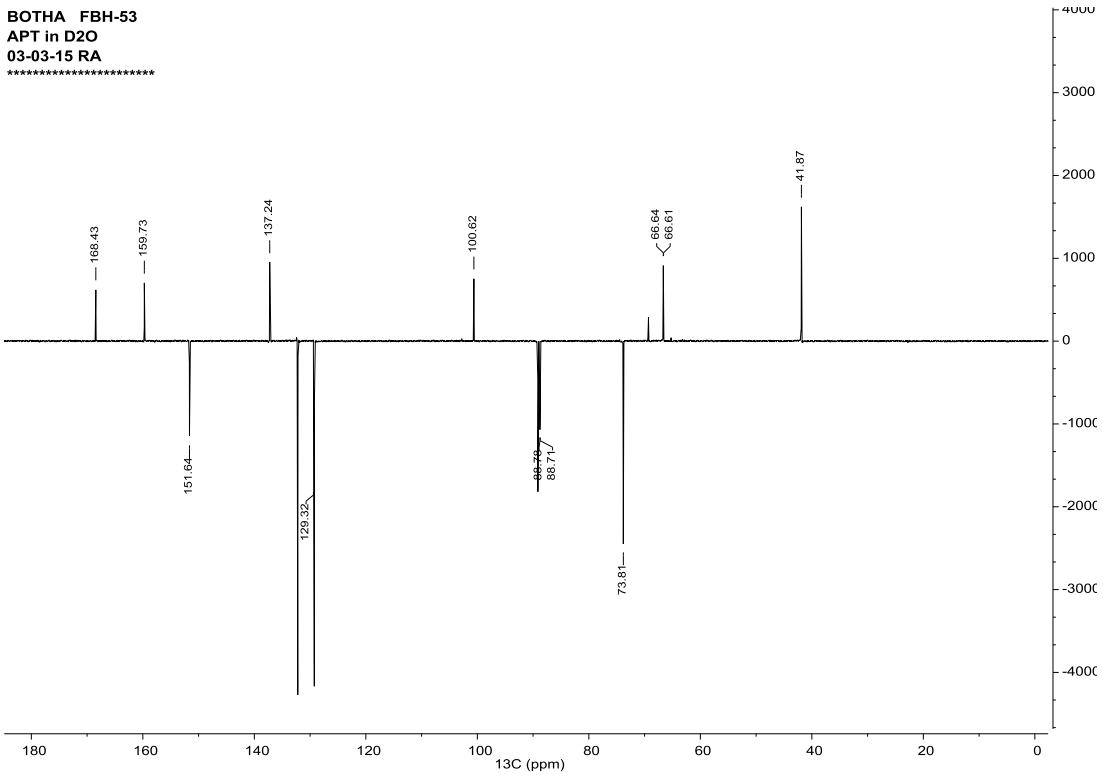


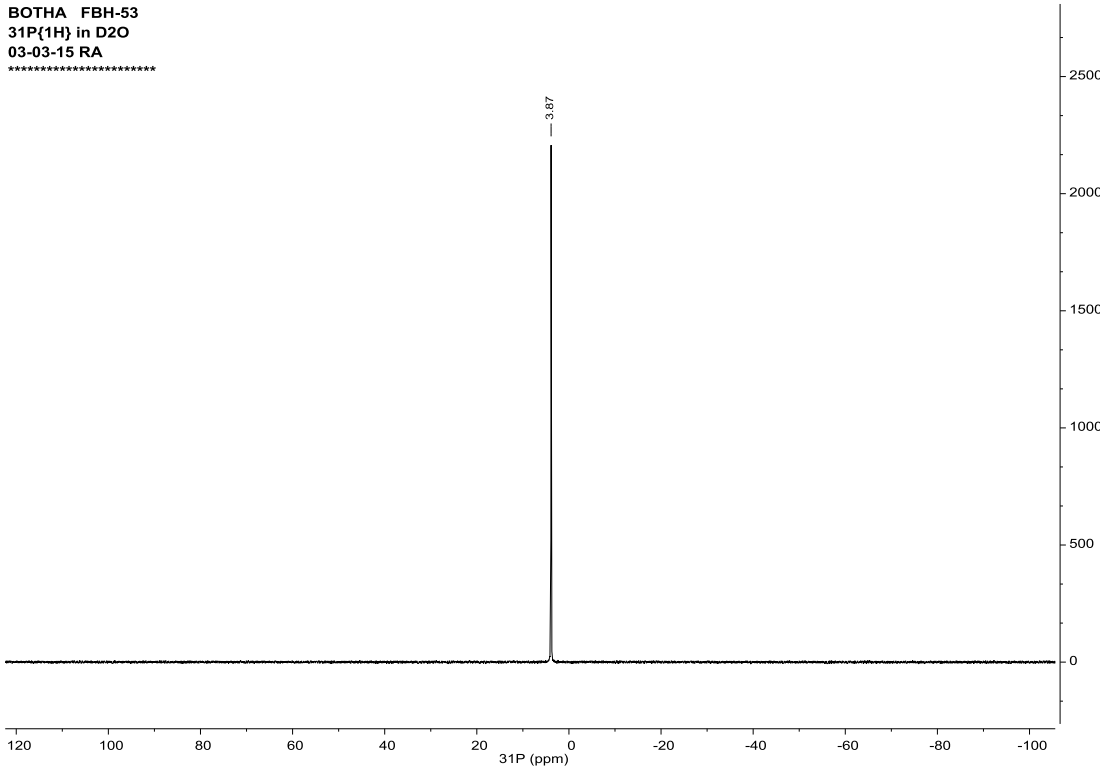



BOTHA FBH-119
77Se NMR in DMSO-d6
11-03-16 RA
****
262.71
15
10
5
0
400
350
300
250
200
150
100
77Se (ppm)

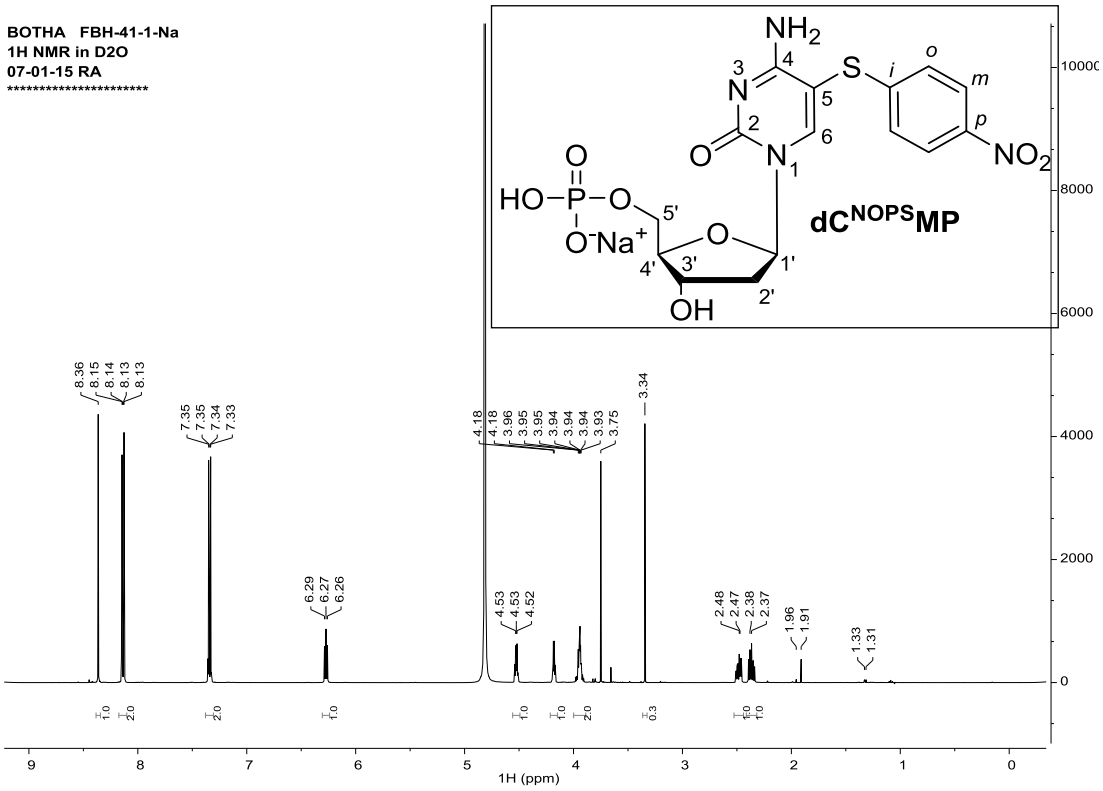
5-Phenylsulfanyl-2'-deoxycytidine 5'-*O*-monophosphate sodium salt

