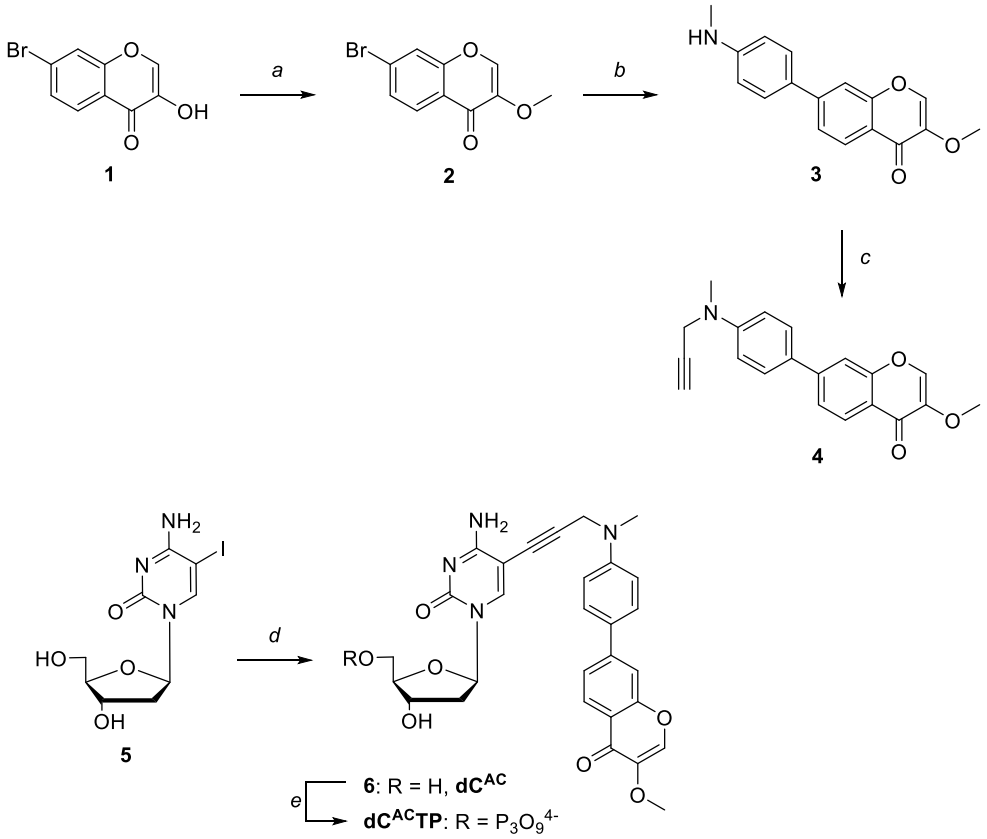
Electronic Supporting Information

Chemical synthesis

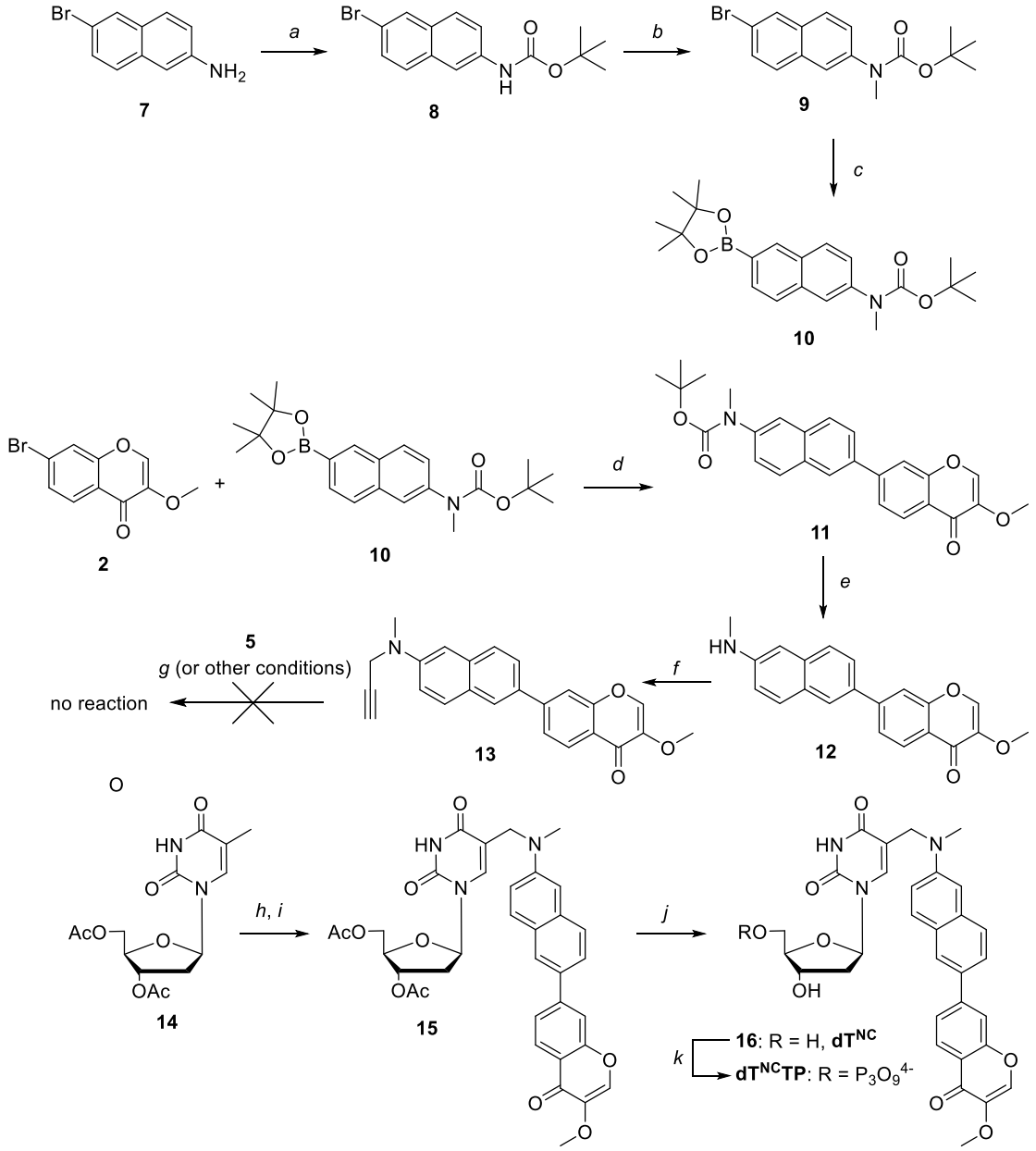
Materials and methods

Reagents and solvents were purchased from commercial suppliers. The reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} plates and visualized by UV (254 nm). Purification by column chromatography was performed using silica gel (40–63 μm). Separations of nucleotides were performed using HPLC (Waters modular HPLC system) on a column packed with C18 reversed phase silica gel. NMR spectra were measured on a Bruker AVANCE III 500 (^{1}H at 500.0 MHz, ^{13}C at 125.7 MHz and ^{31}P at 202.4 MHz) in deuterated solvents at 25 °C. Chemical shifts (δ) are given in ppm, coupling constants (J) are given in Hz. Complete assignment of all NMR signals was achieved by using a combination of H,H-COSY, H,C-HSQC and H,C-HMBC experiments. Mass spectra and high-resolution mass spectra were measured by ESI ionization technique and spectra were measured on LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific). 5-Iodo-2'-deoxycytidine (dC^I) and thymidine (dT) were purchased from Santiago. Compound **1**[1] and compound **14**[2] were prepared according to literature.



Scheme 1. Chemical synthesis of **dC^{AC}TP**. Reagents and conditions: a) MeI, K_2CO_3, DMF, 22 °C, overnight, 71%; b) 4-(methylamino)phenylboronic acid pinacol ester, K_2CO_3, $Pd(PPh_3)_4$, DMF/H_2O, 70 °C, 2.5 h, 73%; c) propargyl bromide, DBU, CH_3CN, 60 °C, 24 h, 85%; d) **dC^I**,

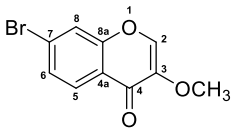
$PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 22 °C, overnight, 70%; e) 1. $POCl_3$, $PO(OMe)_3$, 0 °C, 3 h; 2. $(n\text{-}Bu_3NH)_2H_2P_2O_7$, NBu_3, DMF, 0°C, 1 h; 3. 1M TEAB, 0 °C – rt, 1 min, 51%.



Scheme 2. Chemical synthesis of **$dT^{NC}TP$**. Reagents and conditions: a) Boc_2O, THF, 70 °C, overnight, 96%; b) MeI, KOtBu, THF, 22 °C, overnight, 87%; c) B_2pin_2, KOAc, $PdCl_2dppf.CH_2Cl_2$, DME, 80 °C, overnight, 72%; d) $PdCl_2dppf.CH_2Cl_2$**,** Cs_2CO_3, CH_3CN, 80 °C, overnight, 95%; e) TFA, CH_2Cl_2, 0 - 22 °C, 2.5 h, 96%; f) propargyl bromide, K2CO3, CH3CN, 70 °C, 16 h 94%; g) **dC^I**, $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 22 °C, overnight; h) NBS, AIBN, benzene, 60 °C, 3h; i) **12**, Cs_2CO_3, DMF, 22 °C, overnight 57%; j) K_2CO_3, MeOH, 22 °C, 1 h, 76%; k) 1. $POCl_3$, $PO(OMe)_3$, 0 °C, 3 h; 2. $(n\text{-}Bu_3NH)_2H_2P_2O_7$, NBu_3, DMF, 0°C, 1 h; 3. 1M TEAB, 0 °C – rt, 1 min, 41%.

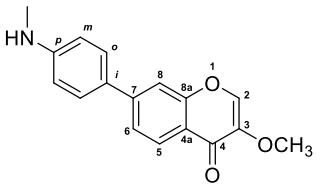
7-bromo-3-methoxy-4H-chromen-4-one (2)

7-bromo-3-hydroxy-4H-chromen-4-one **1** (0.64 g, 2.64 mmol) and K_2CO_3 (1.01 g, 7.93 mmol) were suspended in dry DMF (26 ml). MeI (0.5 ml, 7.93 mmol) was added and the reaction mixture was stirred at 22 °C overnight. Volatiles were evaporated, the residue was dissolved in EtOAc (30 ml) and the organic phase was washed with H_2O (30 ml). Organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The title compound was obtained after column chromatography (0% → 30% EtOAc in PE) as a yellowish solid (480 mg, 71%). R_f: 0.42 (40% EtOAc in PE). ^{1}H NMR (500.0 MHz, $CDCl_3$): 3.84 (s, 3H, CH_3O); 7.49 (dd, 1H, $J_{6,5}$ = 8.6, $J_{6,8}$ = 1.8, H-6); 7.65 (d, 1H, $J_{8,6}$ = 1.8, H-8); 7.70 (s, 1H, H-2); 8.15 (d, 1H, $J_{5,6}$ = 8.6, H-5). ^{13}C NMR (125.7 MHz, $CDCl_3$): 57.19 (CH_3O); 121.09 (CH-8); 122.62 (C-4a); 127.51 (CH-5); 127.84 (C-7); 128.16 (CH-6); 139.13 (CH-2); 145.08 (C-3); 155.75 (C-8a); 172.59 (C-4). MS (EI$^+$): *m/z* (%): 256.0 (97) [^{81}(Br)M+H]$^+$, 254.0 (100) [^{79}(Br)M+H]$^+$. HRMS (EI$^+$): calculated for $C_{10}H_7O_3{}^{79}$(Br): 253.9579; found: 253.9581.



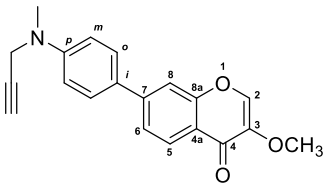
3-methoxy-7-(4-(methylamino)phenyl)-4H-chromen-4-one (3)

A flask charged with starting 7-bromo-3-hydroxy-4H-chromen-4-one **2** (150 mg, 0.60 mmol), K_2CO_3 (325 mg, 2.35 mmol) and 4-(methylamino)phenylboronic acid pinacol ester (165 mg, 0.70 mmol) was put under argon. A mixture of DMF/H_2O (4/1, 12 ml) was added and the solution was purge-and-refilled with argon 5x. $Pd(PPh_3)_4$ (40 mg, 0.04 mmol) was added and the reaction mixture was stirred at 70 °C for 2.5 h. Volatiles were evaporated, the residue was redissolved in MeOH and coevaporated with silica gel. The desired product was purified by column chromatography (0% → 60% EtOAc in PE) affording a yellow solid (121 mg, 73%). R_f: 0.24 (40% EtOAc in PE). ^{1}H NMR (500.0 MHz, $CDCl_3$): 2.90 (s, 3H, CH_3N); 3.85 (s, 3H, CH_3O); 6.69 – 6.72 (m, 2H, H-*m*-phenylene); 7.52 – 7.56 (m, 2H, H-*o*-phenylene); 7.56 (d, 1H, $J_{8,6}$ = 1.7, H-8); 7.58 (dd, 1H, $J_{6,5}$ = 8.4, $J_{6,8}$ = 1.7, H-6); 7.72 (s, 1H, H-2); 8.27 (d, 1H, $J_{5,6}$ = 8.4, H-5). ^{13}C NMR (125.7 MHz, $CDCl_3$): 30.55 (CH_3N); 57.20 (CH_3O); 112.61 (CH-*m*-phenylene); 114.00 (CH-8); 121.43 (C-4a); 122.82 (CH-6); 126.29 (CH-5); 127.29 (C-*i*-phenylene); 128.28 (CH-*o*-phenylene); 139.16 (CH-2); 144.90 (C-3); 146.69 (C-7); 149.83 (C-*p*-phenylene); 156.38 (C-8a); 173.11 (C-4). MS (EI$^+$): *m/z* (%): 281.1 (100) [M]$^+$. HRMS (EI$^+$): calculated for $C_{17}H_{15}O_3N$: 281.1052; found: 281.1039.



3-methoxy-7-(4-(methyl(propargyl)amino)phenyl)-4H-chromen-4-one (4)

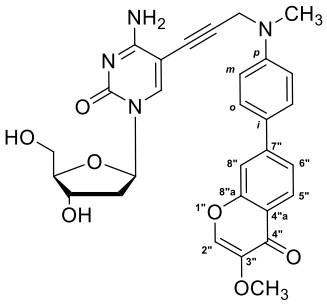
To a suspension of 3-methoxy-7-(4-(methylamino)phenyl)-4H-chromen-4-one **3** (116 mg, 0.41 mmol) in dry CH_3CN (8.5 ml) DBU (120 µl, 0.83 mmol) and propargyl bromide (140 µl, 1.24 mmol, 80% wt in toluene) were added. Reaction mixture was stirred at 60 °C for 24h. After cooling H_2O (10 ml) and $CHCl_3$ (15 ml) were added. Phases were separated, aqueous phase was extracted with $CHCl_3$ (2x15 ml). Collected organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. Crude product was purified by column chromatography (0% → 50% EtOAc in PE) affording a yellow solid (111 mg, 85%). R_f: 0.28 (40% EtOAc in PE). ^{1}H NMR (600.0 MHz, $CDCl_3$): 2.22 (t, 1H, ^4J = 2.4, **H**C≡C-CH_2); 3.06



(s, 3H, CH_3N); 3.86 (s, 3H, CH_3O); 4.13 (d, 2H, ^4J = 2.4, HC≡C-C**H**$_2$); 6.91 – 6.96 (m, 2H, H-*m*-phenylene); 7.58 – 7.60 (m, 2H, H-6,8); 7.60 – 7.63 (m, 2H, H-*o*-phenylene); 7.73 (s, 1H, H-2); 8.29 (dd, 1H, $J_{5,6}$ = 8.3, $J_{5,8}$ = 0.3, H-5). ^{13}C NMR (150.9 MHz, $CDCl_3$): 38.55 (CH_3N); 42.15 (**C**H_2-C≡CH); 57.31 (CH_3O); 72.21 (CH_2-C≡**C**H); 78.87 (CH_2-**C**≡CH); 114.07 (CH-*m*-phenylene); 114.31 (CH-8); 121.70 (C-4a); 122.95 (CH-6); 126.37 (CH-5); 128.06 (C-*i*-phenylene); 128.14 (CH-*o*-phenylene); 139.41 (CH-2); 144.96 (C-3); 146.41 (C-7); 149.28 (C-*p*-phenylene); 156.37 (C-8a); 173.11 (C-4). MS (EI$^+$): *m/z* (%): 319.1 (100) [M]$^+$. HRMS (EI$^+$): calculated for $C_{20}H_{17}O_3N$: 319.1208; found: 319.1210.

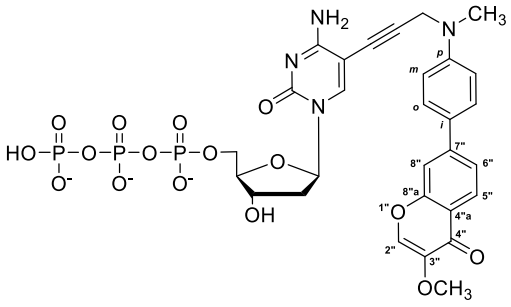
{[(4-(3-methoxy-4H-chromen-4-one-7-yl)phenyl)methylamino]prop-1-yn-1-yl}-2'-deoxycitidine (6)

Dry DMF (1.4 ml) was added to a flask containing 5-iodo-2'-deoxycitidine **dC^I** (70 mg, 0.20 mmol), acetylene **4** (76 mg, 0.24 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 25% mol), CuI (2 mg, 5% mol) and the resulting mixture was purge-and-refilled with argon 5 times. DIPEA (70 µl, 0.40 mmol) was added via syringe and the mixture was stirred at 22 °C overnight. Volatiles were evaporated; the residue was dissolved in MeOH and coevaporated with silica gel. The desired nucleoside was purified by column chromatography (0% → 10% MeOH in DCM) and further purified by RP-FPLC (0% → 100% MeOH in H_2O). The product was obtained as a yellow solid (76 mg, 70%). R_f: 0.22 (10% MeOH in $CHCl_3$). ^{1}H NMR (500.0 MHz, DMSO-d_6): 1.95 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.9, $J_{2'b,3'}$ = 6.1, H-2′b); 2.10 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.6, H-2′a); 3.02 (s, 3H, CH_3N); 3.52, 3.58 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.2, $J_{5',4'}$ = 3.6, H-5′); 3.74 (s, 3H, CH_3O); 3.76 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.6, H-4′); 4.17 (ddt, 1H, $J_{3',2'}$ = 6.2, 3.6, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.6, H-3′); 4.44 (s, 2H, CH_2N); 5.06 (t, 1H, $J_{OH,5'}$ = 5.2, OH-5′); 5.20 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3′); 6.07 (dd, 1H, $J_{1',2'}$ = 6.8, 6.2, H-1′); 6.70 (bs, 1H, NH_a**H$_b$**); 6.99 – 7.03 (m, 2H, H-*m*-phenylene); 7.71 – 7.75 (m, 2H, H-*o*-phenylene); 7.75 (dd, 1H, $J_{6'',5''}$ = 8.5, $J_{6'',8''}$ = 1.7, H-6″); 7.79 (bs, 1H, N**H$_a$**H$_b$); 7.84 (d, 1H, $J_{8'',6''}$ = 1.7, H-8″); 8.08 (d, 1H, $J_{5'',6''}$ = 8.5, H-5″); 8.11 (s, 1H, H-6); 8.30 (s, 1H, H-2″). ^{13}C NMR (125.7 MHz, DMSO-d_6): 38.41 (CH_3N); 40.96 (CH_2-2′); 42.53 (CH_2N); 57.00 (CH_3O); 61.13 (CH_2-5′); 70.23 (CH-3′); 75.59 (CH_2-C≡**C**-C5); 85.56 (CH-1′); 87.62 (CH-4′); 89.56 (C-5); 91.61 (CH_2-**C**≡C-C5); 113.99 (CH-8″); 114.06 (CH-*m*-phenylene); 121.21 (C-4″a); 122.65 (CH-6″); 125.80 (CH-5″); 126.37 (C-*i*-phenylene); 128.15 (CH-*o*-phenylene); 140.96 (CH-2″); 144.49 (CH-6); 144.54 (C-3″); 145.66 (C-7″); 149.62 (C-*p*-phenylene); 153.62 (C-2); 156.16 (C-8″a); 164.60 (C-4); 171.76 (C-4″). MS (ESI$^+$): *m/z* (%): 567.1 (100) [M+Na]$^+$. HRMS (ESI$^+$): calculated for $C_{29}H_{28}O_7N_4Na$: 567.18502; found: 567.18526.



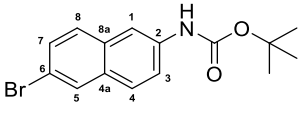
{[(4-(3-methoxy-4H-chromen-4-one-7-yl)phenyl)methylamino]prop-1-yn-1-yl}-2'-deoxycitidine-5'-*O*-triphosphate (dC^{AC}TP)

Starting nucleoside **5** (18 mg, 0.03 mmol) was dried in vacuo overnight. Then it was dissolved in dry $PO(OMe)_3$ (0.4 ml) and cooled down to 0 °C. Freshly distilled $POCl_3$ (4 µl, 0.04 mmol) was added dropwise and reaction mixture was stirred at 0 °C for 3 h. Then an ice-cold solution of (*n*-$Bu_3NH)_2H_2P_2O_7$ (92 mg, 0.17 mmol) and *n*-Bu_3N (35 µl, 0.13 mmol) in dry DMF (0.3 ml) was added dropwise. The reaction mixture was stirred for another 1 h at 0 ºC and then quenched by the addition of cold 2M TEAB solution (0.3 ml). The mixture was concentrated on a rotavap; the residue was co-evaporated with distilled water three times. The crude product was dissolved in water (ca 3 ml); the aqueous solution was purified by semi-preparative HPLC using a linear gradient of methanol (5→100%) in 0.1 M TEAB buffer. The appropriate fractions were combined and evaporated on a rotavap. The viscous oil was coevaporated with distilled water three times. The product was converted to sodium salt on an ion-exchange column (Dowex 50WX8 in Na^+ cycle) and freeze-dried. The title compound was obtained as a yellow solid (15 mg, 51%). ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 1.87 (bm, 1H, H-2′b); 2.17 (bm, 1H, H-2′a); 3.12 (bs, 3H, CH_3N); 3.77 (bs, 3H, CH_3O); 3.98 – 4.11 (bm, 3H, H-4′,5′); 4.30 (bm, 1H, H-3′); 4.37, 4.56 (2 × d, 2 × 1H, J_{gem} = 18.5, CH_2N); 5.73 (bm, 1H, H-1′); 6.57 (bm, 1H, H-8″); 6.84 (bm, 1H, H-6″); 7.01 – 7.08 (bm, 2H, H-*m*-phenylene); 7.13 – 7.22 (bm, 2H, H-*o*-phenylene); 7.52 (bm, 1H, H-5″); 7.66 (s, 1H, H-6); 7.87 (bs, 1H, H-2″). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 41.47 (CH_3N); 41.71 (CH_2-2′); 45.72 (CH_2N); 59.24 (CH_3O); 67.62 (d, $J_{C,P}$ = 4.4, CH_2-5′); 72.53 (CH-3′); 76.95 (CH_2-C≡**C**-C5); 87.90 (d, $J_{C,P}$ = 8.5, CH-4′); 88.97 (CH-1′); 94.59, 94.86 (C-5, CH_2-**C**≡C-C5); 116.47 (CH-8″); 117.94 (CH-*m*-phenylene); 122.17 (C-4″a); 125.54 (CH-6″); 127.32 (CH-5″); 130.30 (C-*i*-phenylene); 131.31 (CH-*o*-phenylene); 142.71 (CH-2″); 146.42 (CH-6); 146.65 (C-3″); 148.64 (C-7″); 152.24 (C-*p*-phenylene); 157.93 (C-2); 158.27 (C-8″a); 167.06 (C-4); 176.62 (C-4″). ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.35 (bs, P$_\beta$); -10.49 (d, J = 19.2, P$_\alpha$); -7.10 (bs, P$_\gamma$). MS (ESI$^-$): *m/z* (%): 391.0 (100) $[M–H_2PO_3]^{2-}$. HRMS (ESI$^-$): calculated for $C_{29}H_{30}O_{16}N_4P_3$: 783.08751; found: 783.08629.



tert-Butyl (6-bromonaphthalen-2-yl)carbamate (8)

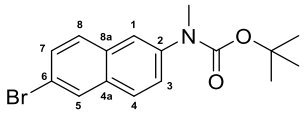
To a stirring solution of compound **7** (800 mg, 3.62 mmol) in dry THF (18 ml), Boc_2O (1.7 ml, 7.24 mmol) was added. The resulting solution was stirred at 70 °C overnight. After cooling, the volatiles were evaporated and residue was purified by column chromatography (0% → 10% EtOAc in cyclohexane). Compound **LT-22** (1.12 g, 96%) was obtained as a white solid.
Rf: 0.17 (50% toluene in CHX). ^{1}H NMR (500.0 MHz, $CDCl_3$) δ (ppm): 1.55 (s, 9H, $(CH_3)_3C$); 6.63 (bs, 1H NH); 7.32 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.2, H-3); 7.50 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.0, H-7); 7.63 (d, 1H, $J_{8,7}$ = 8.8, H-8); 7.66 (d, 1H, $J_{4,3}$ = 8.8, H-4); 7.91 (d, 1H, $J_{5,7}$ = 2.0, H-5); 8.00 (bm, 1H, H-1).^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 28.34 ((**C**$H_3)_3$C); 80.93 ($(CH_3)_3$**C**); 114.31 (CH-1); 118.09 (C-6); 120.04 (CH-3); 127.84 (CH-4); 129.03 (CH-8); 129.53 (CH-5); 129.79 (CH-7);



130.96 (C-4a); 132.50 (C-8a); 136.20 (C-2); 152.68 (NCOO). HRMS (ESI+) calculated for $C_{15}H_{16}O_2NBrNa$: 344.02566, found: 344.02527.

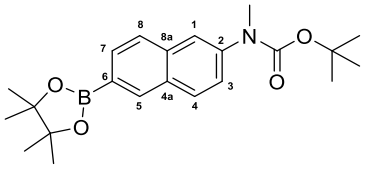
tert-Butyl (6-bromonaphthalen-2-yl)(methyl)carbamate (9)

Starting carbamate **8** (950 mg, 2.96 mmol) and potassium tert-butoxide (840 mg, 5.92 mmol) were suspended in dry THF (15 ml). MeI (0.4 ml, 5.92 mmol) was added and the resulting suspension was vigorously stirred at 22 °C overnight. Reaction was stopped by the addition of H_2O (15 ml) and DCM (50 ml) was added. Phases were separated, the aqueous phase was extracted with DCM (3x30 ml) and the collected organic phases were dried over anhydrous $NaSO_4$, filtered and evaporated. The title compound **9** (860 mg, 87%) was isolated, after column chromatography (0% → 10% EtOAc in CHX), as a white-off solid. Rf: 0.10 (50% toluene in CHX). ^{1}H NMR (500.0 MHz, $CDCl_3$) δ (ppm): 1.46 (s, 9H, $(CH_3)_3C$); 3.36 (s, 3H, CH_3N); 7.45 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.2, H-3); 7.53 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 1.9, H-7); 7.60 (d, 1H, $J_{1,3}$ = 2.2, H-1); 7.65 (d, 1H, $J_{8,7}$ = 8.8, H-8); 7.69 (d, 1H, $J_{4,3}$ = 8.8, H-4); 7.96 (d, 1H, $J_{5,7}$ = 1.9, H-5). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 28.32 (($\mathbf{C}H_3)_3C$); 37.39 (CH_3N); 80.67 (($CH_3)_3\mathbf{C}$); 119.37 (C-6); 122.29 (CH-1); 126.12 (CH-3); 127.15 (CH-4); 129.21 (CH-8); 129.57 (CH-5,7); 131.91 (C-8a); 132.17 (C-4a); 141.85 (C-2); 154.68 (NCOO). HRMS (ESI+) calculated for $C_{16}H_{18}O_2NBrNa$: 358.04131, found: 358.04099.



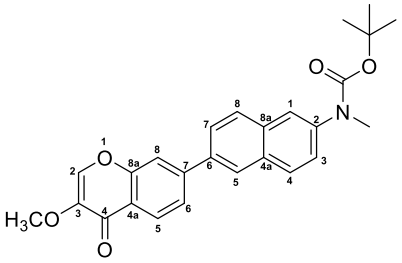
tert-Butyl (methyl)(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)naphthalen-2-yl)carbamate (10)

Disubstituted naphthalene **9** (860 mg, 2.57 mmol), bis(pinacolato)diboron (978 mg, 3.85 mmol), potassium acetate (957 mg, 9.75 mmol) and $PdCl_2$dppf.CH_2Cl_2 (282 mg, 0.39 mmol) were dissolved in anhydrous DME (17 ml) and the resulting mixture was heated at 80 °C overnight. After cooling, the mixture was diluted with DCM (20 ml) and H_2O (20 ml). Phases were separated; aqueous phase was extracted with DCM (2x20 ml). Collected organic phases were dried over anhydrous $NaSO_4$, filtered and evaporated. Column chromatography (0% → 5% EtOAc in CHX) afforded the desired product **10** (705 mg, 72%) as a white-off solid. Rf: 0.31 (10% EtOAc in CHX). ^{1}H NMR (500.0 MHz, $CDCl_3$) δ (ppm): 1.39 (s, 12H, CH_3-piacolboronate); 1.46 (s, 9H, $(CH_3)_3C$); 3.37 (s, 3H, CH_3N); 7.42 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.2, H-3); 7.61 (d, 1H, $J_{1,3}$ = 2.2, H-1); 7.75 (d, 1H, $J_{8,7}$ = 8.4, H-8); 7.81 (d, 1H, $J_{4,3}$ = 8.8, H-4); 7.82 (dd, 1H, $J_{7,8}$ = 8.4, $J_{7,5}$ = 1.1, H-7); 8.32 (d, 1H, $J_{5,7}$ = 1.1, H-5). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 24.91 (CH_3-pinacolboronate); 28.33 (($\mathbf{C}H_3)_3C$); 37.42 (CH_3N); 80.52 (($CH_3)_3\mathbf{C}$); 83.91 (C-pinacolboronate); 122.14 (CH-1); 125.01 (CH-3); 125.75 (C-6); 126.68 (CH-8); 128.82 (CH-4); 130.53 (C-4a); 130.80 (CH-7); 135.15 (C-8a); 135.79 (CH-5); 142.46 (C-2); 154.76 (NCOO). HRMS (ESI+) calculated for $C_{22}H_{30}O_4NBNa$: 406.21601, found: 406.21604.



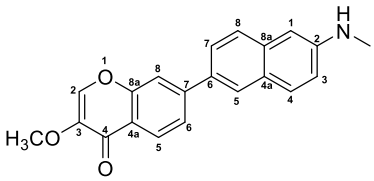
tert-Butyl (6-(3-methoxy-4-oxo-4H-chromen-7-yl)naphthalen-2-yl)(methyl)carbamate (11)

A flask charged with starting 7-bromo-3-hydroxy-4H-chromen-4-one **2** (420 mg, 1.65 mmol), Cs_2CO_3 (808 mg, 2.48 mmol), $PdCl_2dppf.CH_2Cl_2$ (121 mg, 0.17 mmol) and phenylboronic acid pinacol ester **10** (697 mg, 1.82 mmol) was put under argon. Dry MeCN (11 ml) was added and the solution was stirred at 80 °C overnight. Water (20 ml) and DCM (20 ml) were added and phases were separated. The aqueous phase was extracted with DCM (2x20 ml); collected organic phases were dried over anhydrous $NaSO_4$, filtered and evaporated. The desired product was purified by column chromatography (0% → 50% EtOAc in cyclohexane) affording **11** as a yellowish solid (603 mg, 95%). Rf: 0.30 (50% EtOAc in CHX). ^{1}H NMR (500.0 MHz, $CDCl_3$) δ (ppm): 1.49 (s, 9H, $(CH_3)_3C$); 3.39 (s, 3H, CH_3N); 3.88 (s, 3H, CH_3O); 7.51 (dd, 1H, $J_{3,4}$ = 8.7, $J_{3,1}$ = 2.2, H-3-naphth); 7.68 (d, 1H, $J_{1,3}$ = 2.2, H-1-naphth); 7.74 – 7.78 (m, 3H, H-6,8-chromene, H-7-naphth); 7.78 (s, 1H, H-2-chromene); 7.87 (d, 1H, $J_{4,3}$ = 8.7, H-4-naphth); 7.90 (d, 1H, $J_{8,7}$ = 8.9, H-8-naphth); 8.10 (d, 1H, $J_{5,7}$ = 2.1, H-5-naphth); 8.39 (dd, 1H, $J_{5,6}$ = 8.1, $J_{5,8}$ = 0.7, H-5-chromene). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 28.36 ((**C**$H_3)_3$C); 37.44 (CH_3N); 57.29 (CH_3O); 80.68 ($(CH_3)_3$**C**); 116.06 (CH-8-chromene); 122.05 (CH-1-naphth); 122.55 (C-4a-chromene); 123.81 (CH-6-chromene); 125.40 (CH-7-naphth); 125.92 (CH-3-naphth); 126.35 (CH-5-naphth); 126.69 (CH-5-chromene); 128.57 (CH-4,8-naphth); 131.20 (C-4a-naphth); 133.33 (C-8a-naphth); 136.00 (C-6-naphth); 139.52 (CH-2-chromene); 142.32 (C-2-naphth); 145.10 (C-3-chromene); 146.39 (C-7-chromene); 154.71 (NCOO); 156.22 (C-8a-chromene); 173.08 (C-4-chromene). HRMS (ESI+) calculated for $C_{26}H_{25}O_5NNa$: 454.16249, found: 454.16257.



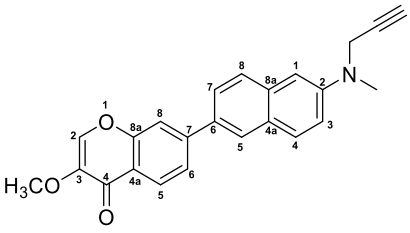
3-methoxy-7-(6-(methylamino)naphthalen-2-yl)-4H-chromen-4-one (12)

To an ice-cooled solution of compound **11** (690 mg, 1.60 mmol) in DCM (11 mL), TFA (1.2 ml, 16.00 mmol) was added dropwise. The mixture was stirred for 30 minutes at 0 °C and for an additional 2 h at room temperature (22 °C). The reaction was neutralized by careful addition of saturated $NaHCO_3$ solution (CO_2 generation!). The phases were separated and the aqueous phase was extracted with DCM (3x30 ml). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated at the rotavapor. Compound **12** (510 mg, 96%) was obtained, after column chromatography (0% → 50% EtOAc in cyclohexane), as an orange solid. Rf: 0.17 (40% EtOAc in cyclohexane). ^{1}H NMR (500.0 MHz, $CDCl_3$) δ (ppm): 2.98 (s, 3H, CH_3N); 3.87 (s, 3H, CH_3O); 6.82 (d, 1H, $J_{1,3}$ = 2.4, H-1-naphth); 6.93 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.4, H-3-naphth); 7.67 – 7.75 (m, 5H, H-6,8-chromene, H-4,7,8-naphth); 7.76 (s, 1H, H-2-chromene); 7.98 (d, 1H, $J_{5,7}$ = 2.1, H-5-naphth); 8.35 (dd, 1H, $J_{5,6}$ = 8.0, $J_{5,8}$ = 0.9, H-5-chromene). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 30.62 (CH_3N); 57.29 (CH_3O); 103.20 (CH-1-naphth); 115.31 (CH-8-chromene); 118.60 (CH-3-naphth); 122.06 (C-4a-chromene); 123.57 (CH-6-chromene); 125.36 (CH-7-naphth); 126.45 (CH-5-chromene); 126.58 (CH-5-naphth); 126.80 (CH-8-naphth); 127.36 (C-4a-naphth); 129.46 (CH-4-naphth); 132.04 (C-6-naphth); 135.34 (C-8a-naphth); 139.42 (CH-2-chromene); 145.02 (C-3-chromene); 146.96 (C-7-chromene); 147.77 (C-2-naphth); 156.33 (C-



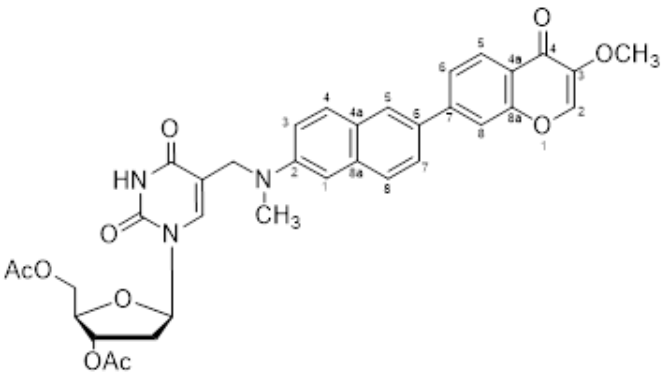
8a-chromene); 173.14 (C-4-chromene). HR TOF MS (EI+) calculated for $C_{21}H_{17}NO_3$: 331.1208, found: 331.1206.

3-methoxy-7-(6-(methyl(prop-2-yn-1-yl)amino)naphthalen-2-yl)-4H-chromen-4-one (13)



To a suspension of secondary amine **12** (61 mg, 0.18 mmol) and K_2CO_3 (265 mg, 1.9 mmol) in dry CH_3CN (3.8 ml), propargyl bromide (70 µl, 0.62 mmol, 80% wt in toluene) was added. Reaction mixture was stirred at 70 °C for 16h. After cooling, H_2O (10 ml) and CH_2Cl_2 (10 ml) were added. Phases were separated, aqueous phase was extracted with CH_2Cl_2 (2x10 ml). Collected organic phases were washed with brine (10 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. Crude product was purified by column chromatography (0% → 50% EtOAc in cyclohexane) affording an orange solid (64 mg, 94%). R_f: 0.31 (50% EtOAc in CHX). ^{1}H NMR (500.0 MHz, $CDCl_3$) δ (ppm): 2.22 (t, 1H, ^4J = 2.4, **H**C≡CCH$_2$); 3.12 (s, 3H, CH_3N); 3.87 (s, 3H, CH_3O); 4.20 (d, 2H, ^4J = 2.4, HC≡CC**H$_2$**); 7.10 (d, 1H, $J_{1,3}$ = 2.6, H-1-naphth); 7.27 (dd, 1H, $J_{3,4}$ = 9.0, $J_{3,1}$ = 2.6, H-3-naphth); 7.71 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.9, H-7-naphth); 7.73 – 7.75 (m, 2H, H-6,8-chromene); 7.76 (s, 1H, H-2-chromene); 7.80 (d, 1H, $J_{8,7}$ = 8.6, H-8-naphth); 7.83 (d, 1H, $J_{4,3}$ = 9.0, H-4-naphth); 8.02 (d, 1H, $J_{5,7}$ = 1.9, H-5-naphth); 8.35 (m, 1H, H-5-chromene). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 38.73 (CH_3N); 42.61 (**C**H$_2$C≡CH); 57.29 (CH_3O); 72.31 (CH$_2$C≡**C**H); 79.00 (CH$_2$**C**≡CH); 108.19 (CH-1-naphth); 115.48 (CH-8-chromene); 117.73 (CH-3-naphth); 122.17 (C-4a-chromene); 123.62 (CH-6-chromene); 125.35 (CH-7-naphth); 126.34 (CH-5-naphth); 126.50 (CH-5-chromene); 127.41 (CH-8-naphth); 127.50 (C-4a-naphth); 129.48 (CH-4-naphth); 132.97 (C-6-naphth); 134.69 (C-8a-naphth); 139.46 (CH-2-chromene); 145.04 (C-3-chromene); 146.83 (C-7-chromene); 147.58 (C-2-naphth); 156.31 (C-8a-chromene); 173.12 (C-4-chromene). HRMS (ESI+) calculated for $C_{24}H_{20}O_3N$: 370.14377, found: 370.14364; calculated for $C_{24}H_{19}O_3NNa$: 392.12571, found: 392.12555.