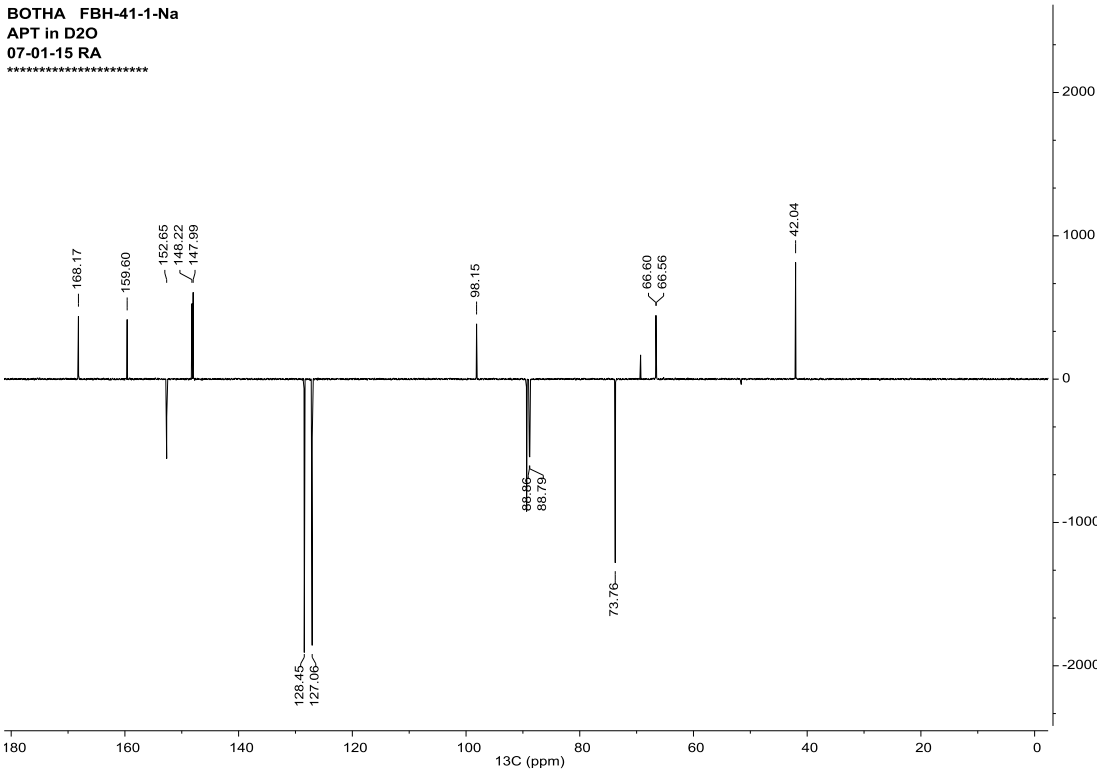


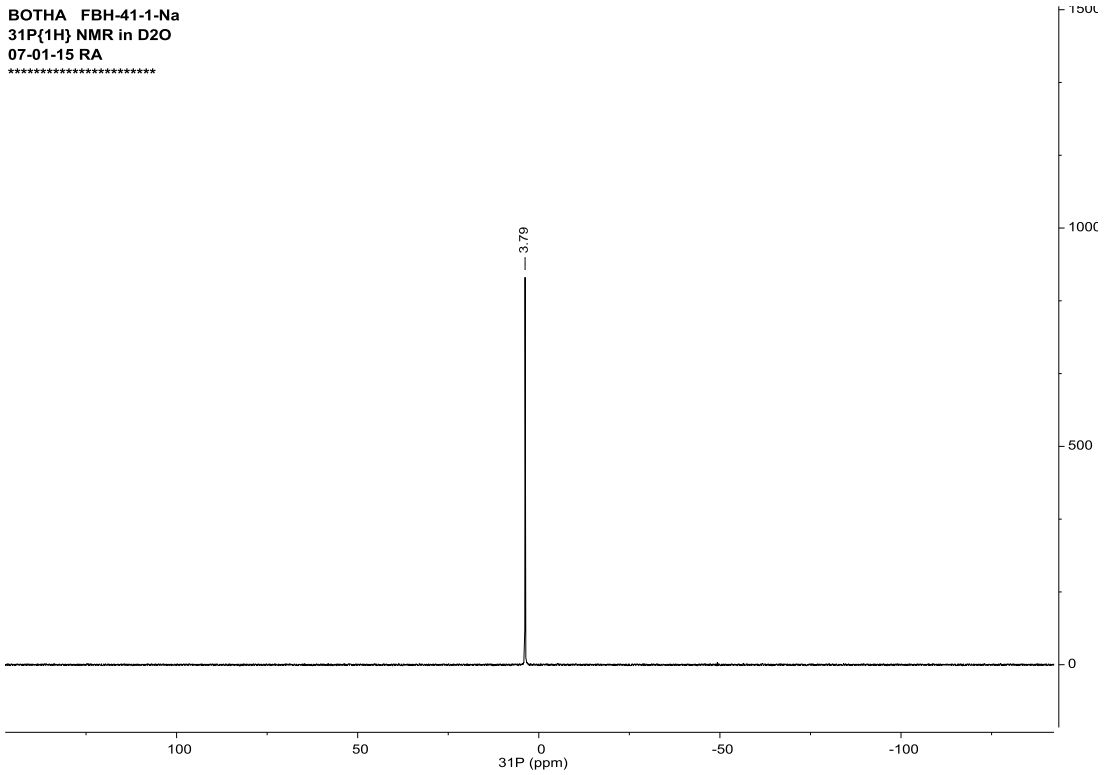



BOTHA FBH-53
31P{1H} in D2O
03-03-15 RA
****
3.87
2500
2000
1500
1000
500
0
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
31P (ppm)

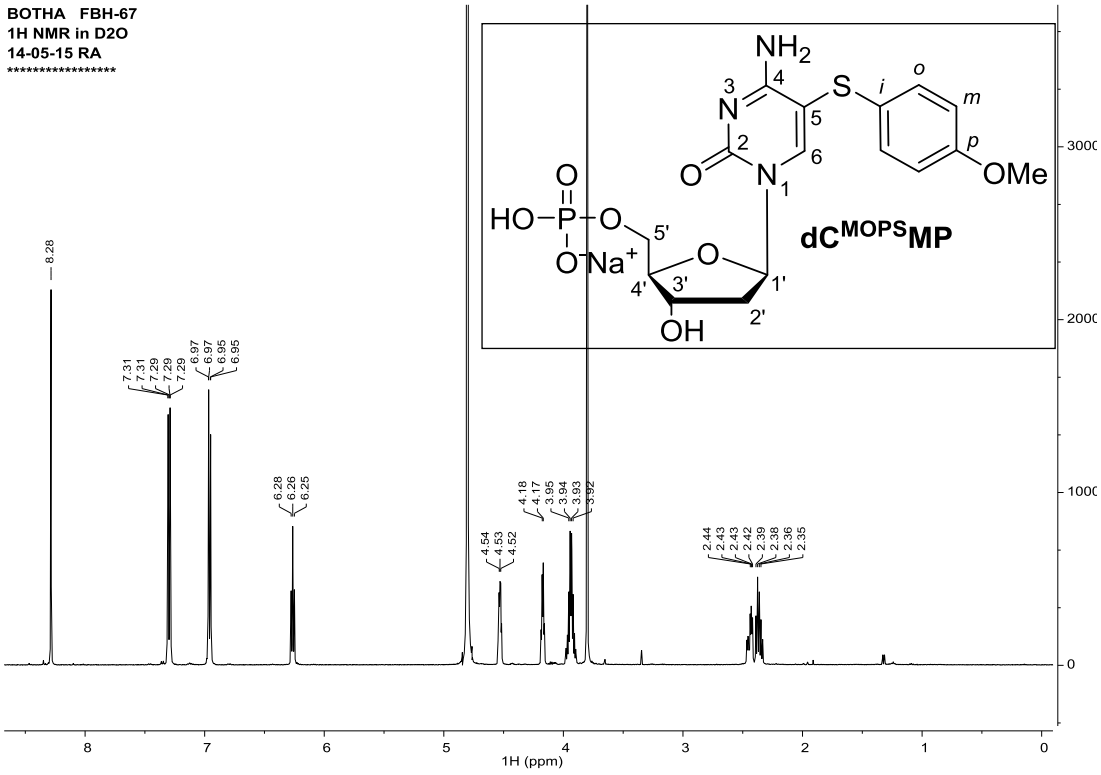
5-[(4-Nitrophenyl)sulfanyl]-2'-deoxycytidine 5'-*O*-monophosphate sodium salt

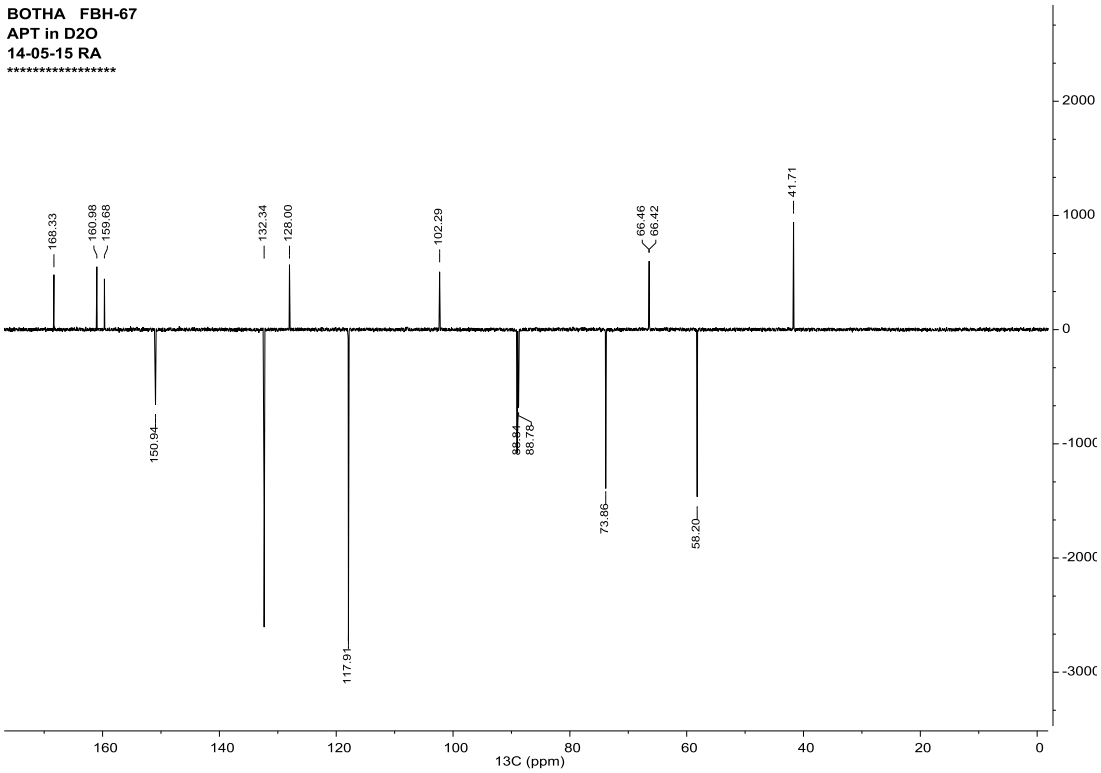


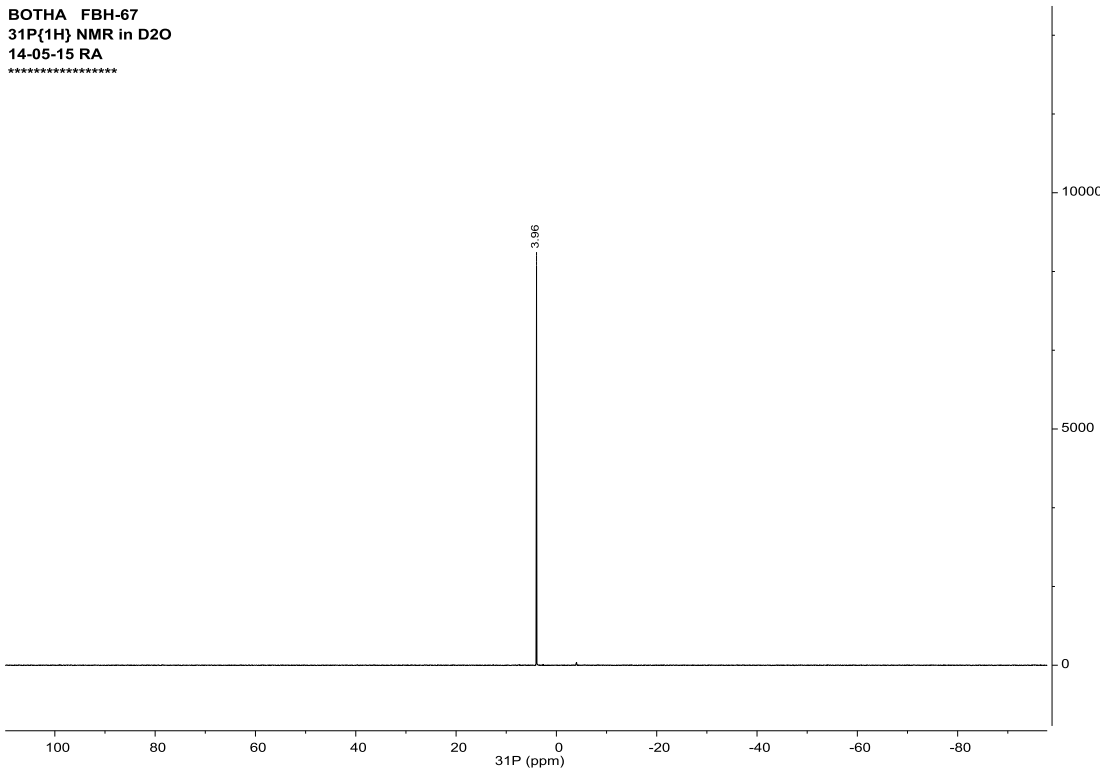



BOTHA FBH-41-1-Na
31P{1H} NMR in D2O
07-01-15 RA
****
3.79
1500
1000
500
0
100
50
0
-50
-100
31P (ppm)