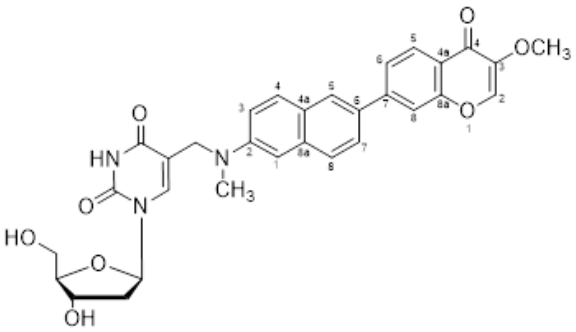
5-(3-{[6-(3-Methoxy-4-oxo-4*H*-chromen-7-yl)naphthalen-2-yl](methyl)amino]methyl})-3',5'-di-*O*-acetyl-2'-deoxyuridine (15)



3',5'-Di-*O*-acetylthymidine **14** (135 mg, 0.51 mmol), freshly recrystallized NBS (108 mg, 0.61 mmol) and AIBN (10 mg, 0.06 mmol) were suspended in benzene (4 ml) under argon; the reaction mixture was stirred at 60°C and the reaction proceeding was monitored by TLC (Eluent: Cyclohexane:EtOAc 1:1). When a complete conversion was reached (ca. 3 h), volatiles were evaporated. Secondary amine **12** (164 mg, 0.51 mmol) and Cs_2CO_3 (242 mg, 0.74 mmol) were added to the residue and anhydrous DMF (5 ml) was added. The mixture was stirred at room temperature (22 °C) overnight. DMF was

removed under reduced pressure and the residue was re-dissolved in MeOH and silica gel was added. After evaporation of all volatiles and column chromatography (0% → 90% EtOAc in CHX), the title compound **15** (184 mg, 57% over 2 steps) was isolated as a yellow solid. Rf: 0.32 (90% EtOAc in CHX). ^{1}H NMR (500.0 MHz, DMSO-d_6): 1.91, 2.04 (2 × s, 2 × 3H, CH_3CO); 2.29 (ddd, 1H, J_{gem} = 14.2, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 2.7, H-2′b); 2.34 (ddd, 1H, J_{gem} = 14.2, $J_{2'a,1'}$ = 8.1, $J_{2'a,3'}$ = 6.3, H-2′a); 3.07 (s, 3H, CH_3N); 3.77 (s, 3H, CH_3O); 3.97 – 4.11 (m, 3H, H-4′,5′); 4.32 (s, 2H, CH_2N); 5.11 (dt, 1H, $J_{3',2'}$ = 6.3, 2.7, $J_{3',4'}$ = 2.7, H-3′); 6.11 (dd, 1H, $J_{1',2'}$ = 8.1, 6.3, H-1′); 7.04 (d, 1H, $J_{1,3}$ = 2.6, H-1-naphth); 7.37 (dd, 1H, $J_{3,4}$ = 9.2, $J_{3,1}$ = 2.6, H-3-naphth); 7.47 (s, 1H, H-6); 7.78 (d, 1H, $J_{8,7}$ = 8.9, H-8-naphth); 7.81 – 7.87 (m, 2H, H-4,7-naphth); 7.90 (dd, 1H, $J_{6,5}$ = 8.5, $J_{6,8}$ = 1.7, H-6-chromene); 8.01 (d, 1H, $J_{8,6}$ = 1.7, H-8-chromene); 8.16 (d, 2H, $J_{5,6}$ = 8.5, H-5-chromene); 8.24 (d, 1H, $J_{5,7}$ = 2.0, H-5-naphth); 8.35 (s, 1H, H-2-chromene); 11.54 (s, 1H, NH-3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 20.62, 20.92 (**C**H_3CO); 35.76 (CH_2-2′); 38.57 (CH_3N); 48.87 (CH_2N); 57.04 (CH_3O); 63.74 (CH_2-5′); 74.10 (CH-3′); 81.27 (CH-4′); 84.83 (CH-1′); 105.79 (CH-1-naphth); 110.43 (C-5); 115.11 (CH-8-chromene); 117.30 (CH-3-naphth); 121.74 (C-4a-chromene); 123.22 (CH-6-chromene); 125.07 (CH-7-naphth); 125.90 (CH-5-chromene); 126.36 (CH-5-naphth); 126.50 (C-4a-naphth); 127.02 (CH-8-naphth); 129.58 (CH-4-naphth); 130.95 (C-6-naphth); 134.91 (C-8a-naphth); 137.29 (CH-6); 141.19 (CH-2-chromene); 144.58 (C-3-chromene); 145.83 (C-7-chromene); 147.88 (C-2-naphth); 150.31 (C-2); 156.06 (C-8a-chromene); 163.29 (C-4); 170.16, 170.21 (CH_3**C**O); 171.76 (C-4-chromene). HRMS (ESI+) calculated for $C_{35}H_{33}O_{10}N_3Na$: 678.20582, found: 678.20600.

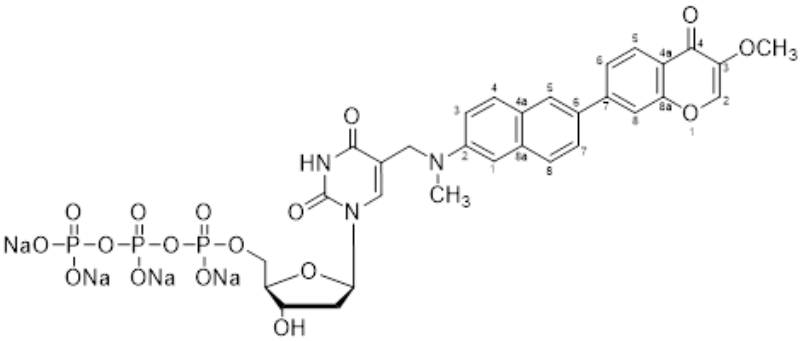
5-(3-{[6-(3-Methoxy-4-oxo-4*H*-chromen-7-yl)naphthalen-2-yl](methyl)amino]methyl})-2'-deoxyuridine (16)



Protected nucleoside **15** (180 mg, 0.28 mmol) and K_2CO_3 (95 mg, 0.69 mmol) were suspended in MeOH (5.5 ml). After stirring of the mixture at room temperature (25 °C) for 1 h, silica gel was added and volatiles were evaporated. Desired modified nucleoside **15** (120 mg, 76%) was isolated as a yellow solid after column chromatography (0% → 10% MeOH in DCM). Rf: 0.26 (10% EtOAc in cyclohexane). ^{1}H NMR (500.0 MHz, DMSO-d_6): 1.93 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.6, $J_{2'b,3'}$ = 5.8, H-2′b); 2.04 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.1, H-2′a); 3.08 (s, 3H, CH_3N); 3.43 (dd, 2H, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.9, H-5′); 3.72 (td, 1H, $J_{4',5'}$ = 3.9, $J_{4',3'}$ = 2.6, H-4′); 3.76 (s, 3H, CH_3O); 4.15 (dddd, 1H, $J_{3',2'}$ = 5.8, 3.1, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 2.6, H-3′); 4.28, 4.31 (2 × dd, 2 × 1H, J_{gem} = 17.3, ^{4}J = 1.0, CH_2N); 4.99 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.19 (d, 1H, $J_{OH,3'}$ = 4.2, OH-3′); 6.13 (dd, 1H, $J_{1',2'}$ = 7.6, 6.1, H-1′); 6.99 (d, 1H, $J_{1,3}$ = 2.6, H-1-naphth); 7.32 (dd, 1H, $J_{3,4}$ = 9.2, $J_{3,1}$ = 2.6, H-3-naphth); 7.77 (t, 1H, ^{4}J = 1.0, H-6); 7.78 (d, 1H, $J_{8,7}$ = 8.8, H-8-naphth); 7.83 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.0, H-7-naphth); 7.84 (d, 1H, $J_{4,3}$ = 9.2, H-4-naphth); 7.90 (dd, 1H, $J_{6,5}$ = 8.5, $J_{6,8}$ = 1.7, H-6-chromene); 8.01 (d, 1H, $J_{8,6}$ = 1.7, H-8-chromene); 8.15 (d, 2H, $J_{5,6}$ = 8.5, H-5-chromene); 8.24 (d, 1H, $J_{5,7}$ = 2.0, H-5-naphth); 8.34 (s, 1H, H-2-chromene); 11.42

(s, 1H, NH-3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 38.57 (CH_3N); 39.70 (CH_2-2′); 48.82 (CH_2N); 57.04 (CH_3O); 61.59 (CH_2-5′); 70.75 (CH-3′); 84.32 (CH-1′); 87.58 (CH-4′); 105.49 (CH-1-naphth); 109.84 (C-5); 115.09 (CH-8-chromene); 117.13 (CH-3-naphth); 121.72 (C-4a-chromene); 123.23 (CH-6-chromene); 125.03 (CH-7-naphth); 125.89 (CH-5-chromene); 126.36 (C-4a-naphth); 126.38 (CH-5-naphth); 127.02 (CH-8-naphth); 129.55 (CH-4-naphth); 130.80 (C-6-naphth); 134.95 (C-8a-naphth); 137.64 (CH-6); 141.19 (CH-2-chromene); 144.58 (C-3-chromene); 145.86 (C-7-chromene); 147.66 (C-2-naphth); 150.38 (C-2); 156.08 (C-8a-chromene); 163.42 (C-4); 171.77 (C-4-chromene). MS (ESI$^+$): *m/z* (%): 572.2 (100) $[M]^+$, 594.2 $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{31}H_{29}O_8N_3Na$: 594.18469; found: 594.18443.

5-(3-{[6-(3-Methoxy-4-oxo-4*H*-chromen-7-yl)naphthalen-2-yl](methyl)amino]methyl})-2'-deoxyuridine-5'-*O*-triphosphate (dT^{NC}TP)



Nucleotide **dT^{NC}TP** was prepared from nucleoside **16** (29 mg, 0.05 mmol) according to the procedure described for the preparation of **dC^{AC}TP**. The desired triphosphate was isolated (18.3 mg, 41%) as a yellow lyophilizate. ^{1}H NMR (500.0 MHz, D_2O, ref(*t*BuOH) = 1.24 ppm): 1.90 – 2.06 (bm, 2H, H-2′); 3.02 (bs, 3H, CH_3N); 3.68 (bs, 3H, CH_3O); 3.85 (bm, 1H, H-4′); 4.01 – 4.16 (bm, 2H, H-5′); 4.32 (bs, 2H, CH_2N); 4.37 (bm, 1H, H-3′); 5.56 (bm, 1H, H-1′); 6.66 – 6.89 (bm, 3H, H-1,7-naphth, H-8-chromene); 7.02 (bm, 1H, H-6-chromene); 7.11 – 7.32 (bm, 3H, H-3,5,8-naphth); 7.35 (bm, 1H, H-4-naphth); 7.52 (bs, 1H, H-6); 7.58 (bd, 1H, $J_{5,6}$ = 7.5, H-5-chromene); 7.62 (bs, 1H, H-2-chromene). ^{13}C NMR (125.7 MHz, D_2O, ref(*t*BuOH) = 32.43 ppm): 41.22 (CH_3N); 41.27 (CH_2-2′); 51.52 (CH_2N); 59.12 (CH_3O); 68.01 (d, $J_{C,P}$ = 5.7, CH_2-5′); 72.99 (CH-3′); 88.20 (bd, $J_{C,P}$ = 7.5, CH-4′); 88.59 (CH-1′); 110.55 (b, CH-1-naphth); 112.93 (C-5); 116.89 (CH-8-chromene); 120.70 (b, CH-3-naphth); 122.66 (C-4a-chromene); 125.76 (CH-6-chromene); 127.40 (CH-7-naphth); 127.57 (CH-5-chromene); 128.57 (CH-5-naphth); 129.45 (C-4a-naphth); 129.58 (CH-8-naphth); 132.45 (CH-4-naphth); 133.64 (C-6-naphth); 137.21 (C-8a-naphth); 142.17 (CH-6); 142.48 (b, CH-2-chromene); 146.60 (C-3-chromene); 148.38 (C-7-chromene); 150.14 (C-2-naphth); 153.60 (C-2); 158.25 (C-8a-chromene); 167.96 (C-4); 176.46 (C-4-chromene). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): -21.09 (bs, P$_\beta$); -10.48 (d, J = 18.0, P$_\alpha$); -6.41 (bs, P$_\gamma$). MS (ESI$^-$): *m/z* (%): 404.5 (100) $[M–H_2PO_3]^{2-}$. HRMS (ESI$^-$): calculated for $C_{31}H_{30}O_{17}N_3P_3Na$: 832.06912; found: 832.06795.

Absorption and steady-state fluorescence measurements

Materials and methods

Chemicals and spectroscopy grade solvents were purchased from Sigma-Aldrich, Alfa Aesar, Acros Organics and used as supplied. UV-visible spectra were measured on a Cary 100 UV-Vis spectrometer (Agilent Technologies). Fluorescence spectra were measured on a Fluoromax 4 spectrofluorimeter (HORIBA Scientific).

Determination of fluorescence quantum yields

Relative determination of the fluorescence quantum yields[3,4] (Φ_f) was performed using quinine sulfate in 0.5 M H_2SO_4 (Φ_f = 0.55 at 25 °C) as a standard. Measurements were performed in semi-micro quartz fluorescence cuvettes (Hellma Analytics) on a Fluoromax 4 spectrofluorimeter equiped with a thermostated cuvette holder at 25 °C. The solvents used were either of spectroscopy or HPLC grade. The excitation wavelength was 380 nm and the recorded spectral range was 400 – 700 nm. The absorbance of sample solutions at the excitation wavelength were kept below 0.08 to avoid inner filter effects. The quantum yields were calculated using the following equation:

$$\Phi_{f,x} = \Phi_{f,st} \times \frac{F_x}{F_{st}} \times \frac{1 - 10^{-Abs_{st}}}{1 - 10^{-Abs_x}} \times \frac{n_x^2}{n_{st}^2}$$

Here Φ_f is the quantum yield, *F* is the integrated fluorescence intensity, *Abs* is the absorbance of solution at the excitation wavelength, *n* is the refractive index of solvent; the subscripts *x* and *st* stand for the sample and standard, respectively. Measurements were triplicated; the uncertainty of measured values of quantum yields was ± 0.03.

Table 1 Spectroscopic properties of compounds **4**, **dC^{AC}** and **dC^{AC}TP**.

Compound	Solvent	ε [10^3 M^{-1} cm^{-1}]	λ_{abs} [nm][a]	λ_{em} [nm][b]	φ_f[c]
4	dioxane	17.1[d] 10.8 9.9	346 278 240	433	0.43[e]
	EtOAc	17.1 10.8 9.9	346 277 253	457	0.56
	DMSO	15.8 9.9 8.3	360 283 261	525	0.60
	EtOH	15.0 10.1	359 280	567	<0.05 (~0.024)
	MeOH	15.7 10.6	359 280	-	<0.05 (~0.007)
dC^{AC}	EtOH	8.1 11.5	357 280	567	<0.05 (~0.026)
	MeOH	8.3 11.8	357 280	-	<0.05 (~0.007)
dC^{AC}TP	buffer[f]	3.9 7.4	333 239	-	<0.05 (~0.00035)

[a] Position of the absorption maximum, ± 1 nm. [b] Position of the emission maximum, ± 2 nm. [c] Quantum yield of fluorescence measured using quinine sulfate in 0.5 M H_2SO_4 (Φ_f 0.55 at 25 °C) as a standard. [d] For every value the Confidence Interval did not exceed the value of 0.2 x 10^3 $M^{-1}.cm^{-1}$. [e] For every value (above 0.05) the Confidence Interval did not exceed the value of 0.02. [f] 20 mM phosphate buffer, pH = 7.0, 1 M NaCl.

Table 2 Spectroscopic properties of compounds **12**, **dT^{NC}** and **dT^{NC}TP**.

Compound	Solvent	ε [10^3 M^{-1} cm^{-1}]	λ_{abs} [nm][b]	λ_{em} [nm][c]	φ_f[c]
12	dioxane	24.5[d] 42.4	361 252	457	0.80[e]
	EtOAc	24.0 39.9	362 257	498	0.65
	DMSO	22.6 33.3	381 265	592	0.11
	EtOH	20.7 44.2 58.8	375 251 224	-	<0.05 (~0.007)
	MeOH	21.5 46.2 58.1	373 250 224	-	<0.05 (~0.005)
dT^{NC}	EtOH	21.0 49.3 119.7	379 255 222	-	<0.05 (~0.012)
	MeOH	21.7 51.6 117.7	378 254 223	-	<0.05 (~0.008)
dT^{NC}TP	buffer[f]	17.6 27.2	359 255	-	<0.05 (~0.0007)

[a] Position of the absorption maximum, ± 1 nm. [b] Position of the emission maximum, ± 2 nm. [c] Quantum yield of fluorescence measured using quinine sulfate in 0.5 M H_2SO_4 (Φ_f 0.55 at 25 °C) as a standard. [d] For every value the Confidence Interval did not exceed the value of 0.2 x 10^3 $M^{-1}.cm^{-1}$. [e] For every value (above 0.05) the Confidence Interval did not exceed the value of 0.02. [f] 20 mM phosphate buffer, pH = 7.0, 1 M NaCl.

Biochemistry

Materials and methods

Oligonucleotides used in work (Table S1) were purchased from Generi Biotech. KOD XL DNA polymerase and corresponding reaction buffer were obtained from Merck Millipore. Natural nucleoside triphosphates (dCTP, dATP, dGTP, dTTP) and histone H3.1 human recombinant protein (1mg/mL, 65 μM, *M* = 15273 Da) were purchased from New England Biolabs. Bovine serum albumin (BSA) was purchased from Thermofisher. All solutions for biochemistry experiments were prepared in Milli-Q water. Stop solution contained 80% (*v*/v) formamide, 20 mM EDTA, 0.025% (*w*/*v*) bromophenol blue and 0.025% (*w*/*v*) xylene cyanol in water. Concentration of DNA solutions were measured using Nanodrop 1000 Spectrophotometer. Mass spectra of oligonucleotides were measured by MALDI-TOF, using UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany), with 1 kHz smartbeam II laser. Gels were visualized using a fluorescence scanner Typhoon FLA 9500 from GE Healthcare. UV-visible spectra were measured on a Cary 100 UV-Vis spectrometer (Agilent Technologies). Fluorescence spectra were measured on a Fluoromax 4 spectrofluorimeter (HORIBA Scientific).

List of oligonucleotides

Table S1: Oligonucleotides used in the study. Primer sequences in the template are underlined. Cyanine-5 (Cy5) was used for oligonucleotide labelling at 5'-end.

Oligonucleotide	**Sequence 5'→ 3'**	**Length**
$Primer^{PEX}$-Cy5	Cy5-TCA AGA GAC ATG CCT	15-mer
$Primer^{PEX}$	TCA AGA GAC ATG CCT	15-mer
$Primer1^{PCR}$	GAC ATC ATG AGA GAC ATC GC	20-mer
$Primer2^{PCR}$	CAA GGA CAA AAT ACC TGT ATT CCT T	25-mer
$Template1^{PEX}$	P-ATA ATA AAC ATG TCT <u>AGG CAT GTC TCT TGA</u>	30-mer
$Template2^{PEX}$	P-TTG TTG GGC ATG TCT <u>AGG CAT GTC TCT TGA</u>	30-mer
$Template^{PCR}$	GAC ATC ATG AGA GAC ATC GCC TCT GGG CTA ATA GGA CTA CTT CTA ATC TGT AAG AGC AGA TCC CTG GAC AGG C<u>AA GGA ATA CAG GTA TTT TGT CCT TG</u>	98-mer

Enzymatic synthesis of modified DNA using $dC^{AC}TP$ by primer extension

Reaction mixture (20 μL) contained Cy5 labelled primer ($Primer^{PEX}$-Cy5, 100 μM, 1 μL), template ($Template1^{PEX}$, 100 μM, 1 μL), KOD XL DNA polymerase (0.25 U/μL, 0.12 μL), natural dNTPs

(4 mM mixture of dATP, dGTP and dTTP, 0.3 μL), modified **dC^{AC}TP** (4 mM, 0.3 μL) and corresponding reaction buffer (10×, 2 μL) as supplied by the manufacturer. The reaction mixture was incubated at 60°C for 60 min in thermal cycler. The reaction was stopped by the addition of PAGE stop solution (20 μL) and the reaction mixture was denatured at 95°C for 5 min. The samples were subjected to vertical electrophoresis in 12.5% denaturing polyacrylamide gel containing 1×TBE buffer (pH 8.5) and 7M urea at 42mA for 1 h. The gel was visualized by a fluorescent scanner (PAGE gel is shown in Figure S1).

To prepare modified dsDNA for MALDI and interactions with histone H3.1, multiple PEX reactions were performed as described above using non-labeled primer Primer^{PEX} at 60 °C for 70 min. Subsequently the product of PEX was purified using spin columns (QIAquick® Nucleotide Removal Kit, QIAGEN, following supplier's manual) and eluted by milli-Q water.

Enzymatic synthesis of modified DNA using dT^{NC}TP by primer extension

Reaction mixture (20 μL) contained Cy5 labelled primer (Primer^{PEX}-Cy5, 100 μM, 1 μL), template (Template2^{PEX}, 100 μM, 1 μL), KOD XL DNA polymerase (0.25 U/μL, 0.12 μL), natural dNTPs (4 mM mixture of dATP, dGTP and dCTP, 0.3 μL), modified **dT^{NC}TP** (4 mM, 0.3 μL) and corresponding reaction buffer (10×, 2 μL) as supplied by the manufacturer. The reaction mixture was incubated at 60°C for 35 min in thermal cycler. The reaction was stopped by the addition of PAGE stop solution (20 μL) and the reaction mixture was denatured at 95°C for 5 min. The samples were subjected to vertical electrophoresis in 12.5% denaturing polyacrylamide gel containing 1×TBE buffer (pH 8.5) and 7M urea at 42mA for 1 h. The gel was visualized by a fluorescent scanner (PAGE gel is shown in Figure S1).

To prepare modified dsDNA for MALDI (Figure S2, Figure S3) and interactions with histone H3.1, multiple PEX reactions were performed as described above using non-labeled primer Primer^{PEX} at 60 °C for 45 min. Subsequently the product of PEX was purified using spin columns (QIAquick® Nucleotide Removal Kit, QIAGEN, following supplier's manual) and eluted by milli--Q water.