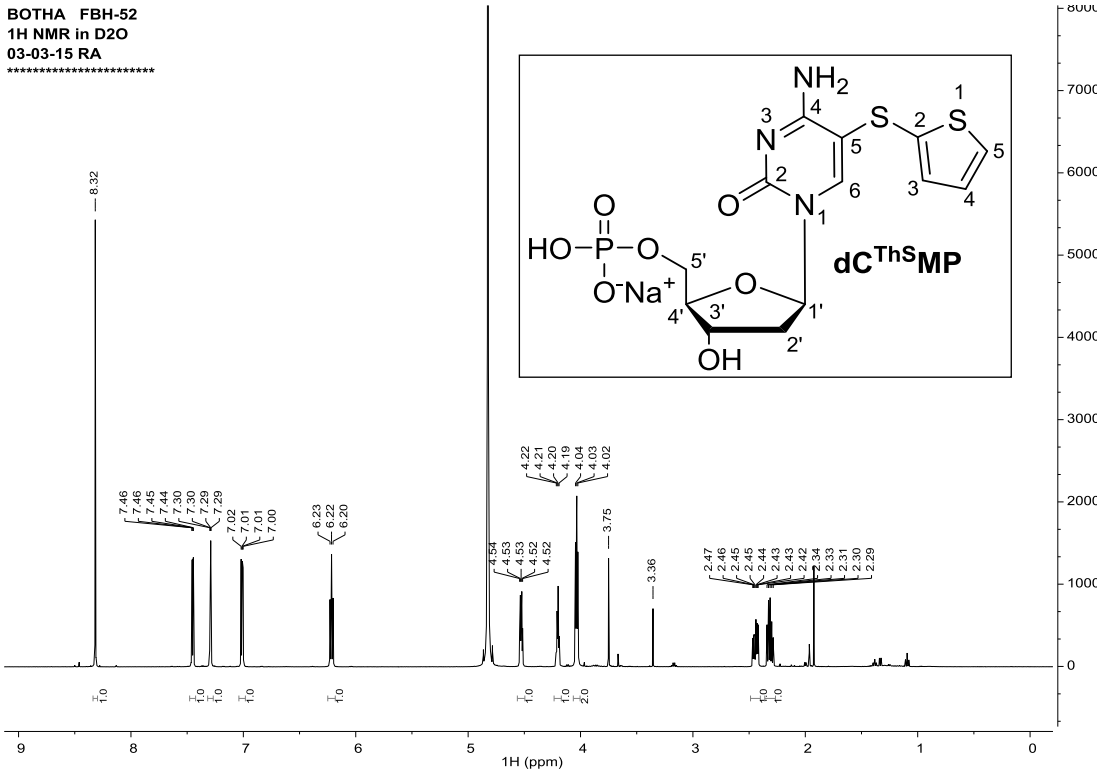
5-[(4-Methoxyphenyl)sulfanyl]-2'-deoxycytidine 5'-*O*-monophosphate sodium salt

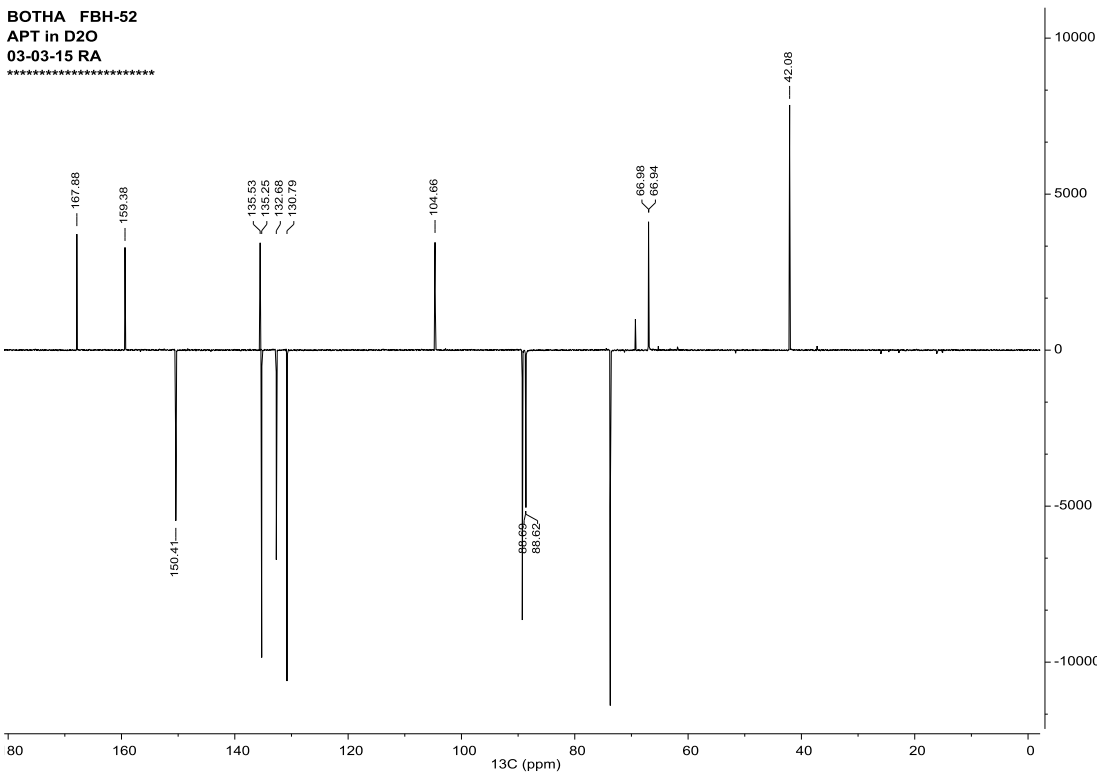


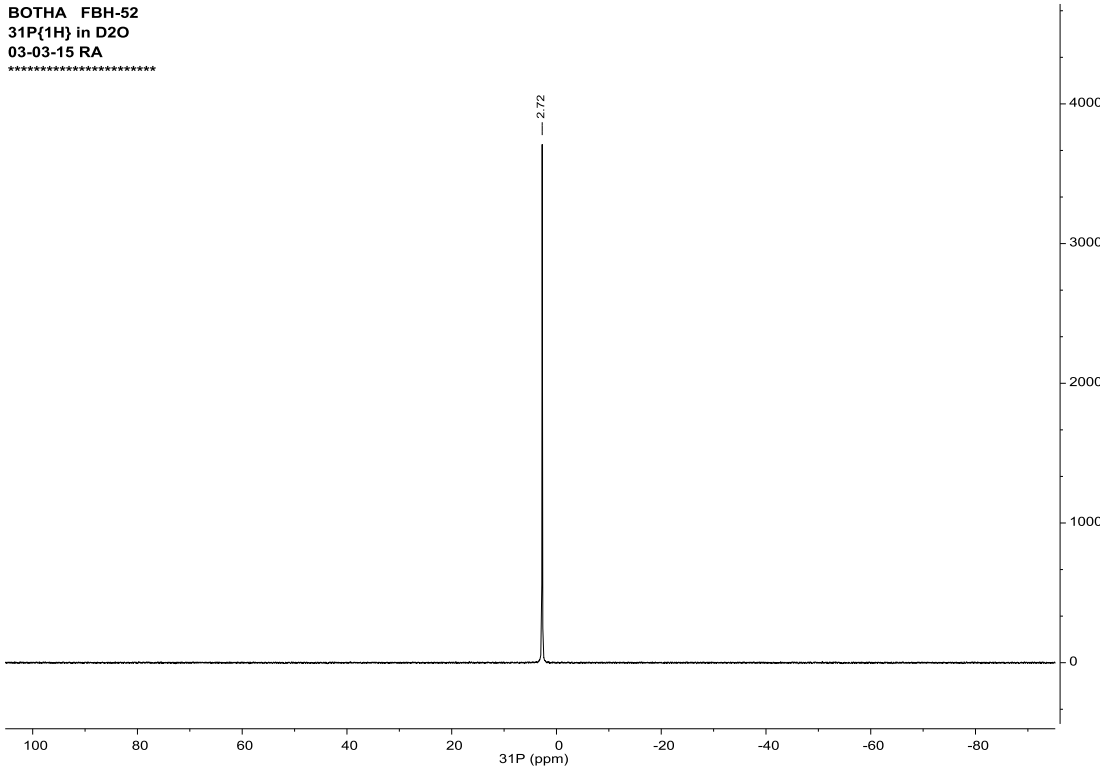



BOTHA FBH-67
31P{1H} NMR in D2O
14-05-15 RA
****
3.96
10000
5000
0
100
80
60
40
20
0
-20
-40
-60
-80
31P (ppm)

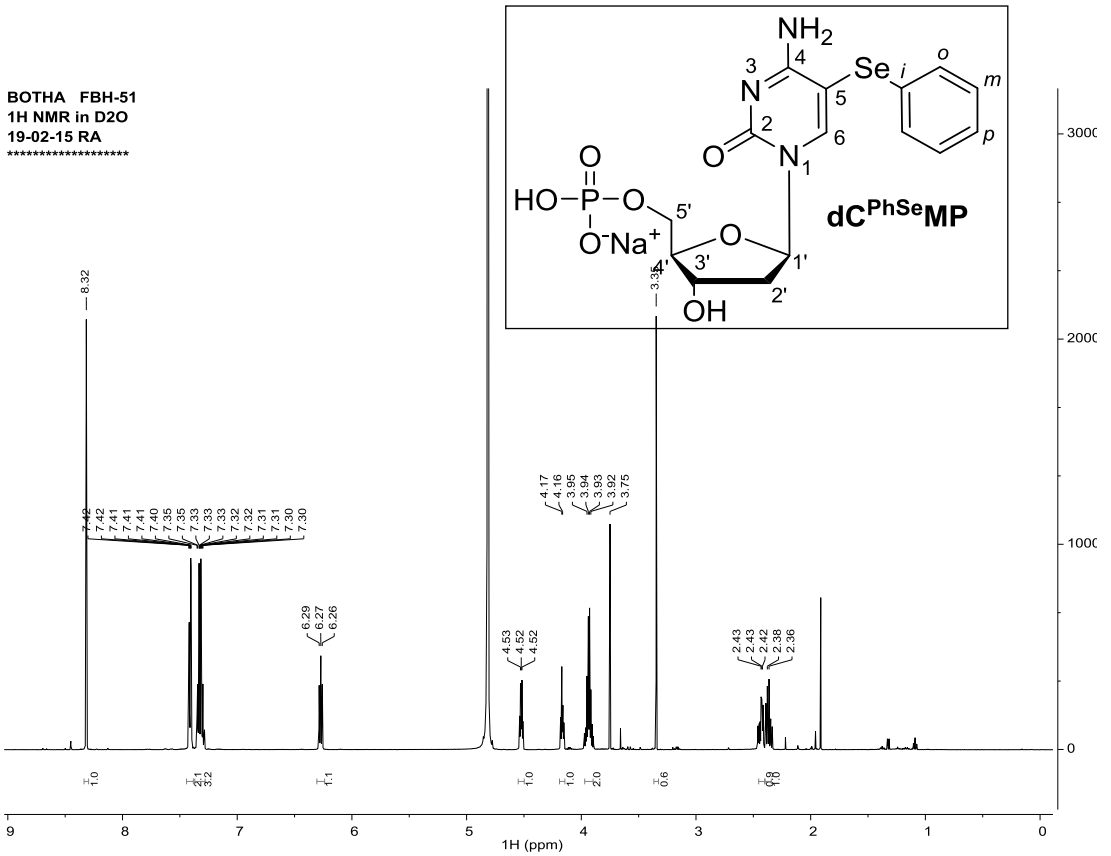
5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-*O*-monophosphate

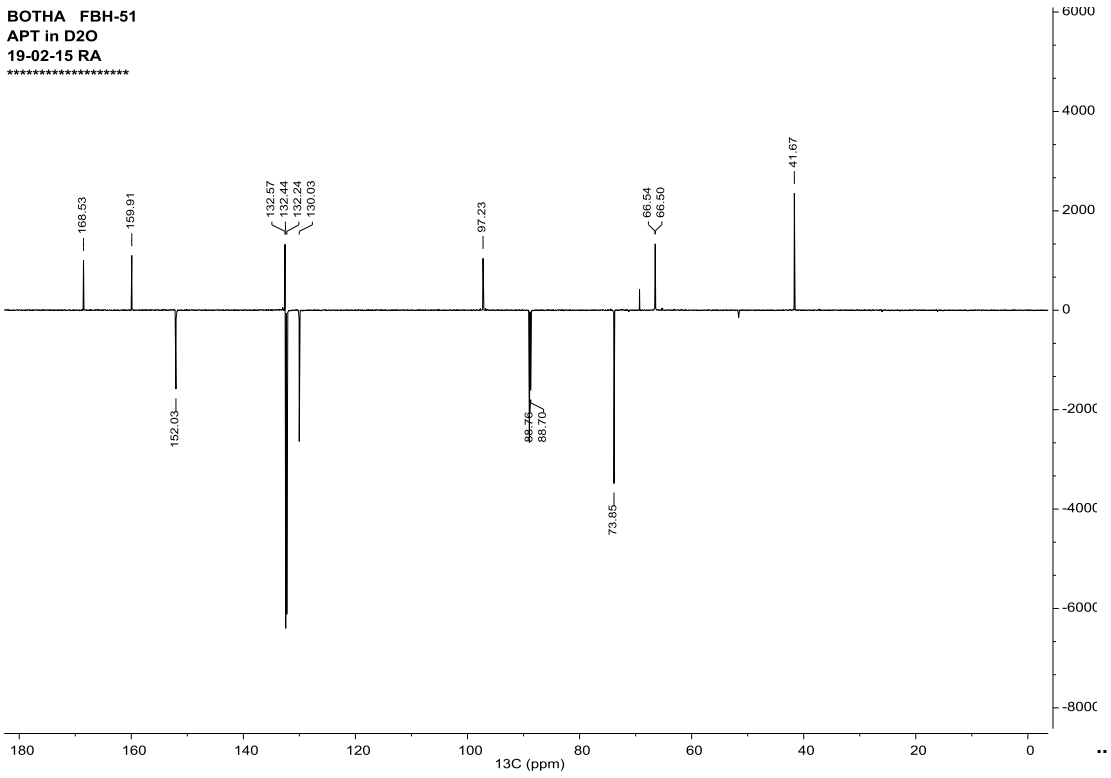


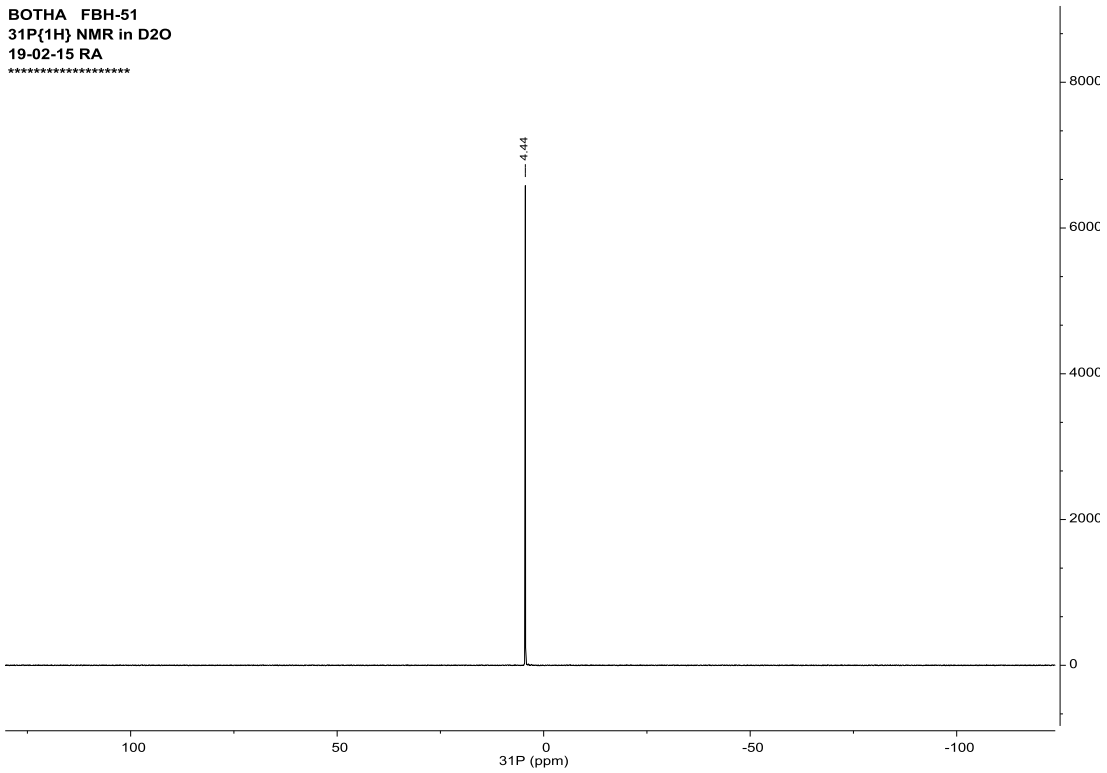


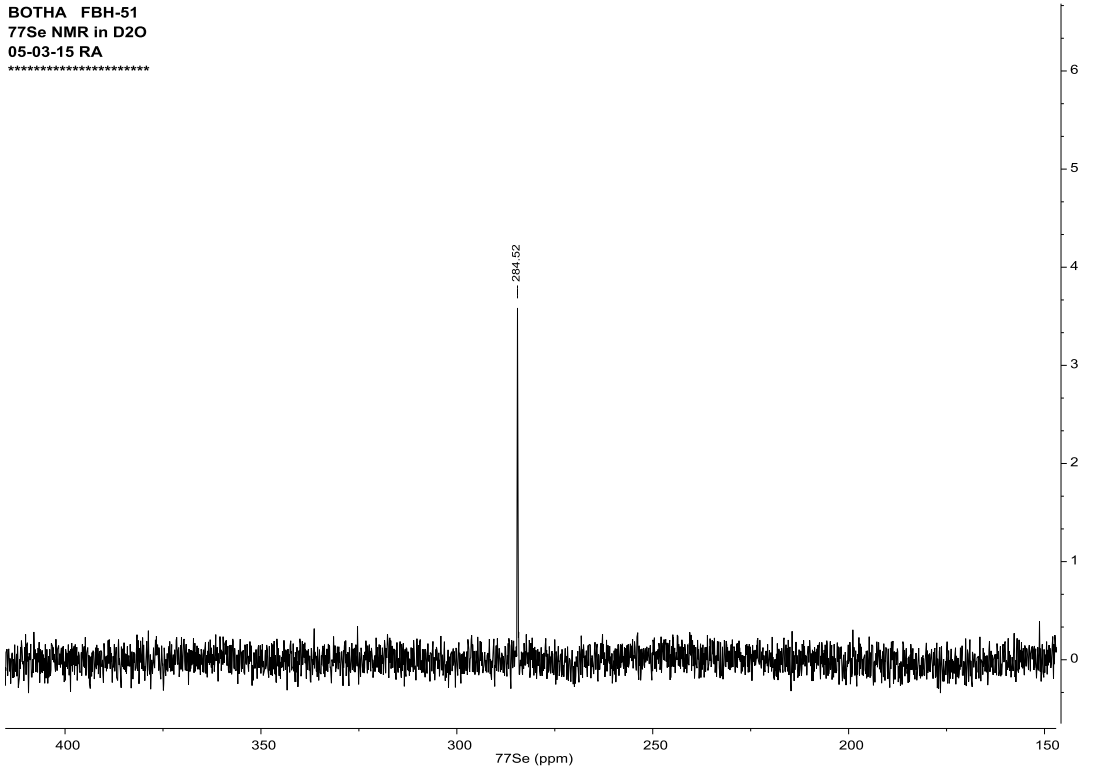

BOTHA FBH-52
31P{1H} in D2O
03-03-15 RA
****
2.72
4000
3000
2000
1000
0
100
80
60
40
20
0
-20
-40
-60
-80
31P (ppm)