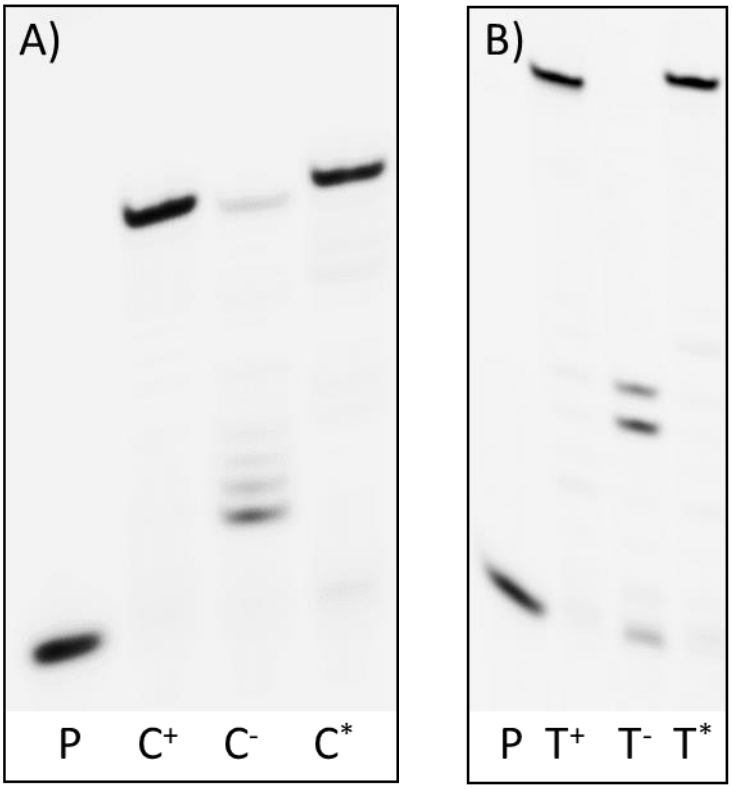


Figure S1. Analysis of PEX by PAGE using A) **dC^{AC}TP** or B) **dT^{NC}TP**. Primer (P), positive control (C^+/T^+), negative control (C^-/T^-), PEX with modified nucleotide (C*/T*).

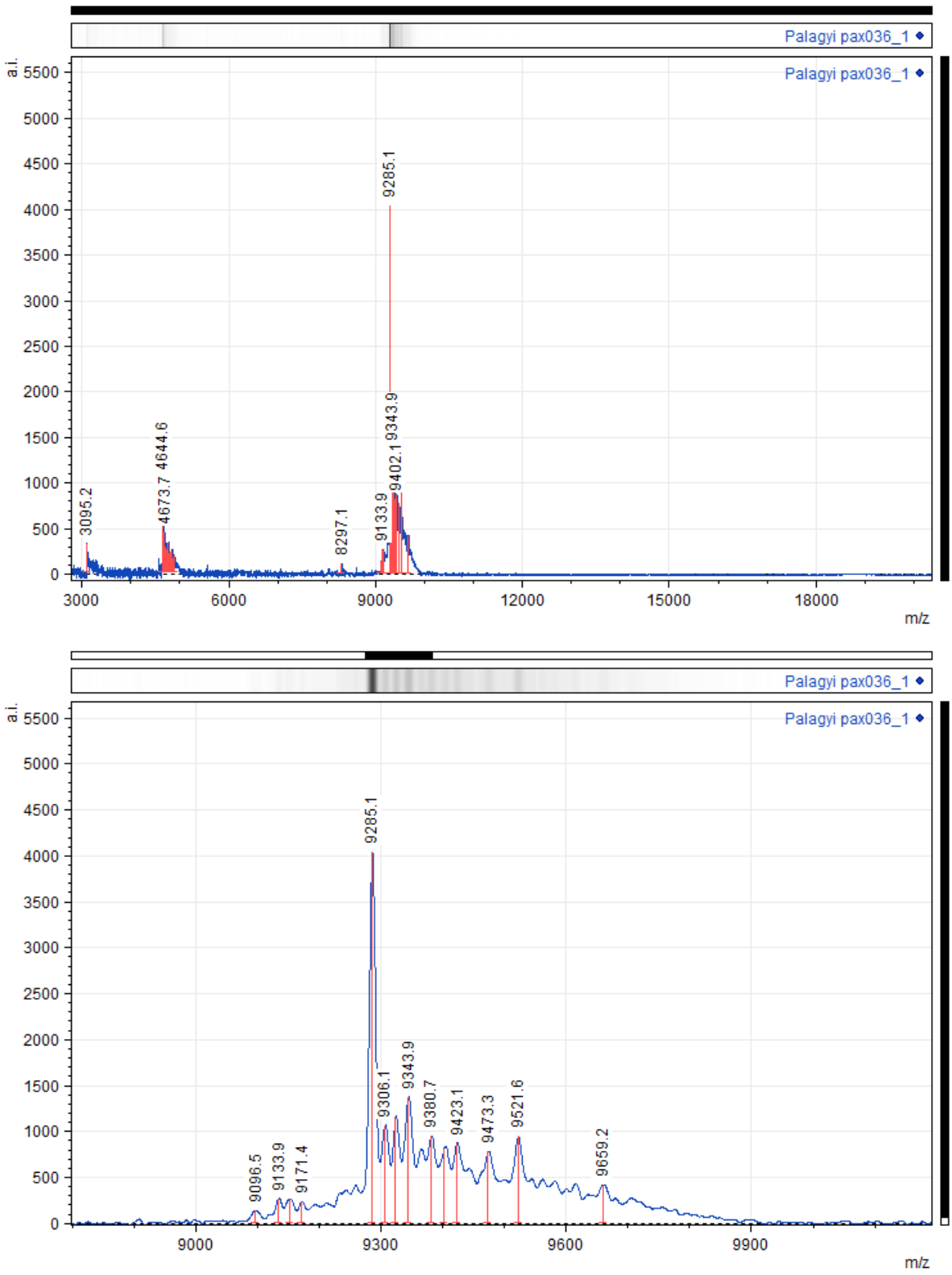


Figure S2. MALDI-TOF MS spectrum of ON **DNA30_1C**^{AC}. Calculated for [M]: 9521.5 Da, found 9521.6 Da. The peak at *m*/*z* = 9285.1 represents template Template1^{PEX}.

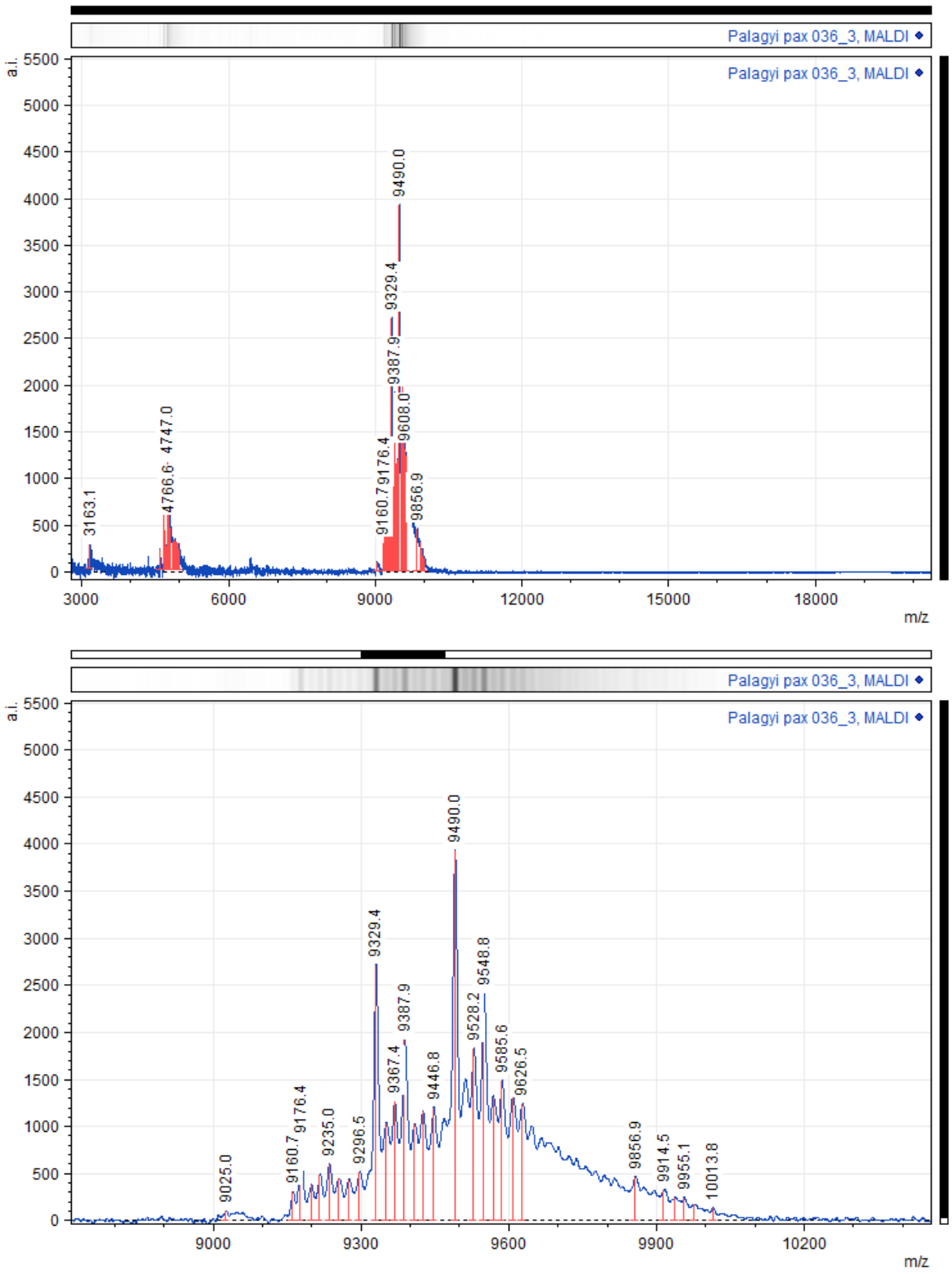


Figure S3. MALDI-TOF MS spectrum of ON **DNA30_1T^{NC}**. Calculated for [M]: 9491.1 Da, found 9490.0 Da. The peak at *m*/*z* = 9329.4 represents template Template2^{PEX}.

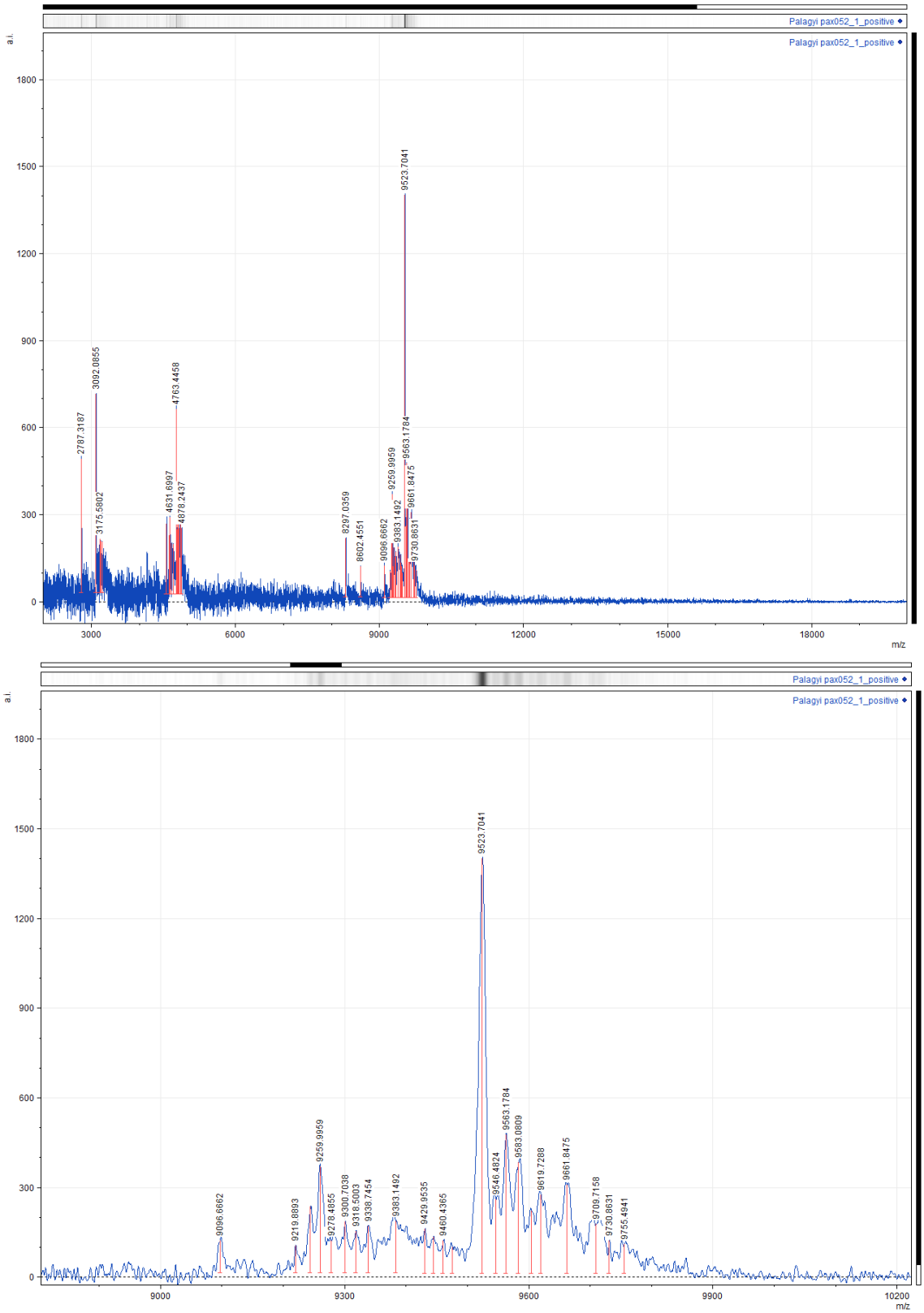


Figure S4. MALDI-TOF MS spectrum of ON **ssDNA30_1C^{AC}**. Calculated for [M]: 9521.5 Da, found 9523.7 Da.

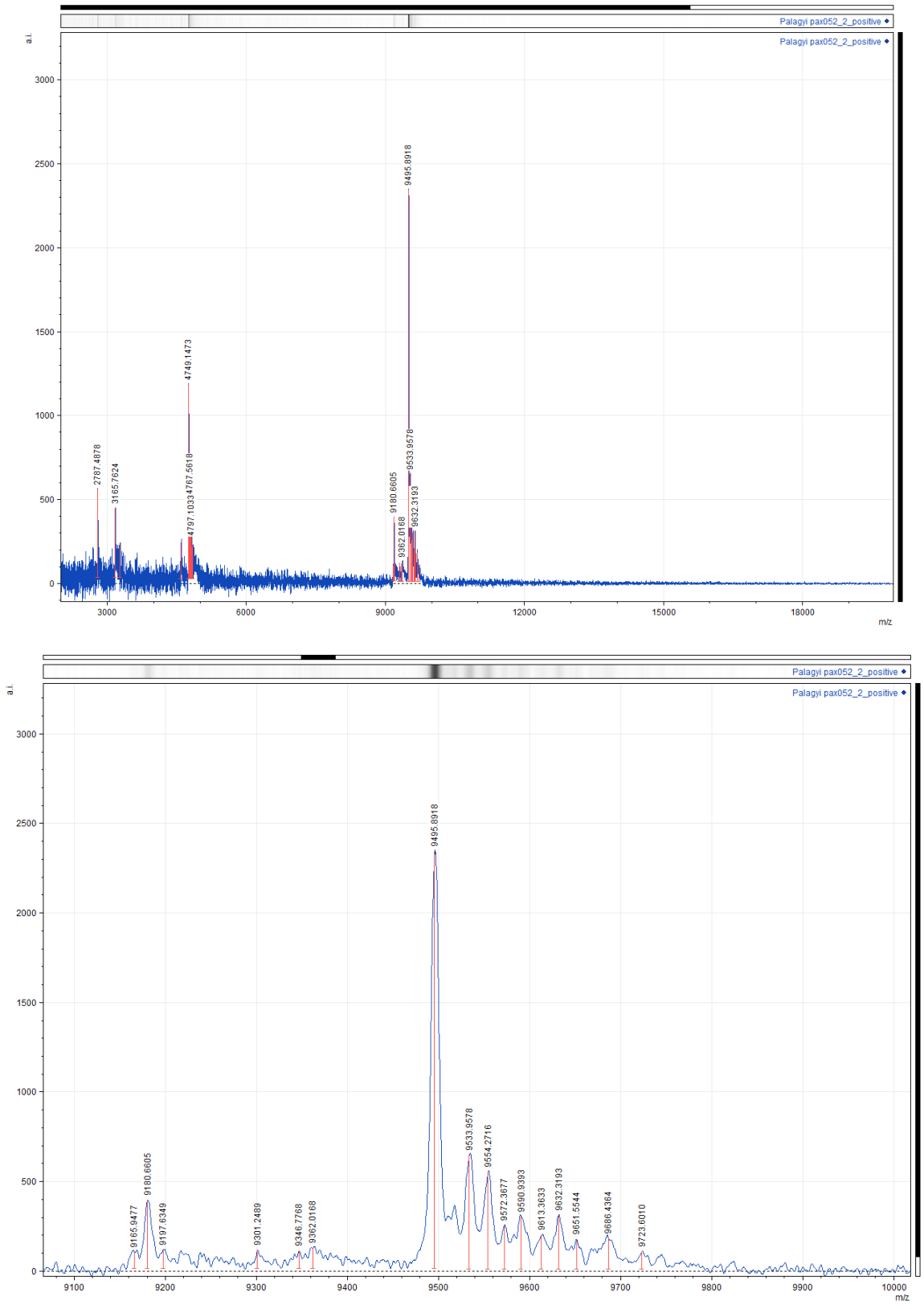


Figure S5. MALDI-TOF MS spectrum of ON **ssDNA30_1T^{NC}**. Calculated for [M]: 9491.1 Da, found 9495.9 Da.

Preparation of single-stranded ON30_1C^{AC} and ON30_1T^{NC} by digestion of phosphorylated strand of DNA using λ exonuclease

For digestion of phosphorylated strands of **DNA30_1C^{AC}** or **DNA30_1T^{NC},** DNA (0.5 nmol) was used with λ exonuclease buffer (5 µL) and λ exonuclease enzyme (2 µL) in 50 µL reaction mixture. Reactions were incubated at 37 °C for 1.5 h and subsequently were purified using QIAquick Nucleotide Removal Kit. Concentration was measured on Nanodrop and the obtained ONs were submitted to MALDI analysis (Figure S4, Figure S5).

Enzymatic incorporation of dC^{AC}TP or dT^{NC}TP by polymerase chain reaction (PCR)

The reaction mixture (20 µL) contained primers (Primer1^{PCR} and Primer2^{PCR}, 10 µM, 4 µL of each), template (Template^{PCR}, 10 µM, 0.5 µL), KOD XL DNA polymerase (2.5 U/µL, 1 µL) and corresponding reaction buffer (10×, 2 µL) supplied by the manufacturer. In the case of PCR with **dC^{AC}TP** the used dNTPs were dATP, dGTP, dTTP (0.4 mM each, 1.5 µL), and either dCTP (0.4 mM, 1.5 uL), **dC^{AC}TP** (0.4 mM, 1.5 uL), or mixture of **dC^{AC}TP** with natural dCTP (5-80%). In the case of PCR with **dT^{NC}TP** the used dNTPs were dATP, dGTP, dCTP (0.4 mM each, 1.5 µL), and either dTTP (0.4 mM, 1.5 uL), **dT^{NC}TP** (0.4 mM, 1.5 uL), or mixture of **dT^{NC}TP** with natural dTTP (5-80%). After the initial denaturation at 94 °C for 3 min, 30 PCR cycles were run under the following conditions: denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min, extension at 72 °C for 2 min. The PCR was terminated with a final extension step at 72°C for 5 min. The reaction was stopped by cooling to 4 °C. The PCR products were analyzed by agarose gel electrophoresis in 2% agarose gel stained with GelRed™ and visualized by a fluorescent scanner (Figure S6).