5-Phenylselanyl-2'-deoxycytidine 5'-*O*-monophosphate sodium salt

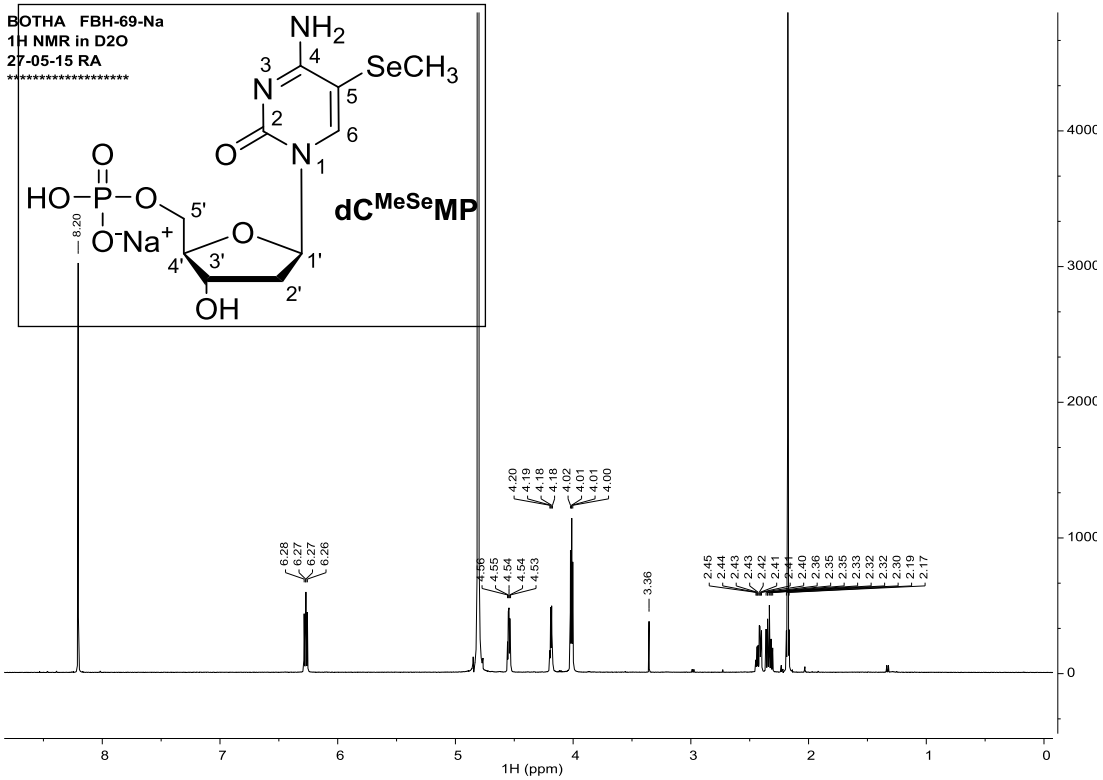


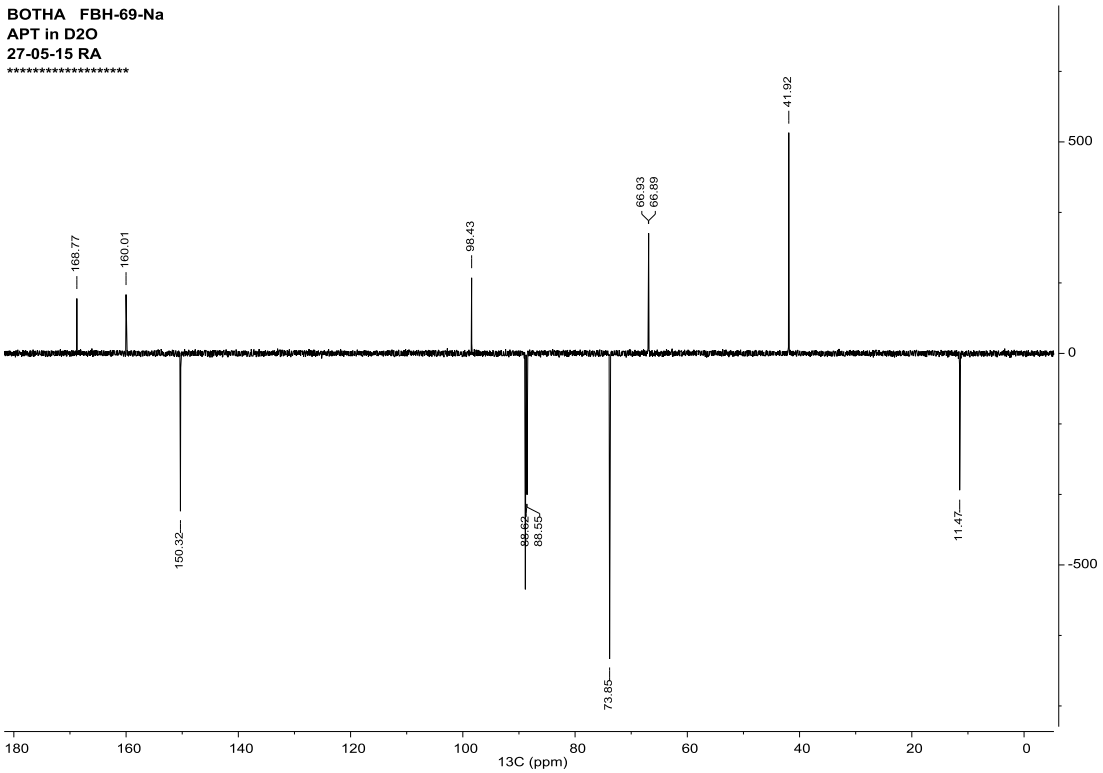


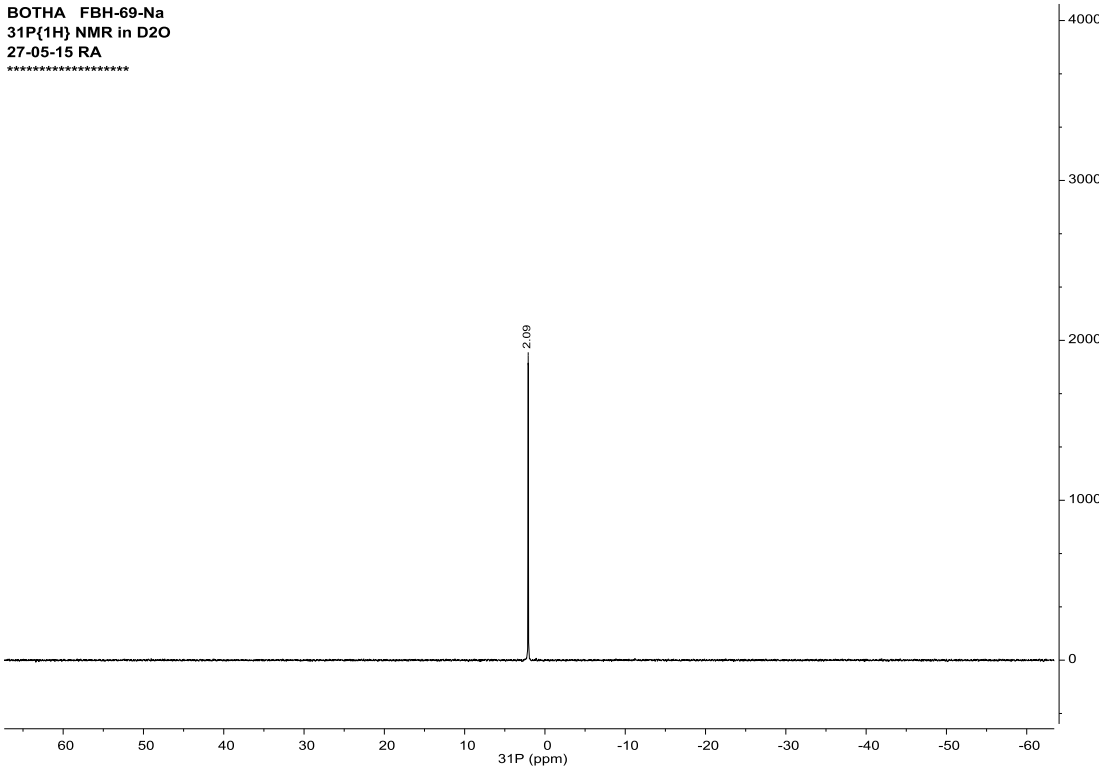

BOTHA FBH-51
31P{1H} NMR in D2O
19-02-15 RA
****
4.44
8000
6000
4000
2000
0
100
50
0
-50
-100
31P (ppm)

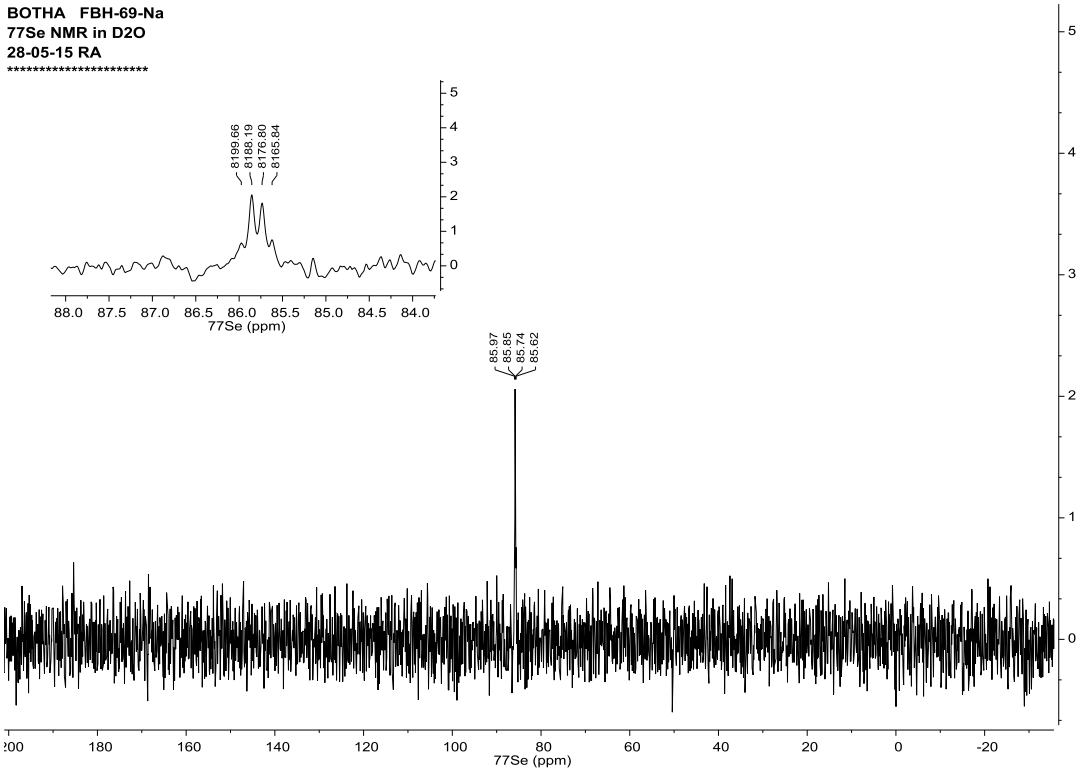

BOTHA FBH-51
77Se NMR in D2O
05-03-15 RA
****
284.52
6
5
4
3
2
1
0
400
350
300
250
200
150
77Se (ppm)