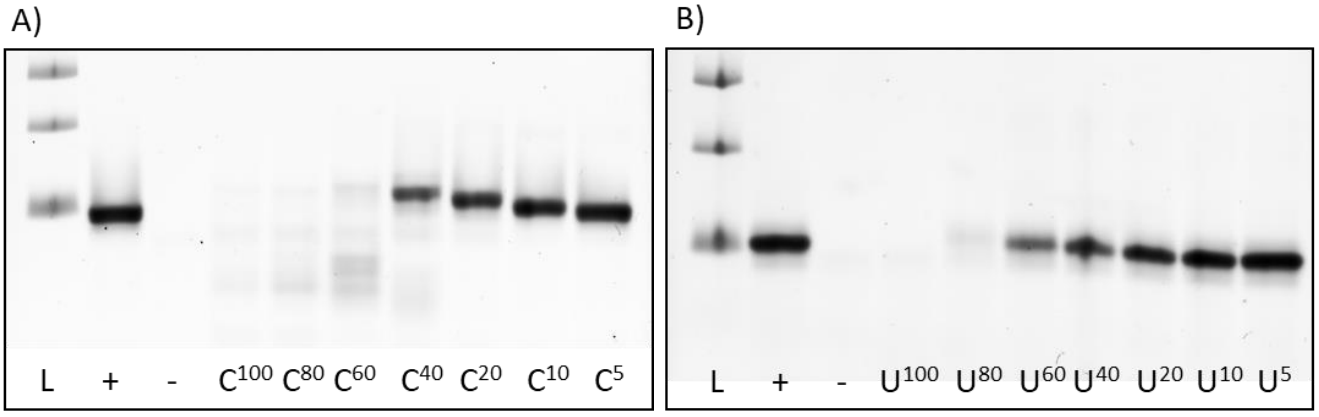


Figure S6. Analysis of PCR amplification using agarose gel stained by GelRed™. PCR experiment was done using different proportions of natural and modified dNTP, A) dCTP/**dC^{AC}TP** (0 – 100%) or B) dTTP/**dT^{NC}TP** (0 – 100%). DNA ladder (L), positive control (+), negative control (-).

Interaction of DNA30_1C^{AC} or DNA30_1T^{NC} with histone H3.1, p53, lysozyme and BSA

Titrations were performed in 100 µL quartz cuvette at 25°C. Solution of dsDNA (100 µL, 1 µM) in PBS buffer (25 mM, pH 7.4) was titrated by histone H3.1, p53 and by lysozyme and BSA (Bovine serum albumin) as control experiments. After every addition (0-20 equiv.) the solution was mixed carefully with a pipette and equilibrated for 1-2 min before recording the fluorescence spectrum (λ_{ex}= 350 nm for **DNA30_1C^{AC}**, λ_{ex}= 370 nm for **DNA30_1T^{NC}**). The samples were used for taking picture (Figure 4E) under irradiation at 366 nm.

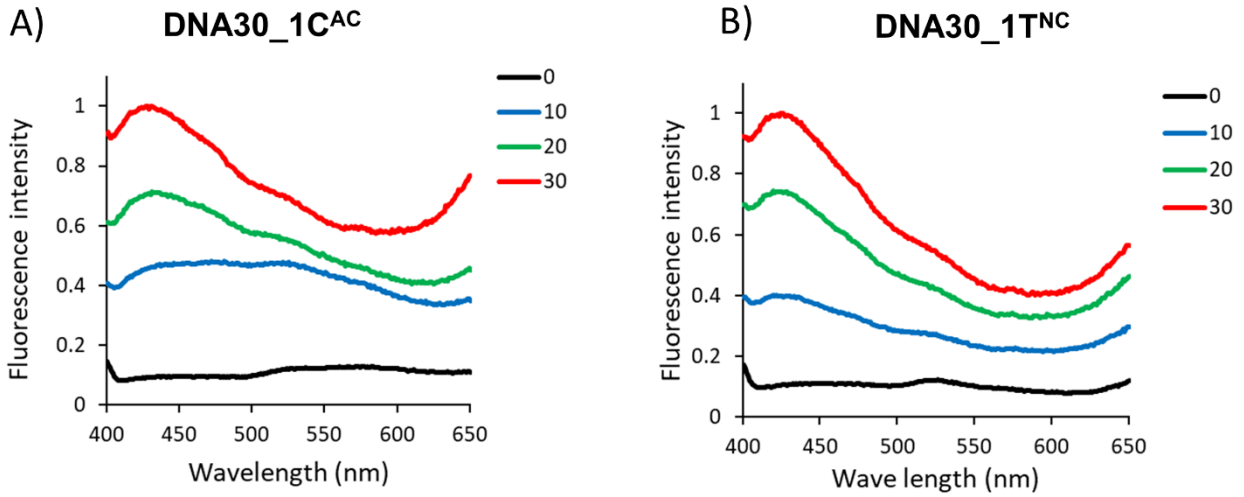


Figure S7. Emission spectra of A) **DNA30_1C^{AC}** and B) **DNA30_1T^{NC}** upon gradual increase of p53 (number of equivalents 0-30). Samples of experiments with **DNA30_1C^{AC}** were excited at 350 nm and with **DNA30_1T^{NC}** were excited at 370 nm at 25 °C.

Interaction of ON30_1C^{AC} or ON30_1T^{NC} with SSB and BSA

Titrations were performed in 100 µL quartz cuvette at 25°C. Solution of ssDNA (100 µL, 1 µM) in H_2O was titrated by SSB and BSA (Bovine serum albumin) as control experiments. After every addition (0-15 equiv.) the solution was mixed carefully with a pipette and equilibrated for 1-2 min before recording the fluorescence spectrum (λ_{ex}= 350 nm for **ON30_1C^{AC}**, λ_{ex}= 370 nm for **ON30_1T^{NC}**). The samples were used for taking picture (Figure 5C,D) under irradiation at 366 nm.

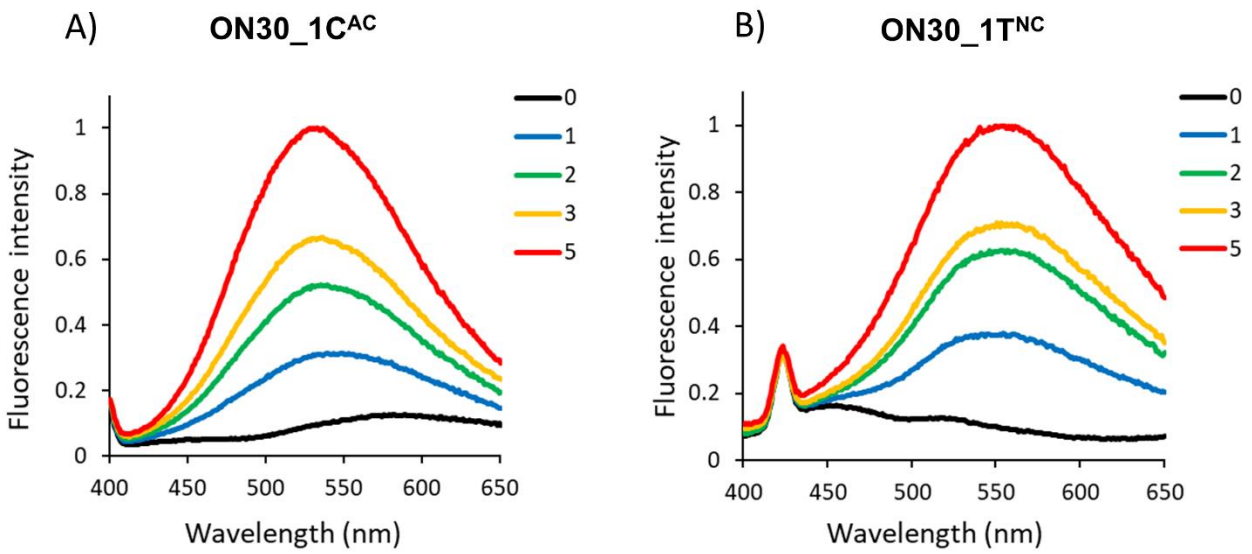


Figure S8. Emission spectra of A) **DNA30_1C^{AC}** and B) **DNA30_1T^{NC}** upon gradual increase of p53 (number of equivalents 0-30). Samples of experiments with **DNA30_1C^{AC}** were excited at 350 nm and with **DNA30_1T^{NC}** were excited at 370 nm at 25 °C.

Cell experiments

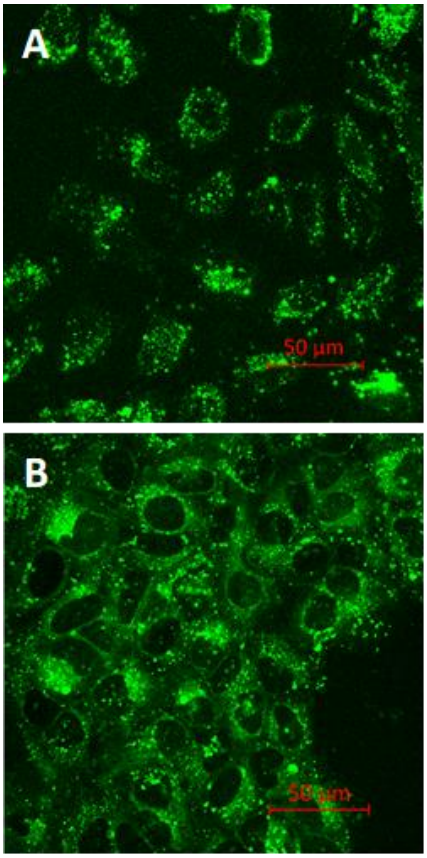
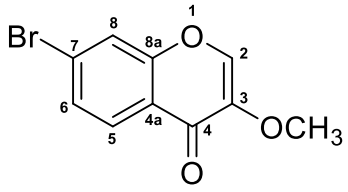


Figure S9. Confocal imaging of cells (U2 OS) after their treatment with 10 µM complexes of the transporter SNTT-1 A) **dC^{AC}TP** and B) **dT^{NC}TP**. Excitation: 405 nm; Detection: 420 – 700 nm.

NMR spectra of compounds

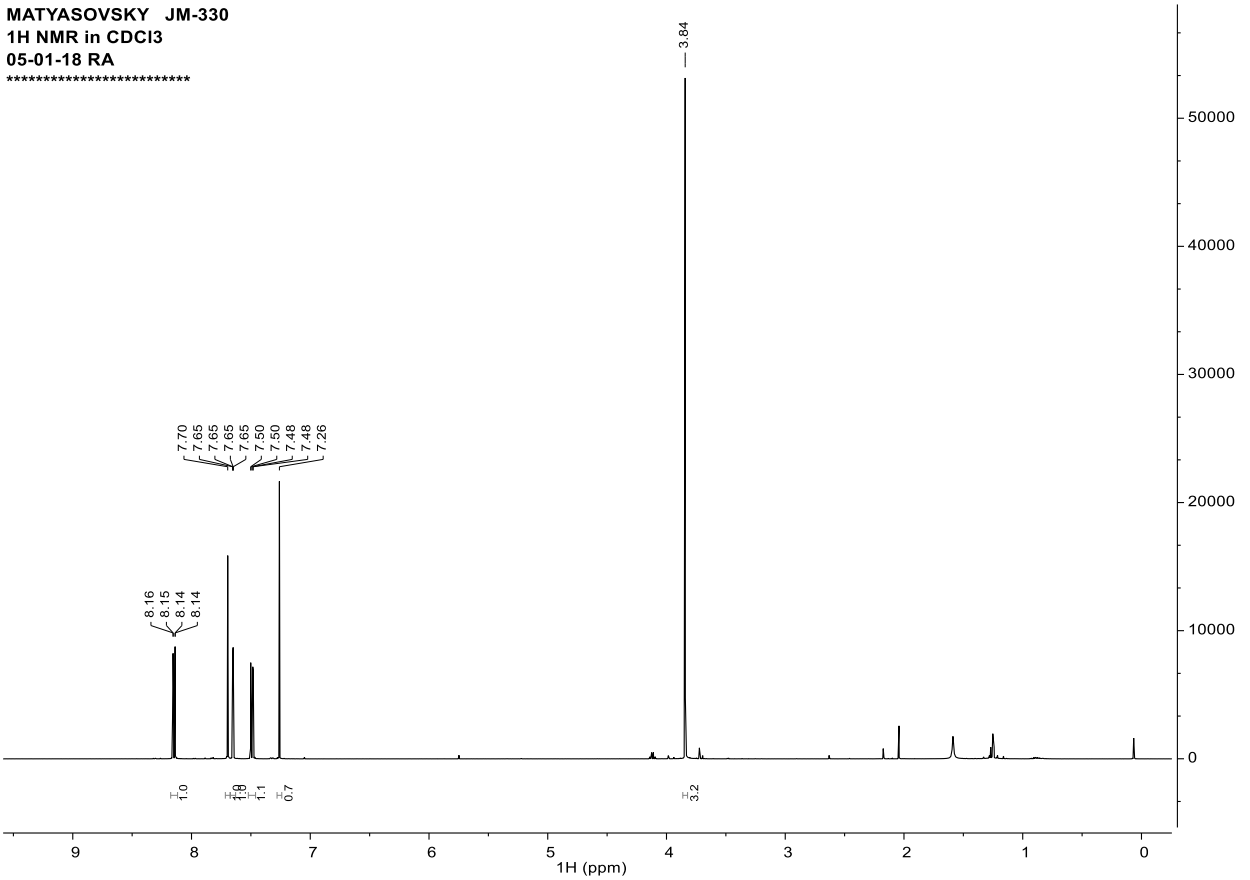
Compound 2

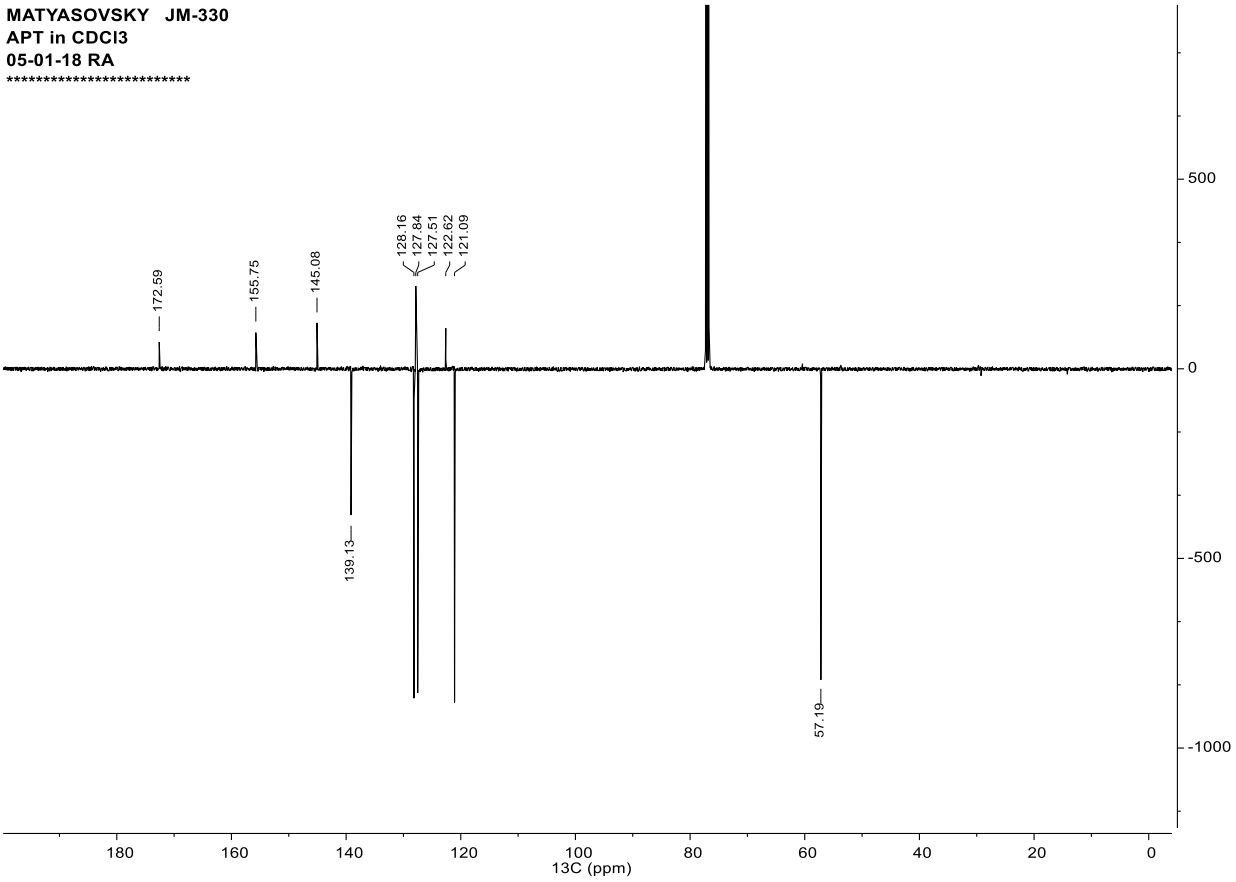


JM-330

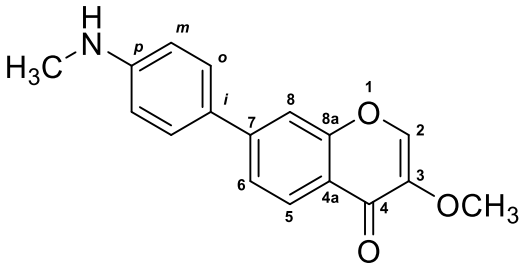
^{1}H NMR (500.0 MHz, $CDCl_3$): 3.84 (s, 3H, CH_3O); 7.49 (dd, 1H, $J_{6,5}$ = 8.6, $J_{6,8}$ = 1.8, H-6); 7.65 (d, 1H, $J_{8,6}$ = 1.8, H-8); 7.70 (s, 1H, H-2); 8.15 (d, 1H, $J_{5,6}$ = 8.6, H-5).

^{13}C NMR (125.7 MHz, $CDCl_3$): 57.19 (CH_3O); 121.09 (CH-8); 122.62 (C-4a); 127.51 (CH-5); 127.84 (C-7); 128.16 (CH-6); 139.13 (CH-2); 145.08 (C-3); 155.75 (C-8a); 172.59 (C-4).





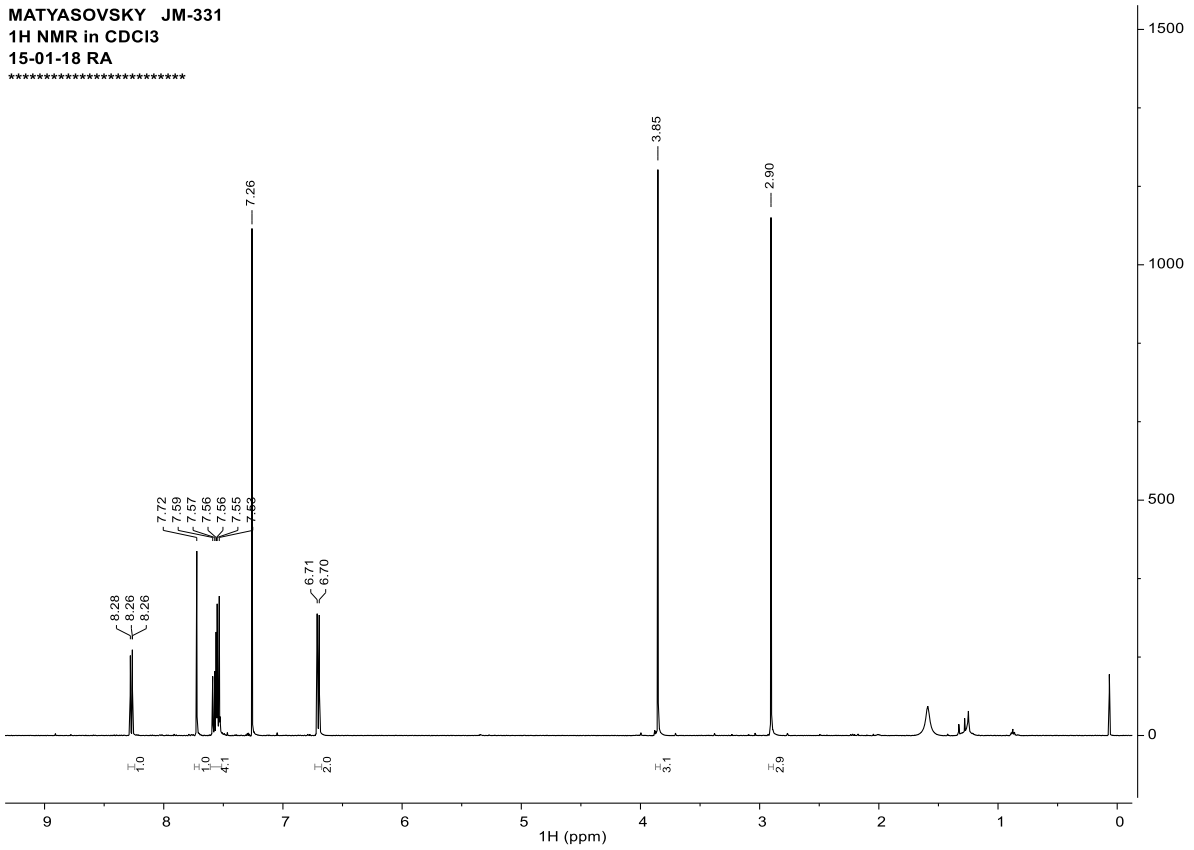
Compound 3

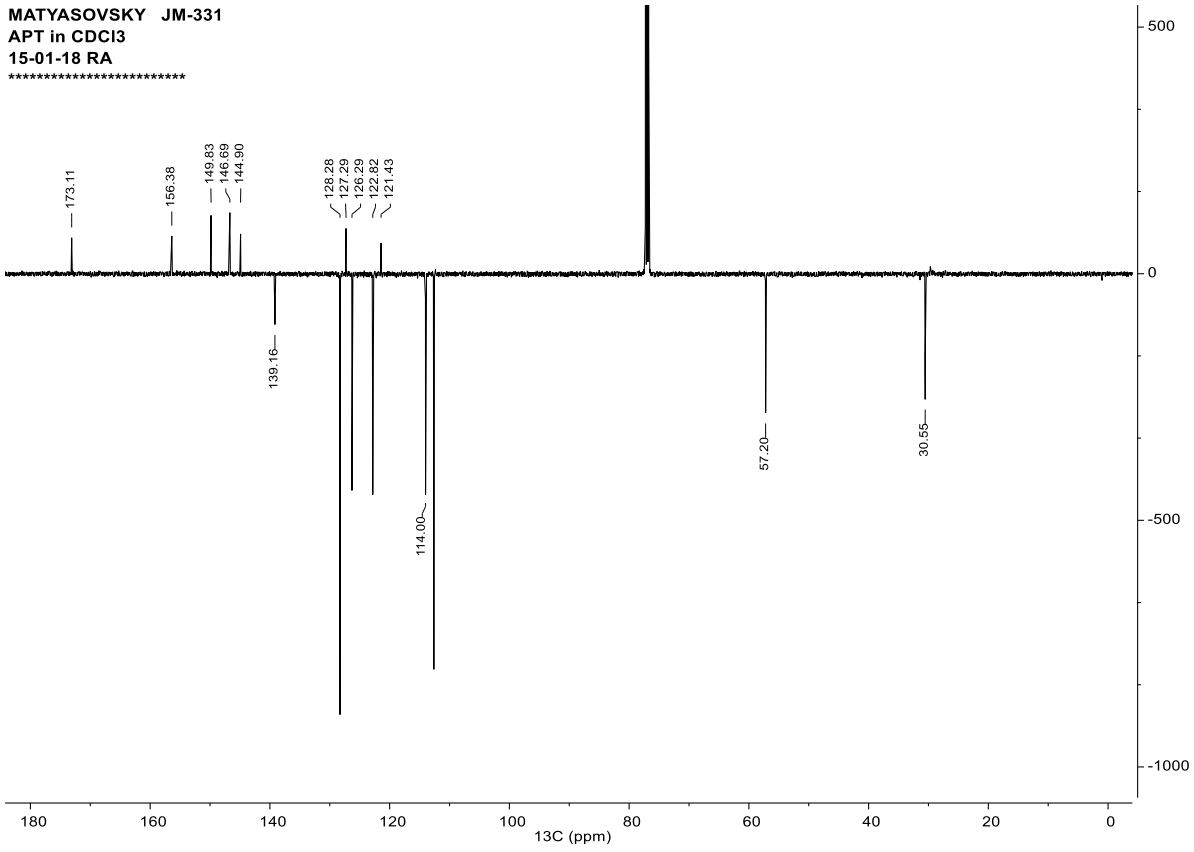


JM-331

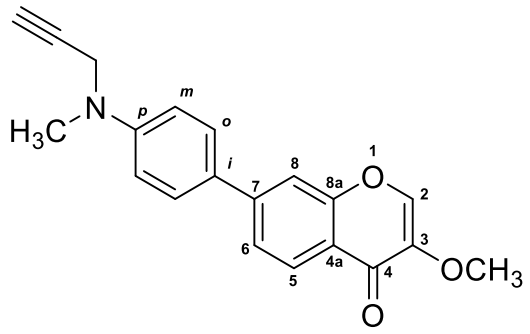
^{1}H NMR (500.0 MHz, $CDCl_3$): 2.90 (s, 3H, CH_3N); 3.85 (s, 3H, CH_3O); 6.69 – 6.72 (m, 2H, H-*m*-phenylene); 7.52 – 7.56 (m, 2H, H-*o*-phenylene); 7.56 (d, 1H, $J_{8,6}$ = 1.7, H-8); 7.58 (dd, 1H, $J_{6,5}$ = 8.4, $J_{6,8}$ = 1.7, H-6); 7.72 (s, 1H, H-2); 8.27 (d, 1H, $J_{5,6}$ = 8.4, H-5).

^{13}C NMR (125.7 MHz, $CDCl_3$): 30.55 (CH_3N); 57.20 (CH_3O); 112.61 (CH-*m*-phenylene); 114.00 (CH-8); 121.43 (C-4a); 122.82 (CH-6); 126.29 (CH-5); 127.29 (C-*i*-phenylene); 128.28 (CH-*o*-phenylene); 139.16 (CH-2); 144.90 (C-3); 146.69 (C-7); 149.83 (C-*p*-phenylene); 156.38 (C-8a); 173.11 (C-4).


MATYASOVSKY JM-331
1H NMR in CDCl3
15-01-18 RA
****
8.28
8.26
8.26
7.72
7.59
7.57
7.56
7.56
7.55
7.53
7.26
6.71
6.70
3.85
2.90
1.0
1.0
4.1
2.0
3.1
2.9
1H (ppm)


MATYASOVSKY JM-331
APT in CDCl3
15-01-18 RA
****
173.11
156.38
149.83
146.69
144.90
139.16
128.28
127.29
126.29
122.82
121.43
114.00
57.20
30.55
13C (ppm)

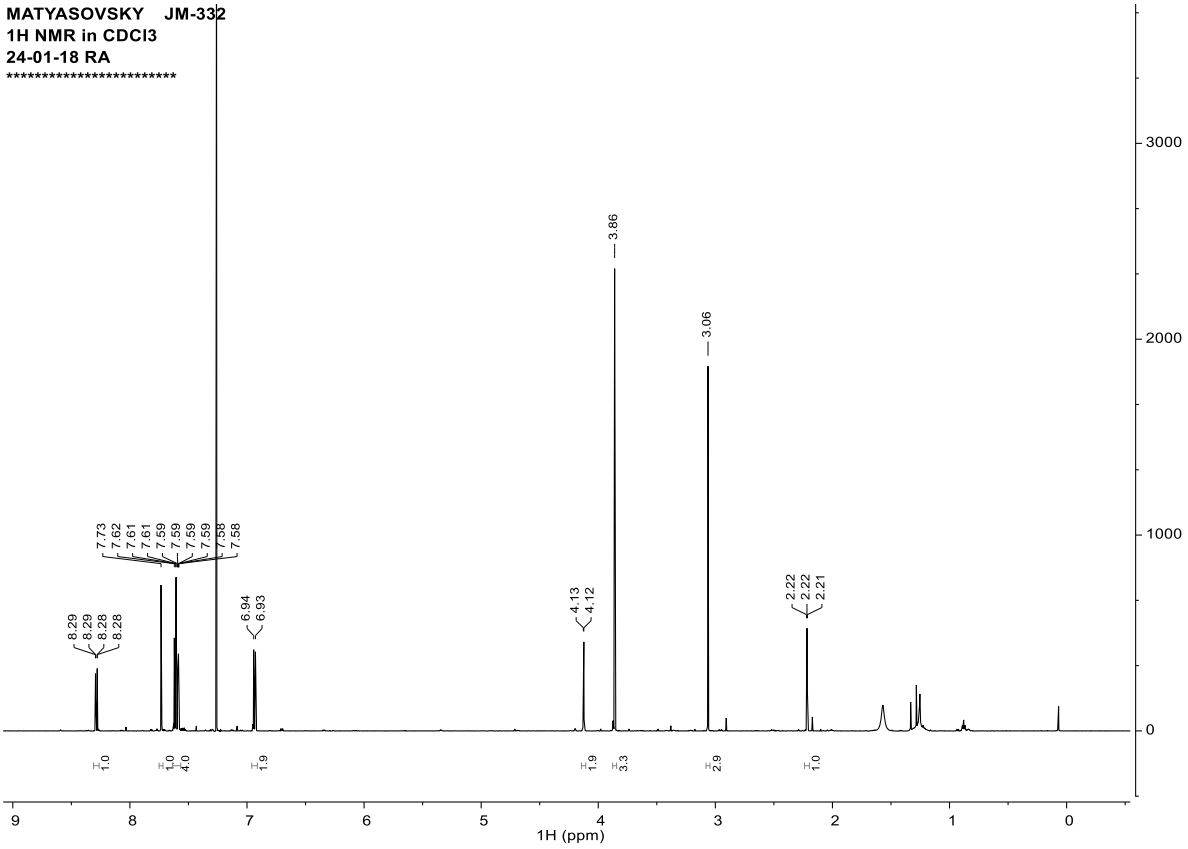
Compound 4

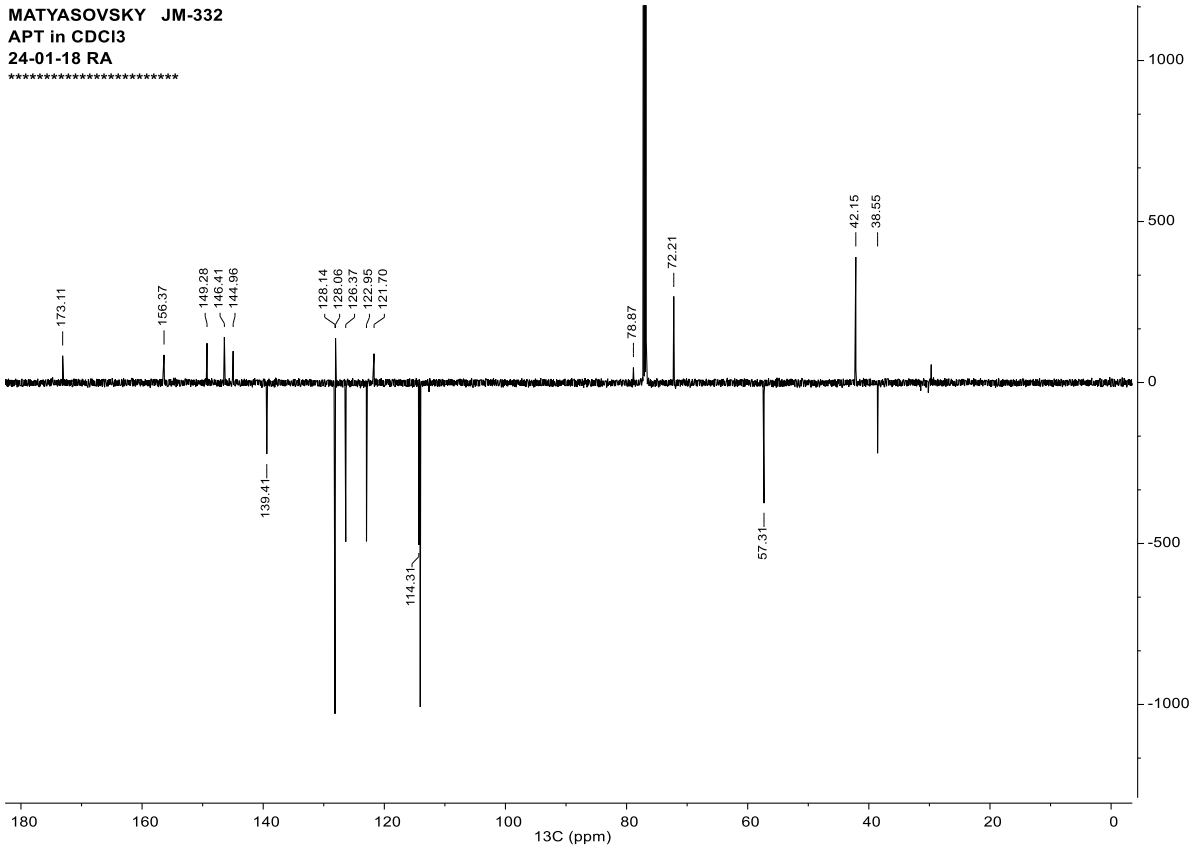


JM-332

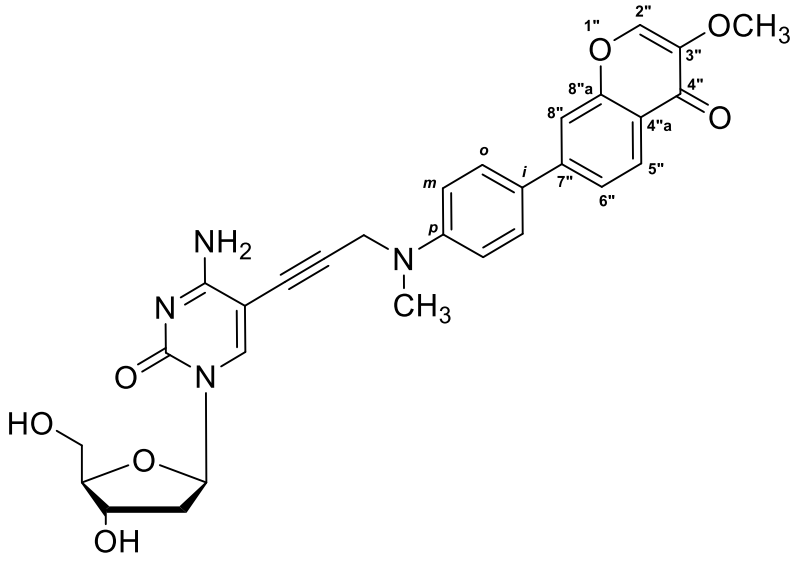
^{1}H NMR (600.0 MHz, $CDCl_3$): 2.22 (t, 1H, ^{4}J = 2.4, **H**C≡C-CH_2); 3.06 (s, 3H, CH_3N); 3.86 (s, 3H, CH_3O); 4.13 (d, 2H, ^{4}J = 2.4, HC≡C-C**H$_2$**); 6.91 – 6.96 (m, 2H, H-*m*-phenylene); 7.58 – 7.60 (m, 2H, H-6,8); 7.60 – 7.63 (m, 2H, H-*o*-phenylene); 7.73 (s, 1H, H-2); 8.29 (dd, 1H, $J_{5,6}$ = 8.3, $J_{5,8}$ = 0.3, H-5).

^{13}C NMR (150.9 MHz, $CDCl_3$): 38.55 (CH_3N); 42.15 (**C**H_2-C≡CH); 57.31 (CH_3O); 72.21 (CH_2-C≡**C**H); 78.87 (CH_2-**C**≡CH); 114.07 (CH-*m*-phenylene); 114.31 (CH-8); 121.70 (C-4a); 122.95 (CH-6); 126.37 (CH-5); 128.06 (C-*i*-phenylene); 128.14 (CH-*o*-phenylene); 139.41 (CH-2); 144.96 (C-3); 146.41 (C-7); 149.28 (C-*p*-phenylene); 156.37 (C-8a); 173.11 (C-4).