5-Methylselanyl-2'-deoxycytidine 5'-*O*-monophosphate sodium salt

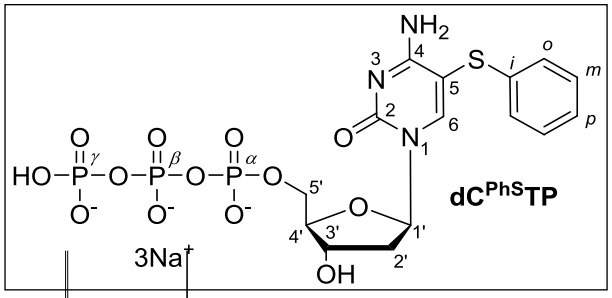


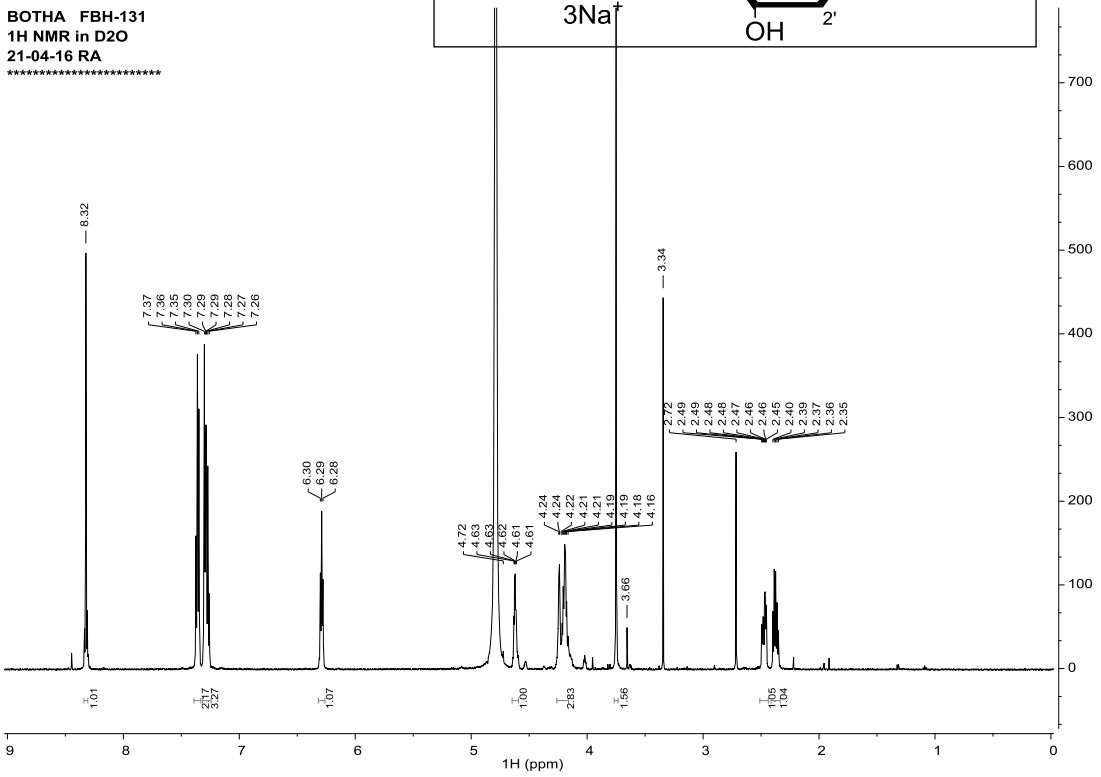


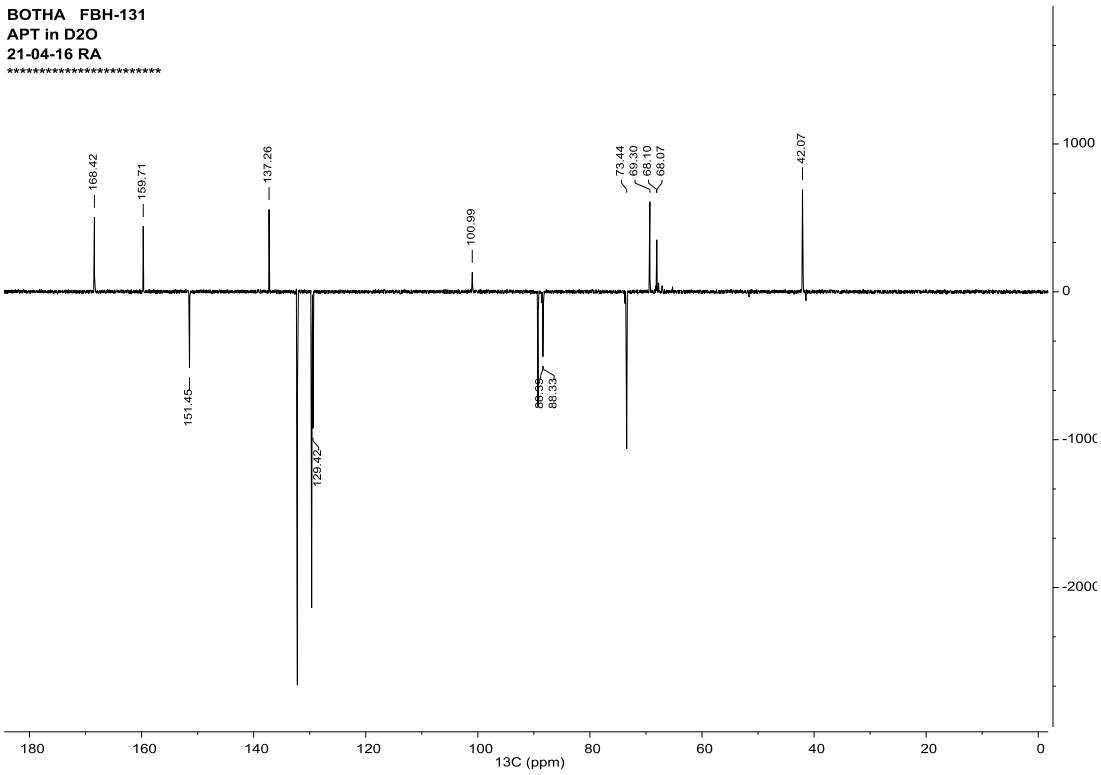

BOTHA FBH-69-Na
31P{1H} NMR in D2O
27-05-15 RA
****
2.09
31P (ppm)

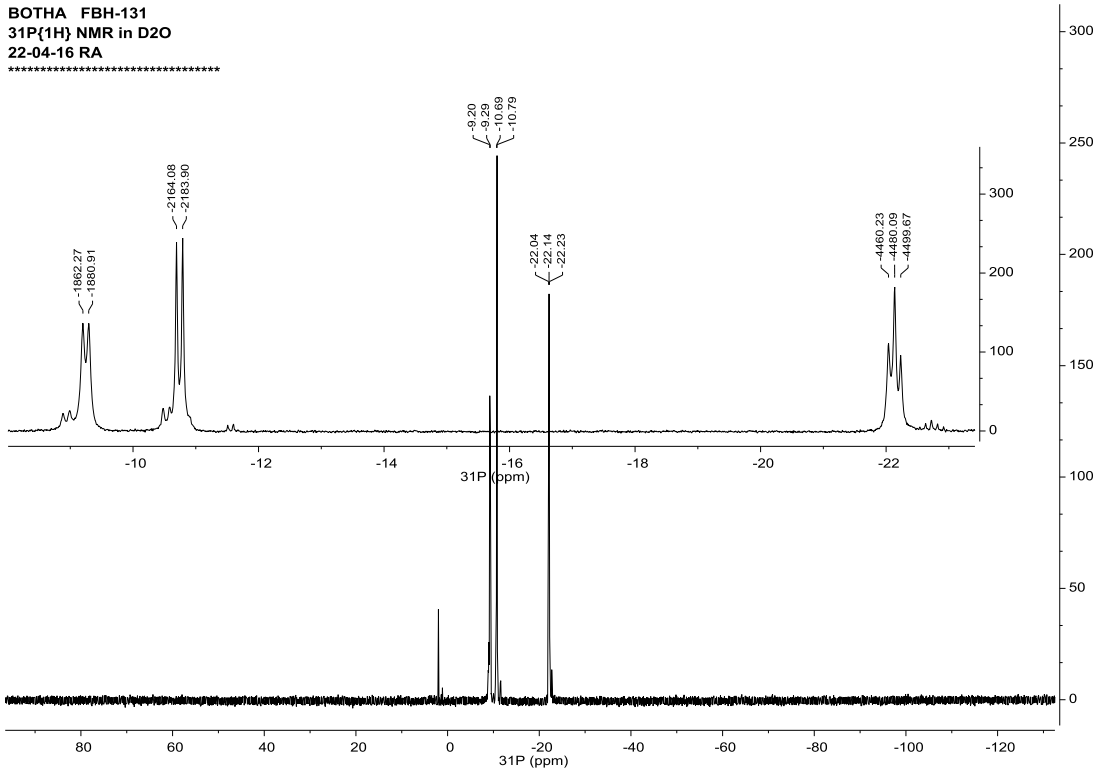

BOTHA FBH-69-Na
77Se NMR in D2O
28-05-15 RA
****
8199.66
8198.19
8176.80
8165.84
77Se (ppm)
85.97
85.85
85.74
85.62
77Se (ppm)

5-Phenylsulfanyl-2'-deoxycytidine 5'-*O*-triphosphate sodium salt

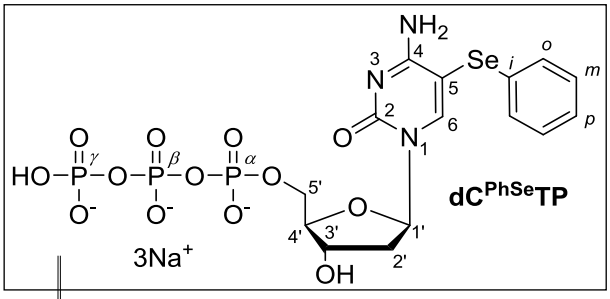


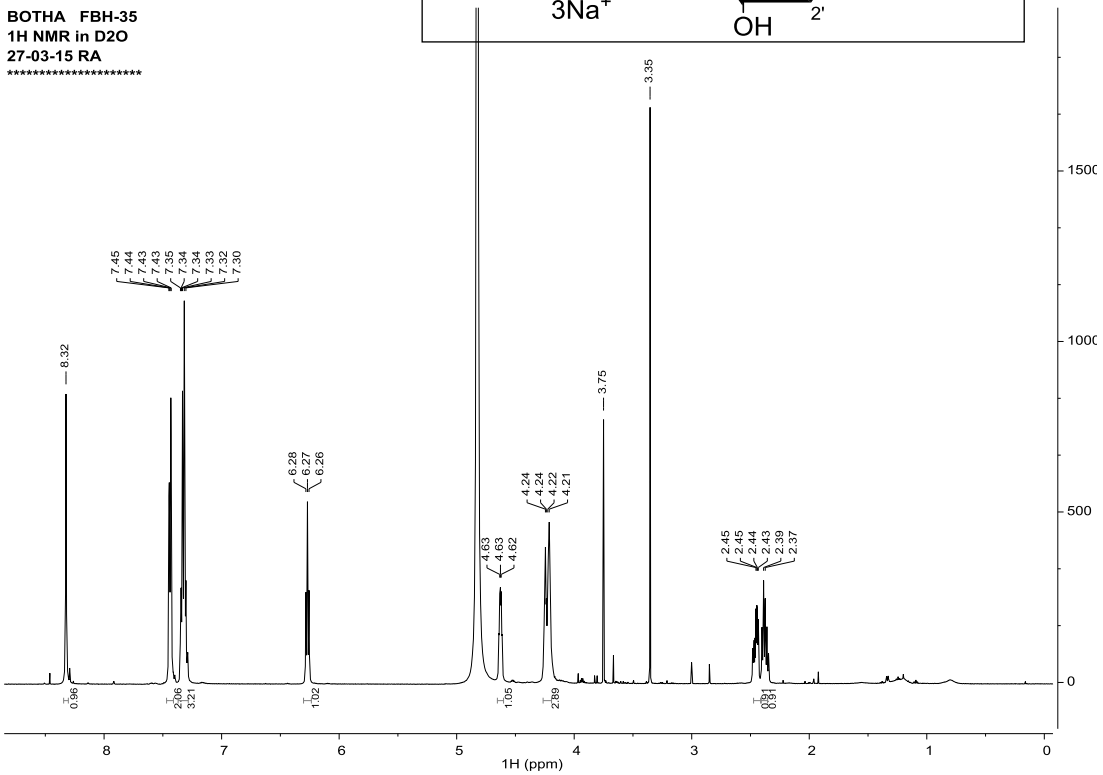


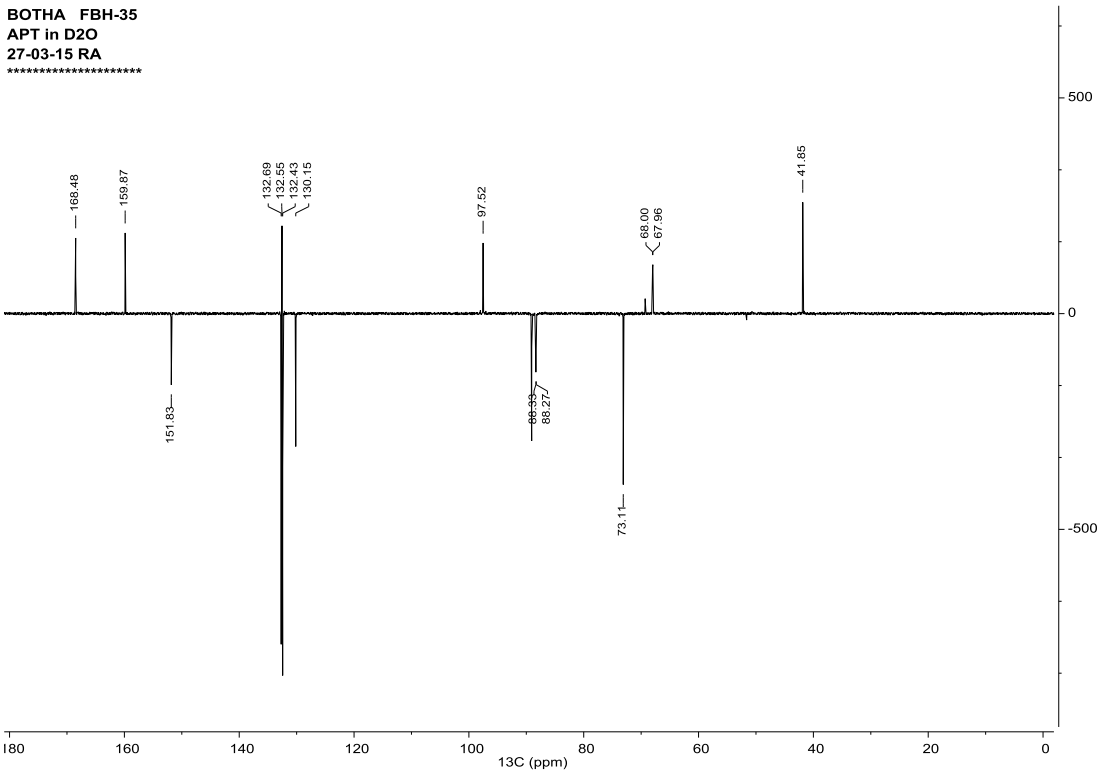


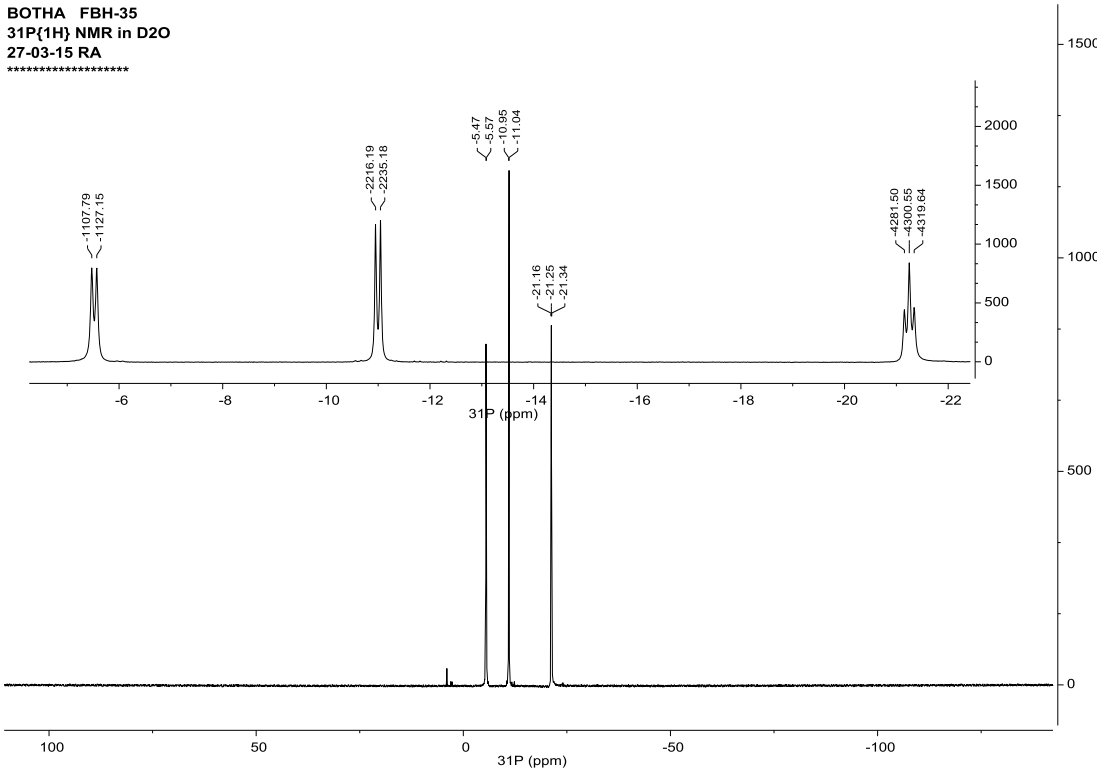

BOTHA FBH-131
31P{1H} NMR in D2O
22-04-16 RA
****
-1862.27
-1880.91
-2164.08
-2183.90
-9.20
-9.28
-10.69
-10.79
-22.04
-22.14
-22.23
-4460.23
-4480.09
-4499.67
31P (ppm)
31P (ppm)

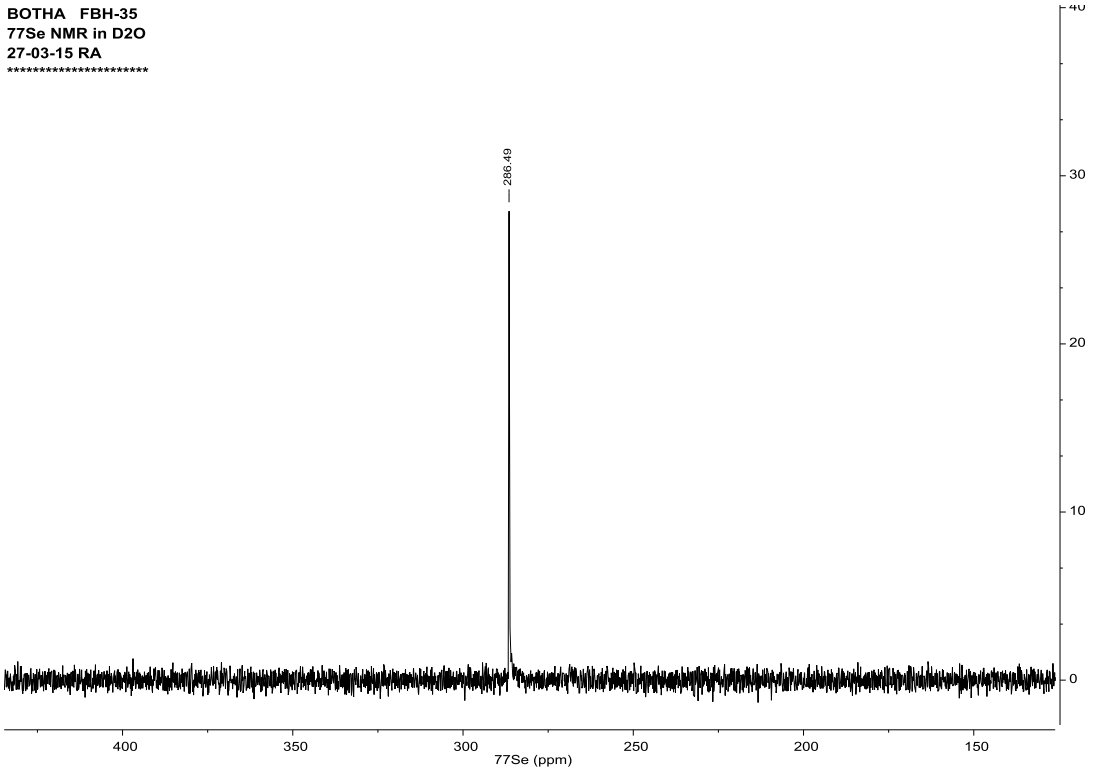
5-Phenylselanyl-2'-deoxycytidine 5'-*O*-triphosphate sodium salt