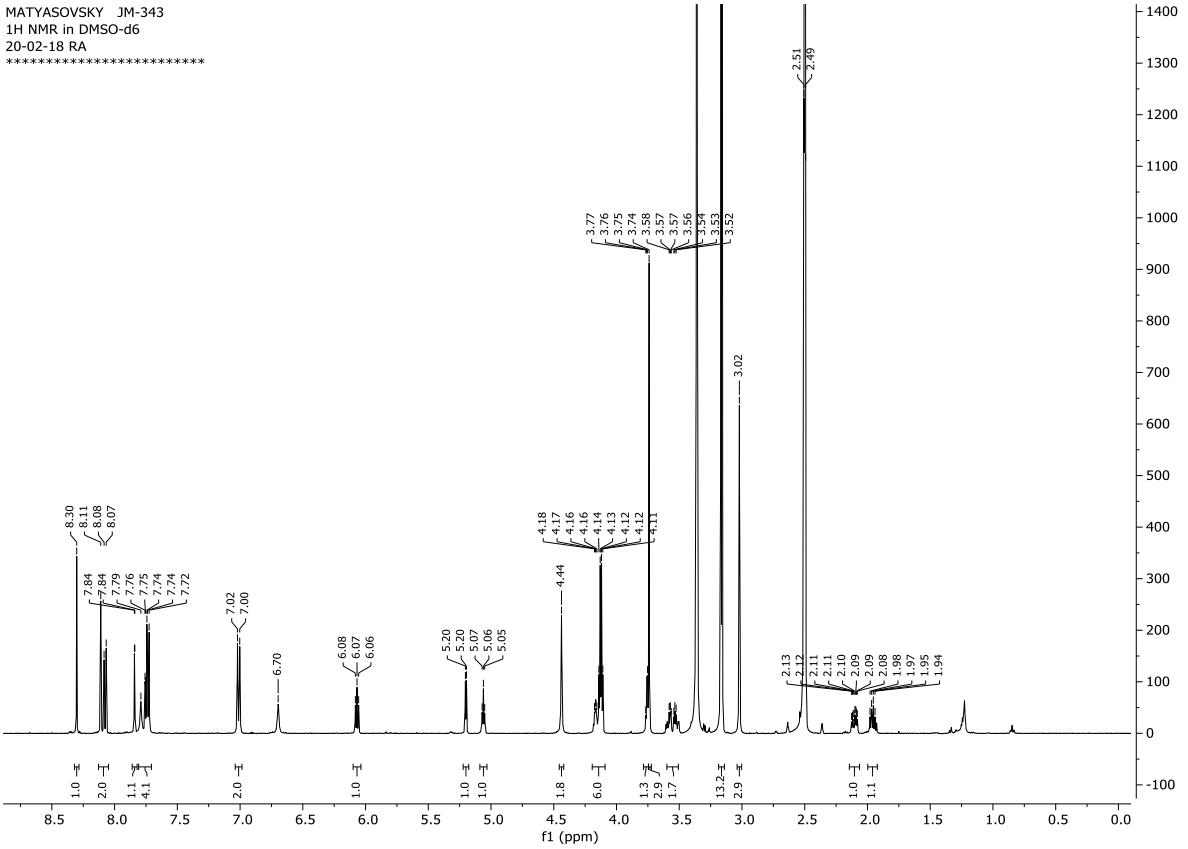
Compound 6

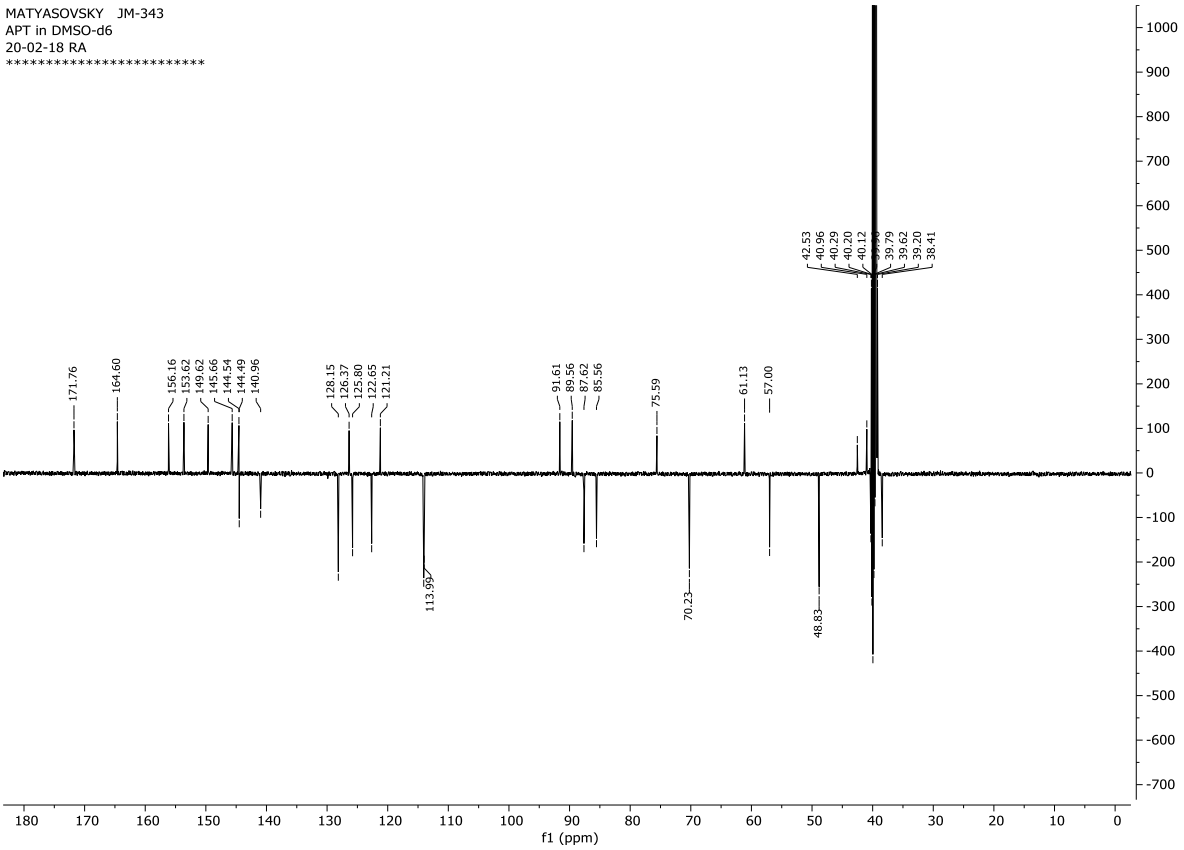


JM-343

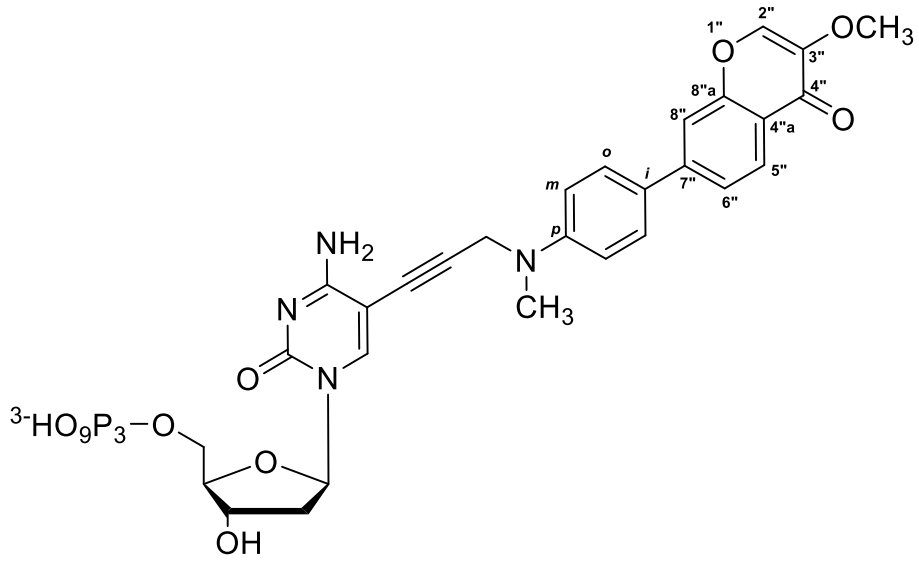
^{1}H NMR (500.0 MHz, DMSO-d_6): 1.95 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.9, $J_{2'b,3'}$ = 6.1, H-2′b); 2.10 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.6, H-2′a); 3.02 (s, 3H, CH_3N); 3.52, 3.58 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.2, $J_{5',4'}$ = 3.6, H-5′); 3.74 (s, 3H, CH_3O); 3.76 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.6, H-4′); 4.17 (ddt, 1H, $J_{3',2'}$ = 6.2, 3.6, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.6, H-3′); 4.44 (s, 2H, CH_2N); 5.06 (t, 1H, $J_{OH,5'}$ = 5.2, OH-5′); 5.20 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3′); 6.07 (dd, 1H, $J_{1',2'}$ = 6.8, 6.2, H-1′); 6.70 (bs, 1H, NH_a**H_b**); 6.99 – 7.03 (m, 2H, H-*m*-phenylene); 7.71 – 7.75 (m, 2H, H-*o*-phenylene); 7.75 (dd, 1H, $J_{6'',5''}$ = 8.5, $J_{6'',8''}$ = 1.7, H-6″); 7.79 (bs, 1H, N**H_a**H_b); 7.84 (d, 1H, $J_{8'',6''}$ = 1.7, H-8″); 8.08 (d, 1H, $J_{5'',6''}$ = 8.5, H-5″); 8.11 (s, 1H, H-6); 8.30 (s, 1H, H-2″).

^{13}C NMR (125.7 MHz, DMSO-d_6): 38.41 (CH_3N); 40.96 (CH_2-2′); 42.53 (CH_2N); 57.00 (CH_3O); 61.13 (CH_2-5′); 70.23 (CH-3′); 75.59 (CH_2-C≡**C**-C5); 85.56 (CH-1′); 87.62 (CH-4′); 89.56 (C-5); 91.61 (CH_2-**C**≡C-C5); 113.99 (CH-8″); 114.06 (CH-*m*-phenylene); 121.21 (C-4″a); 122.65 (CH-6″); 125.80 (CH-5″); 126.37 (C-*i*-phenylene); 128.15 (CH-*o*-phenylene); 140.96 (CH-2″); 144.49 (CH-6); 144.54 (C-3″); 145.66 (C-7″); 149.62 (C-*p*-phenylene); 153.62 (C-2); 156.16 (C-8″a); 164.60 (C-4); 171.76 (C-4″).





Compound dC^{AC}TP



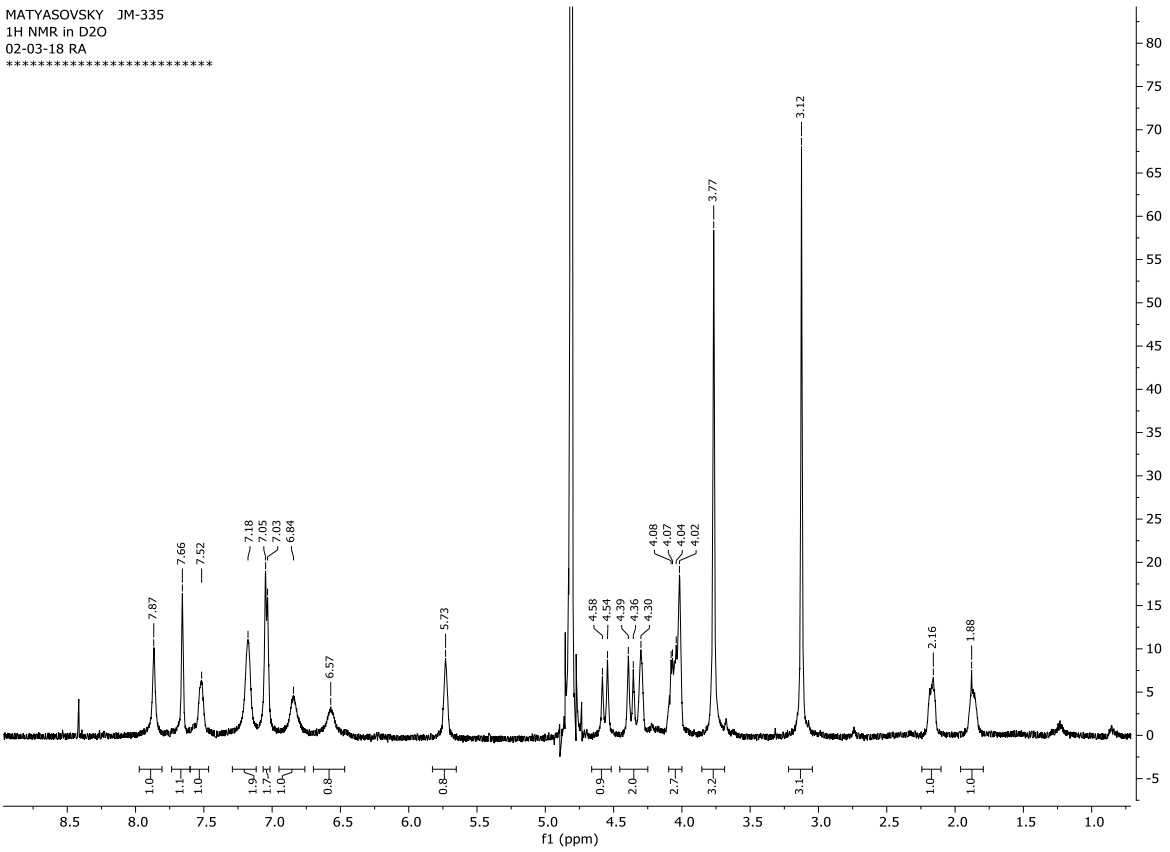
JM-335

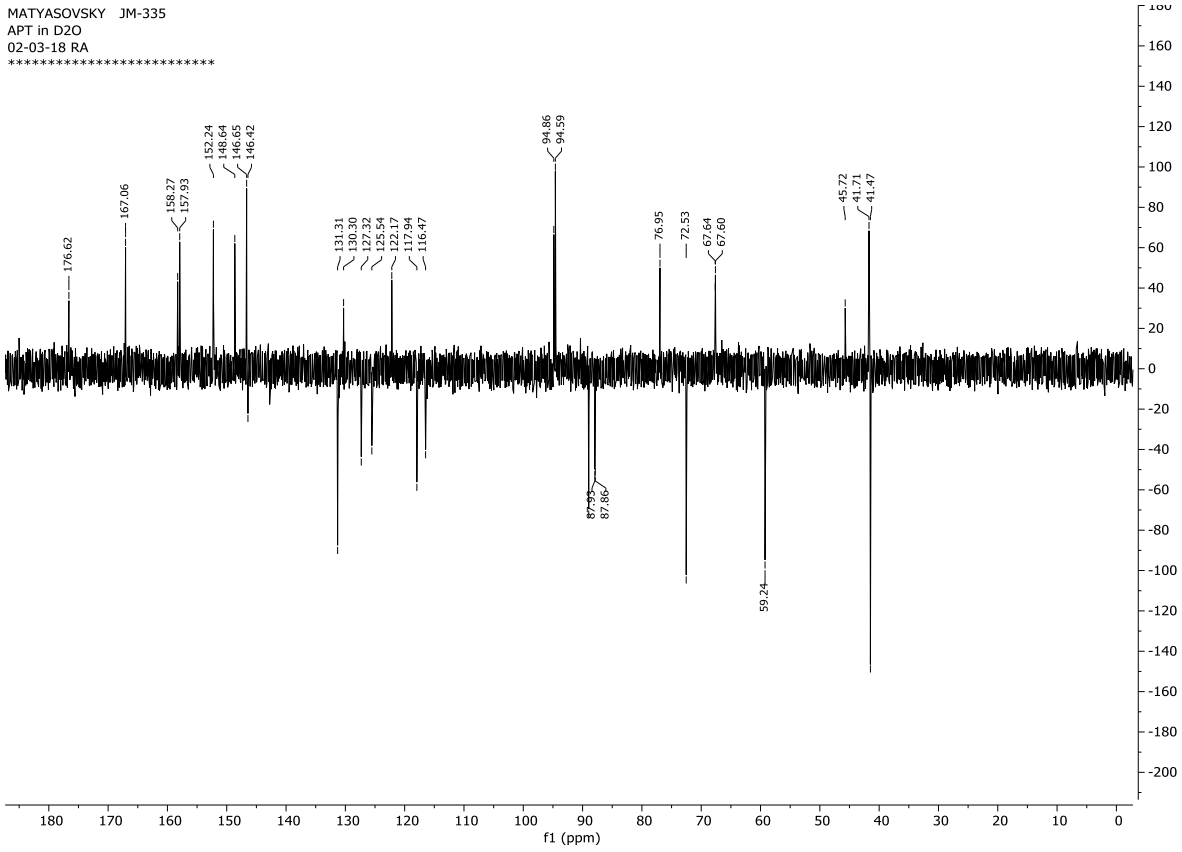
^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 1.87 (bm, 1H, H-2′b); 2.17 (bm, 1H, H-2′a); 3.12 (bs, 3H, CH_3N); 3.77 (bs, 3H, CH_3O); 3.98 – 4.11 (bm, 3H, H-4′,5′); 4.30 (bm, 1H, H-3′); 4.37, 4.56 (2 × d, 2 × 1H, J_{gem} = 18.5, CH_2N); 5.73 (bm, 1H, H-1′); 6.57 (bm, 1H, H-8″); 6.84

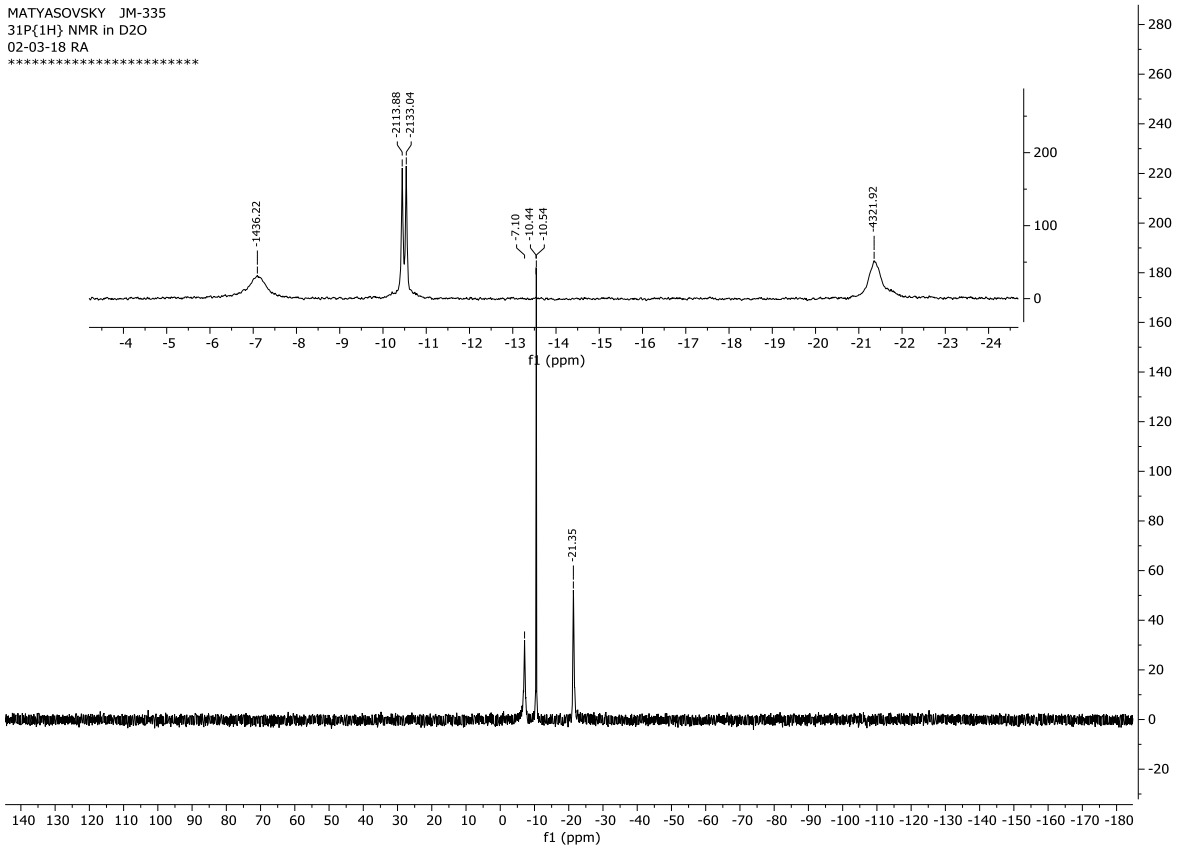
(bm, 1H, H-6″); 7.01 – 7.08 (bm, 2H, H-*m*-phenylene); 7.13 – 7.22 (bm, 2H, H-*o*-phenylene); 7.52 (bm, 1H, H-5″); 7.66 (s, 1H, H-6); 7.87 (bs, 1H, H-2″).

^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 41.47 (CH_3N); 41.71 (CH_2-2′); 45.72 (CH_2N); 59.24 (CH_3O); 67.62 (d, $J_{C,P}$ = 4.4, CH_2-5′); 72.53 (CH-3′); 76.95 (CH_2-C≡**C**-C5); 87.90 (d, $J_{C,P}$ = 8.5, CH-4′); 88.97 (CH-1′); 94.59, 94.86 (C-5, CH_2-**C**≡C-C5); 116.47 (CH-8″); 117.94 (CH-*m*-phenylene); 122.17 (C-4″a); 125.54 (CH-6″); 127.32 (CH-5″); 130.30 (C-*i*-phenylene); 131.31 (CH-*o*-phenylene); 142.71 (CH-2″); 146.42 (CH-6); 146.65 (C-3″); 148.64 (C-7″); 152.24 (C-*p*-phenylene); 157.93 (C-2); 158.27 (C-8″a); 167.06 (C-4); 176.62 (C-4″).

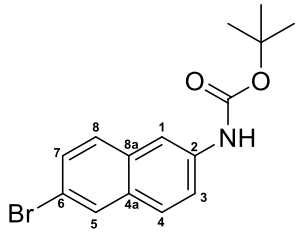
^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.35 (bs, P_β); -10.49 (d, J = 19.2, P_α); -7.10 (bs, P_γ).




MATYASOVSKY JM-335
APT in D2O
02-03-18 RA
****
176.62
167.06
158.27
157.93
152.24
148.64
146.65
146.42
131.31
130.30
127.32
125.54
122.17
117.94
116.47
94.86
94.59
87.93
87.86
76.95
72.53
67.64
67.60
59.24
45.72
41.71
41.47
f1 (ppm)


MATYASOVSKY JM-335
31P{1H} NMR in D2O
02-03-18 RA
****
-2113.88
-2133.04
-1436.22
-7.10
-10.44
-10.54
-4321.92
-21.35
f1 (ppm)