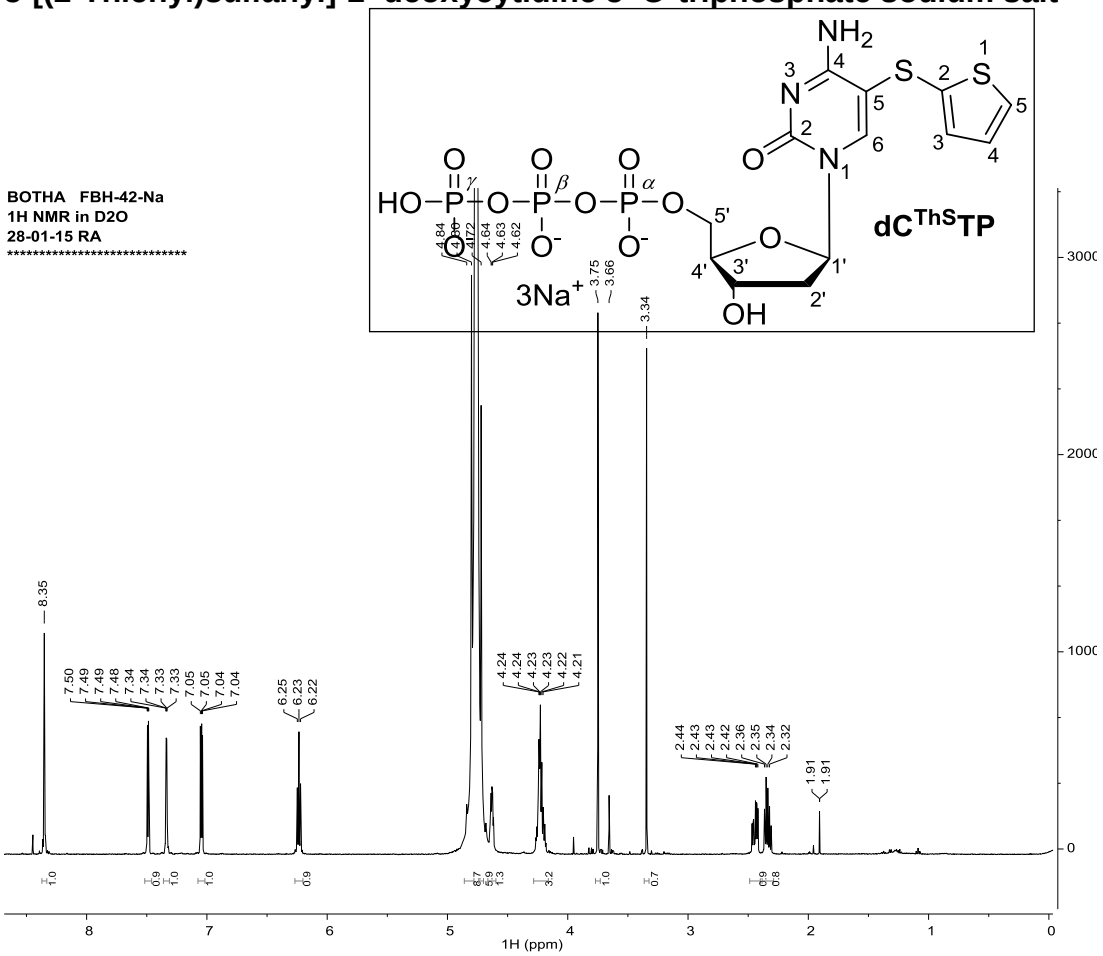


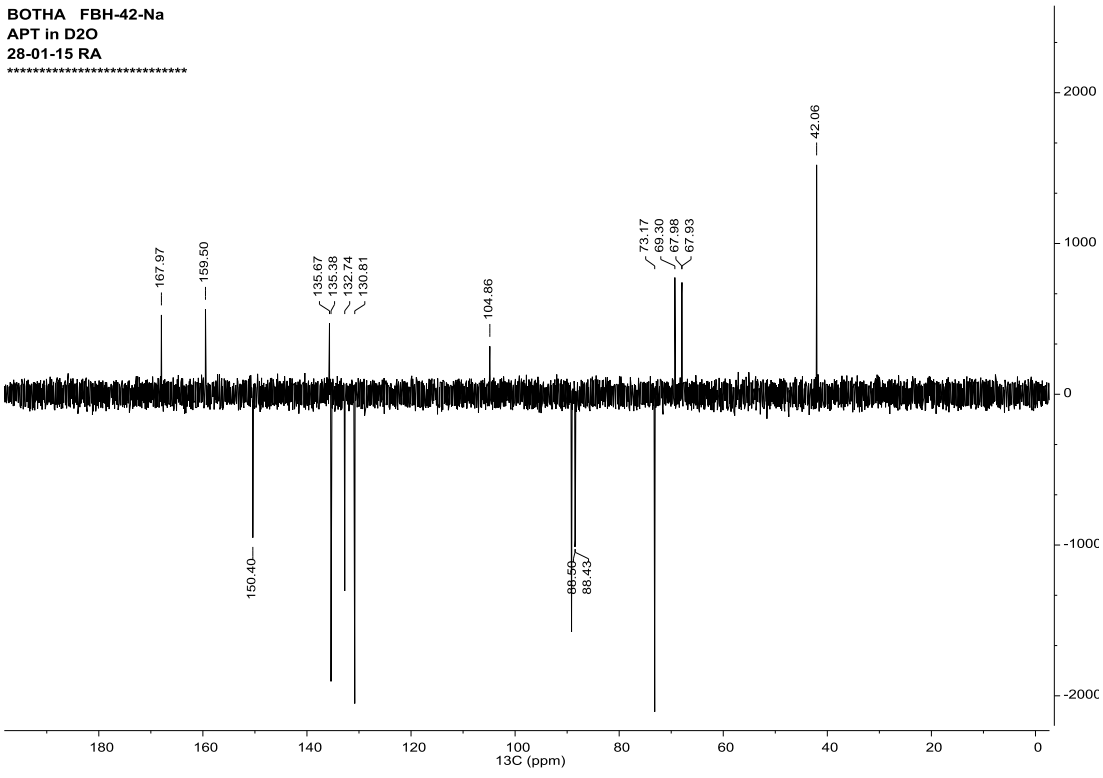


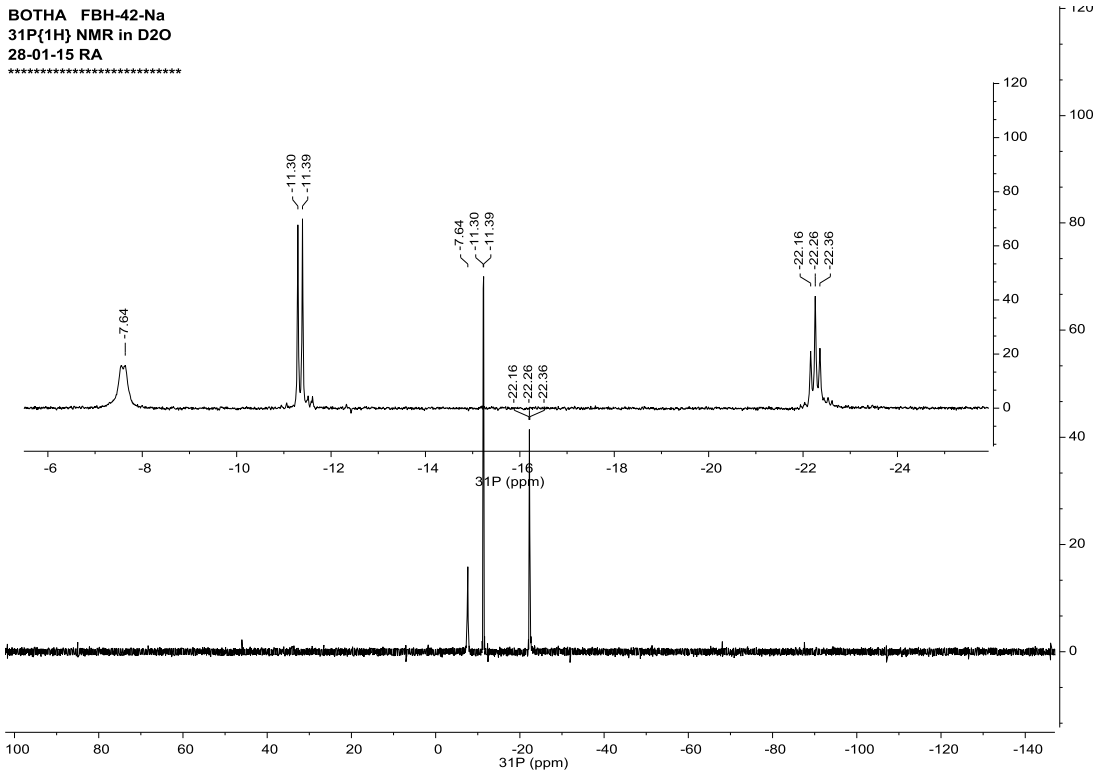

BOTHA FBH-35
31P{1H} NMR in D2O
27-03-15 RA
****
-1107.79
-1127.15
-2216.19
-2235.18
-5.47
-5.57
-10.95
-11.04
-21.16
-21.25
-21.34
-4281.50
-4300.55
-4319.64
31P (ppm)
31P (ppm)


BOTHA FBH-35
77Se NMR in D2O
27-03-15 RA
****
286.49
77Se (ppm)

5-[(2-Thienyl)sulfanyl]-2'-deoxycytidine 5'-*O*-triphosphate sodium salt






BOTHA FBH-42-Na
31P{1H} NMR in D2O
28-01-15 RA
****
-7.64
-11.30
-11.39
-22.16
-22.26
-22.36
31P (ppm)

MALDI-TOF experiments of PEX products for Prb4BaseII

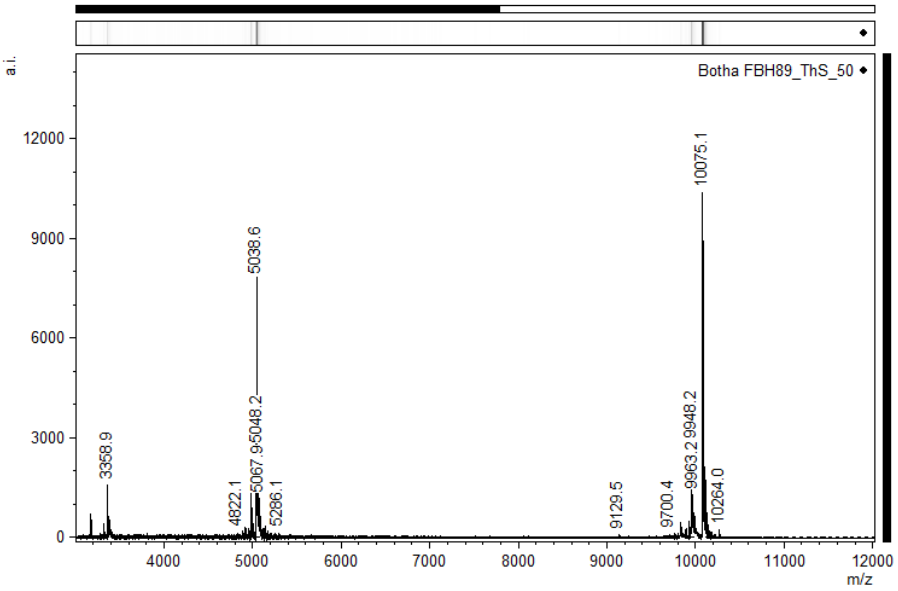


Figure S4: MALDI spectrum of **ON^{4ThS} (dC^{ThS}TP)**. M (calc.) = 10073.2; M (found) = 10075.1 $[M+2H]^+$, 9948.2 $[M-T]^+$

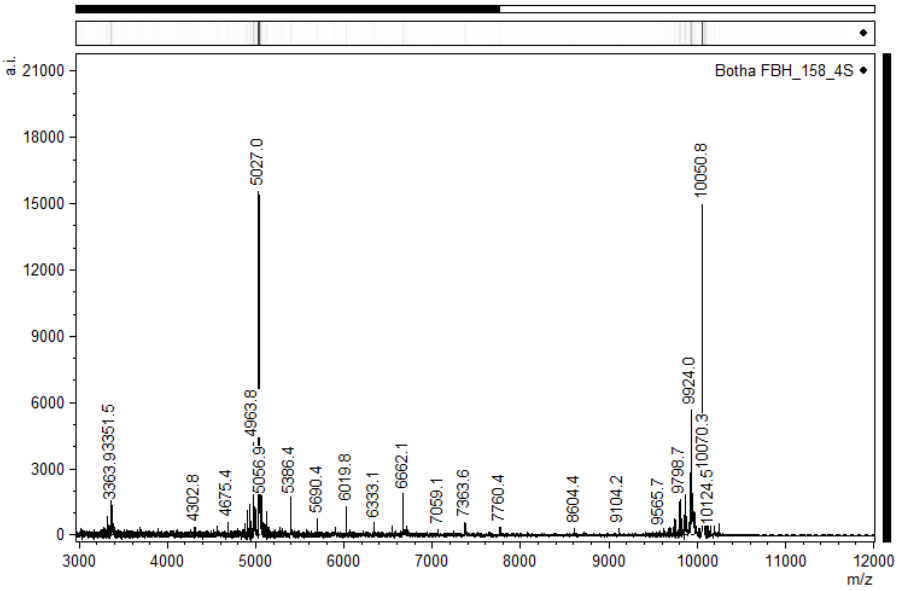


Figure S5: MALDI spectrum of **ON^{4PhS} (dC^{PhS}TP)**. M (calc.) = 10049.3; M (found) = 10050.8 $[M+H]^+$, 9924.0 $[M-T]^+$

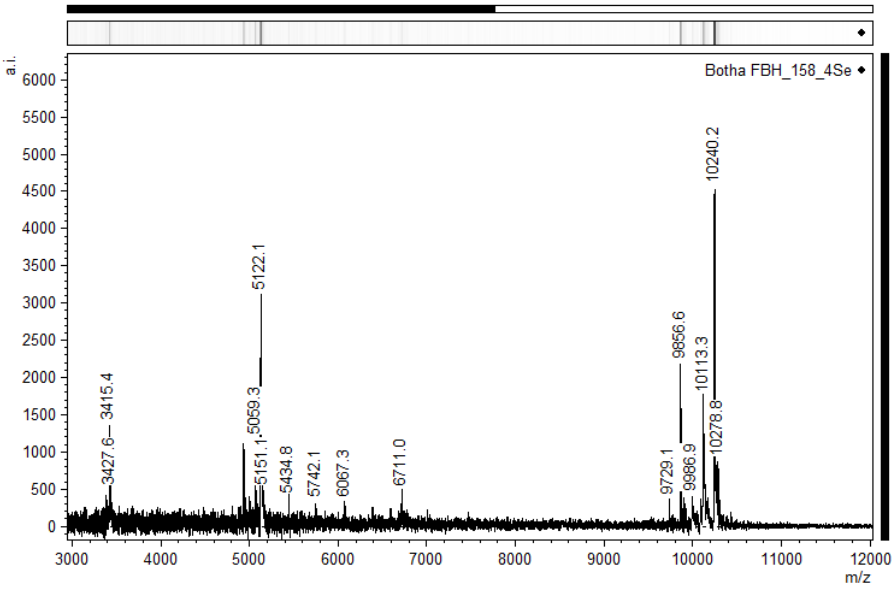


Figure S6: MALDI spectrum of **ON^{4PhSe} (dC^{PhSe}TP)**. M (calc.) = 10241.1; M (found) = 10240.2 $[M]^+$, 10113.3 $[M\text{-}T]^+$, 9856.6 $[template]^+$

References

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S3. Hassan, A. E. A.; Sheng, J.; Jiang, J.; Zhang, W.; Huang, Z.: *Org. Lett.*, **2009**, *11*, 2503-2506.

S4. Sontheimer, G. M.; Kalbitzer, H. R.; Hasselbach, W.: *Biochem.* **1987**, *26*, 2706-2711.