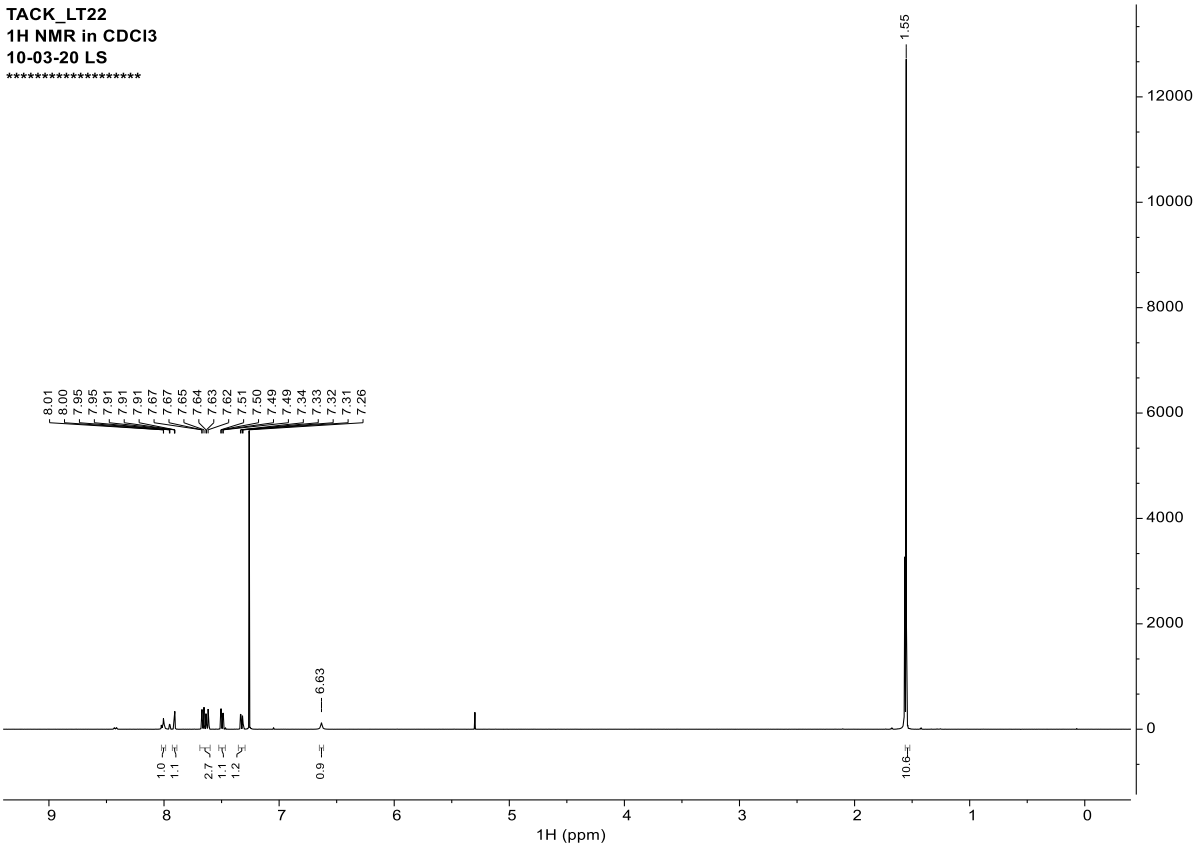
Compound 8

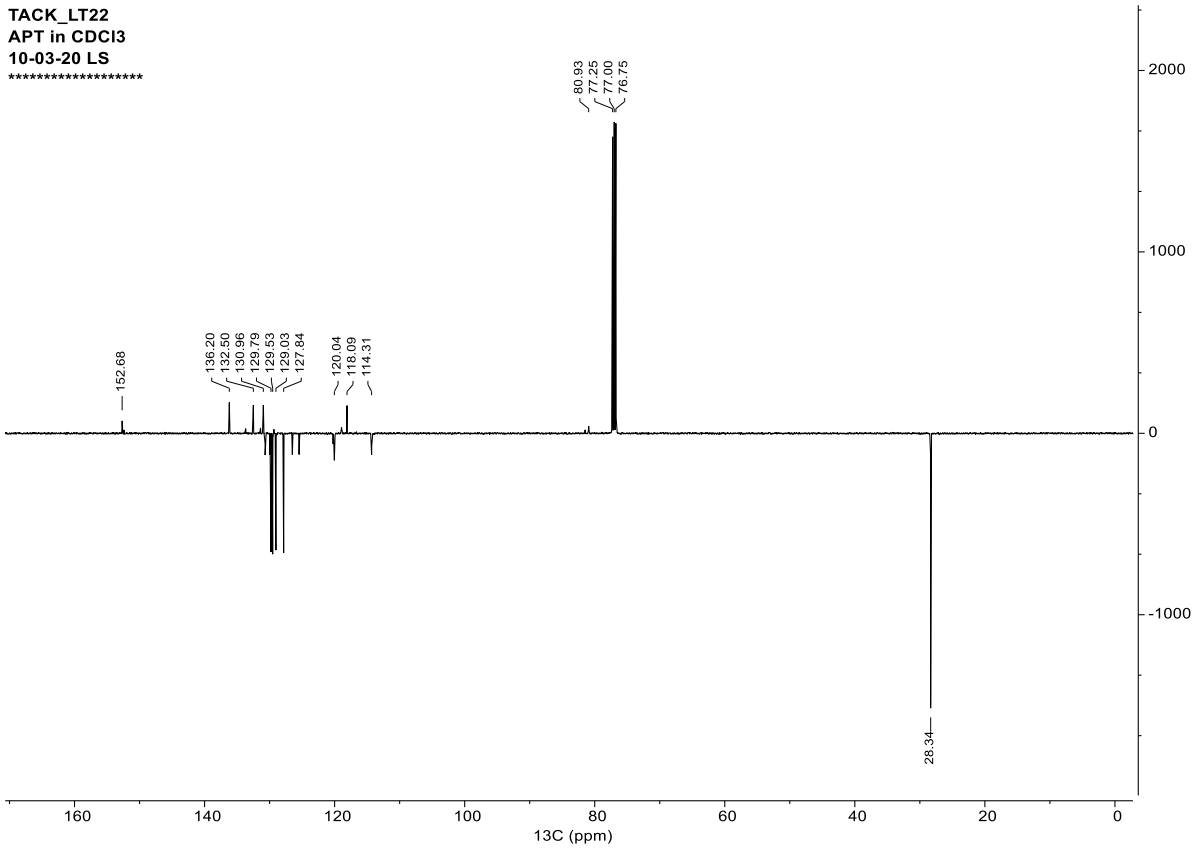


LT22

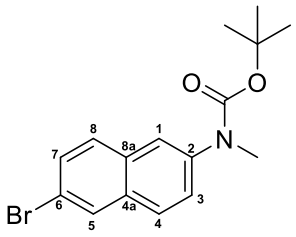
^{1}H NMR (500.0 MHz, $CDCl_3$): 1.55 (s, 9H, $(CH_3)_3C$); 6.63 (bs, 1H NH); 7.32 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.2, H-3); 7.50 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.0, H-7); 7.63 (d, 1H, $J_{8,7}$ = 8.8, H-8); 7.66 (d, 1H, $J_{4,3}$ = 8.8, H-4); 7.91 (d, 1H, $J_{5,7}$ = 2.0, H-5); 8.00 (bm, 1H, H-1).

^{13}C NMR (125.7 MHz, $CDCl_3$): 28.34 ((**C**$H_3)_3$C); 80.93 ($(CH_3)_3$**C**); 114.31 (CH-1); 118.09 (C-6); 120.04 (CH-3); 127.84 (CH-4); 129.03 (CH-8); 129.53 (CH-5); 129.79 (CH-7); 130.96 (C-4a); 132.50 (C-8a); 136.20 (C-2); 152.68 (NCOO).





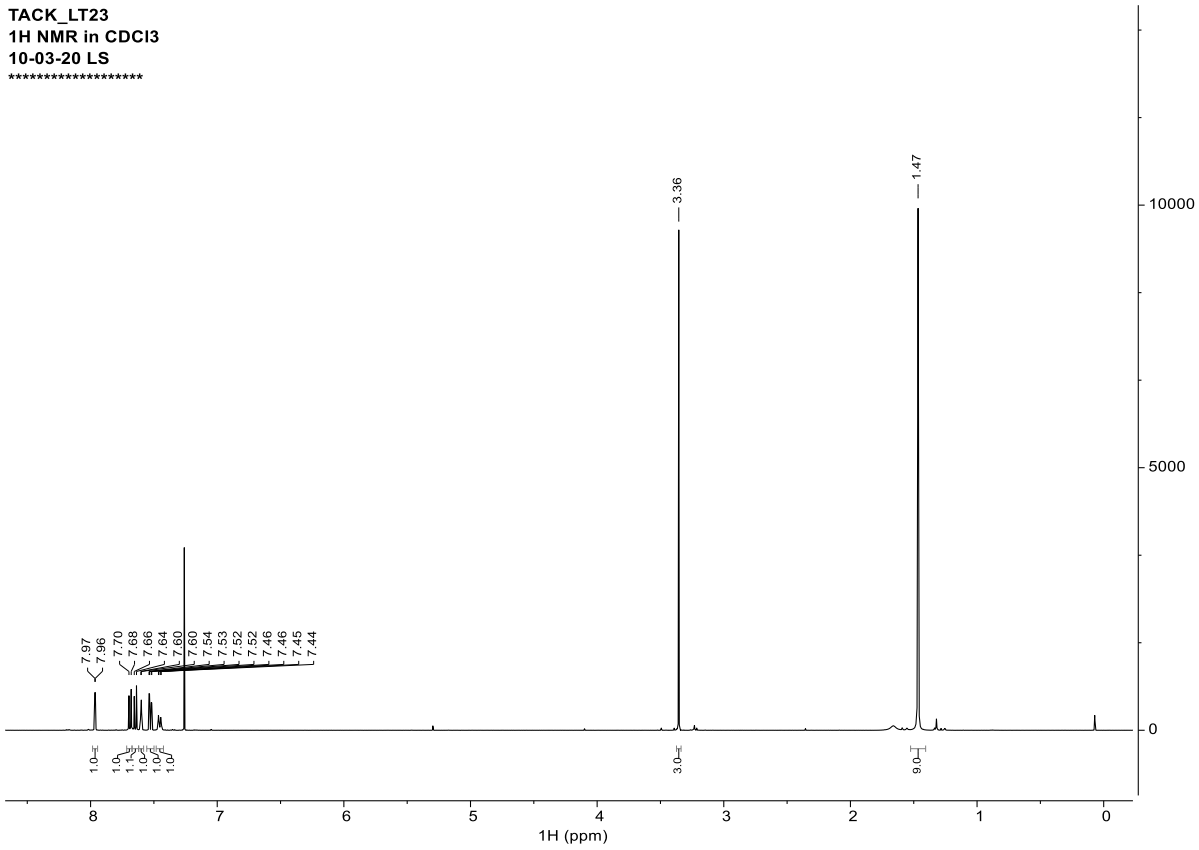
Compound 9

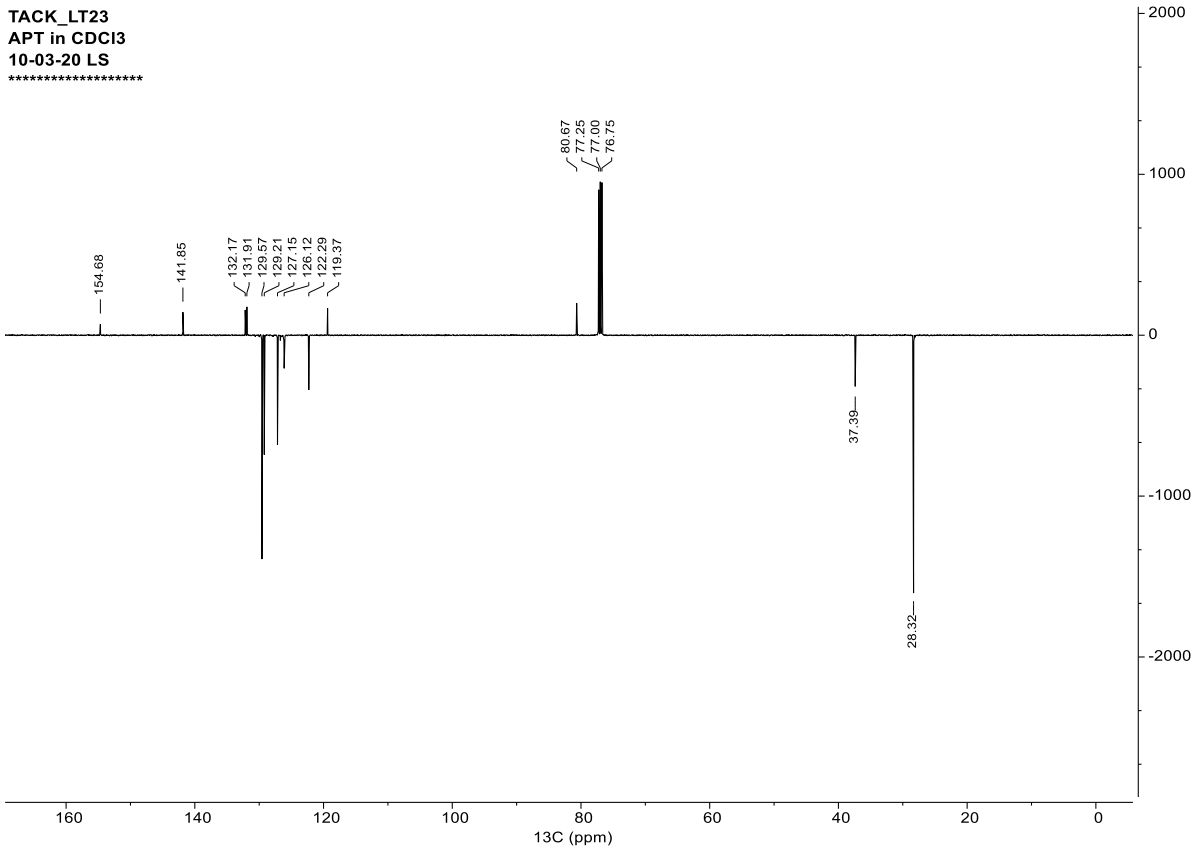


LT23

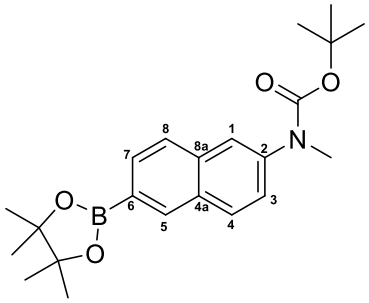
^{1}H NMR (500.0 MHz, $CDCl_3$): 1.46 (s, 9H, $(CH_3)_3C$); 3.36 (s, 3H, CH_3N); 7.45 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.2, H-3); 7.53 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 1.9, H-7); 7.60 (d, 1H, $J_{1,3}$ = 2.2, H-1); 7.65 (d, 1H, $J_{8,7}$ = 8.8, H-8); 7.69 (d, 1H, $J_{4,3}$ = 8.8, H-4); 7.96 (d, 1H, $J_{5,7}$ = 1.9, H-5).

^{13}C NMR (125.7 MHz, $CDCl_3$): 28.32 (($\mathbf{C}H_3)_3C$); 37.39 (CH_3N); 80.67 ($(CH_3)_3\mathbf{C}$); 119.37 (C-6); 122.29 (CH-1); 126.12 (CH-3); 127.15 (CH-4); 129.21 (CH-8); 129.57 (CH-5,7); 131.91 (C-8a); 132.17 (C-4a); 141.85 (C-2); 154.68 (NCOO).


TACK_LT23
1H NMR in CDCl3
10-03-20 LS
****
7.97
7.96
7.70
7.68
7.66
7.64
7.60
7.60
7.54
7.53
7.52
7.52
7.46
7.46
7.45
7.44
3.36
1.47
1.0
1.0
1.1
1.0
1.0
1.0
3.0
9.0
10000
5000
0
8
7
6
5
4
3
2
1
0
1H (ppm)


TACK_LT23
APT in CDCl3
10-03-20 LS
****
154.68
141.85
132.17
131.91
129.57
129.21
127.15
126.12
122.29
119.37
80.67
77.25
77.00
76.75
37.39
28.32
2000
1000
0
-1000
-2000
160
140
120
100
80
60
40
20
0
13C (ppm)

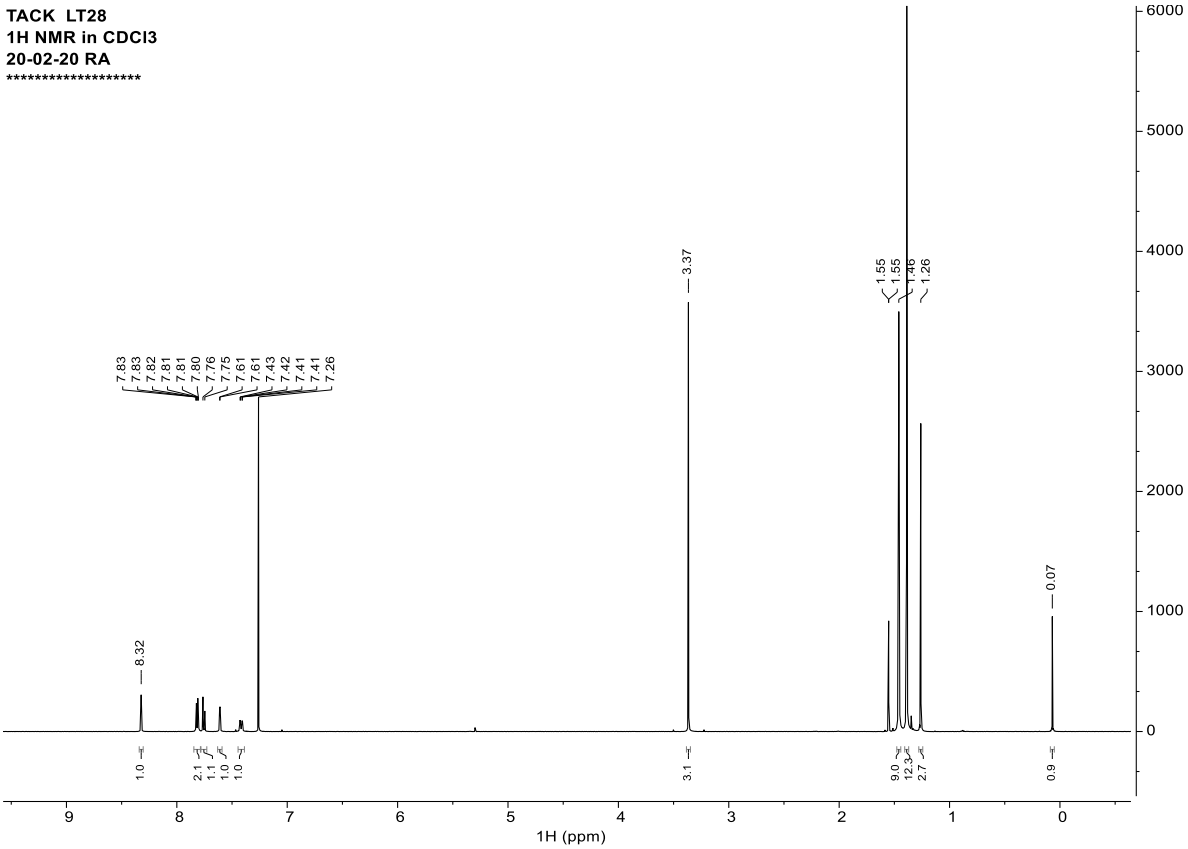
Compound 10

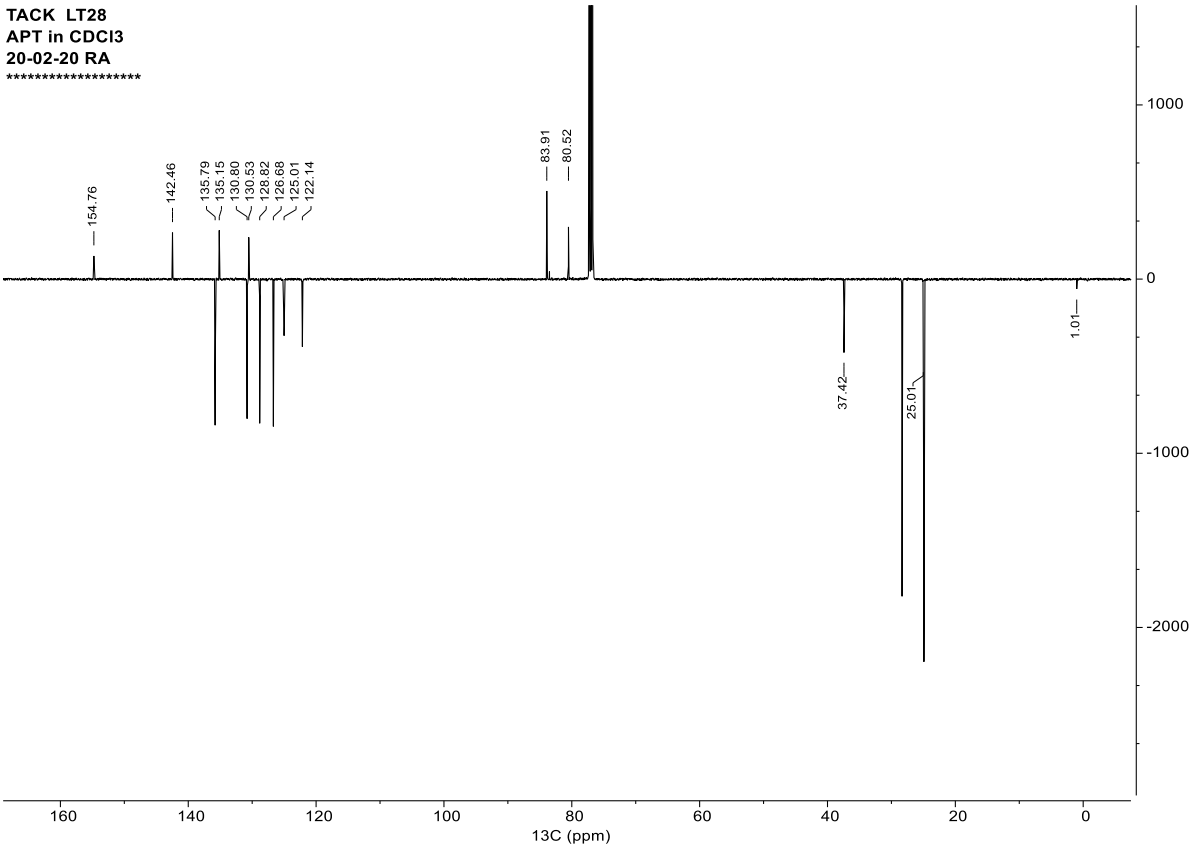


LT28

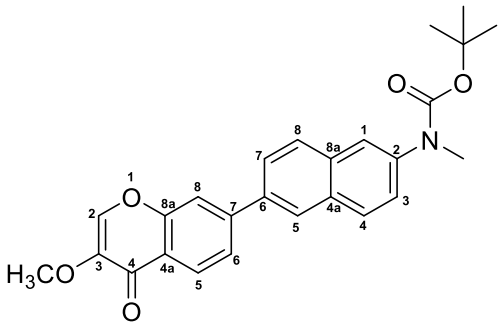
^{1}H NMR (500.0 MHz, $CDCl_3$): 1.39 (s, 12H, CH_3-piacolboronate); 1.46 (s, 9H, $(CH_3)_3C$); 3.37 (s, 3H, CH_3N); 7.42 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.2, H-3); 7.61 (d, 1H, $J_{1,3}$ = 2.2, H-1); 7.75 (d, 1H, $J_{8,7}$ = 8.4, H-8); 7.81 (d, 1H, $J_{4,3}$ = 8.8, H-4); 7.82 (dd, 1H, $J_{7,8}$ = 8.4, $J_{7,5}$ = 1.1, H-7); 8.32 (d, 1H, $J_{5,7}$ = 1.1, H-5).

^{13}C NMR (125.7 MHz, $CDCl_3$): 24.91 (CH_3-pinacolboronate); 28.33 (($\mathbf{C}H_3)_3C$); 37.42 (CH_3N); 80.52 ($(CH_3)_3\mathbf{C}$); 83.91 (C-pinacolboronate); 122.14 (CH-1); 125.01 (CH-3); 125.75 (C-6); 126.68 (CH-8); 128.82 (CH-4); 130.53 (C-4a); 130.80 (CH-7); 135.15 (C-8a); 135.79 (CH-5); 142.46 (C-2); 154.76 (NCOO).





Compound 11

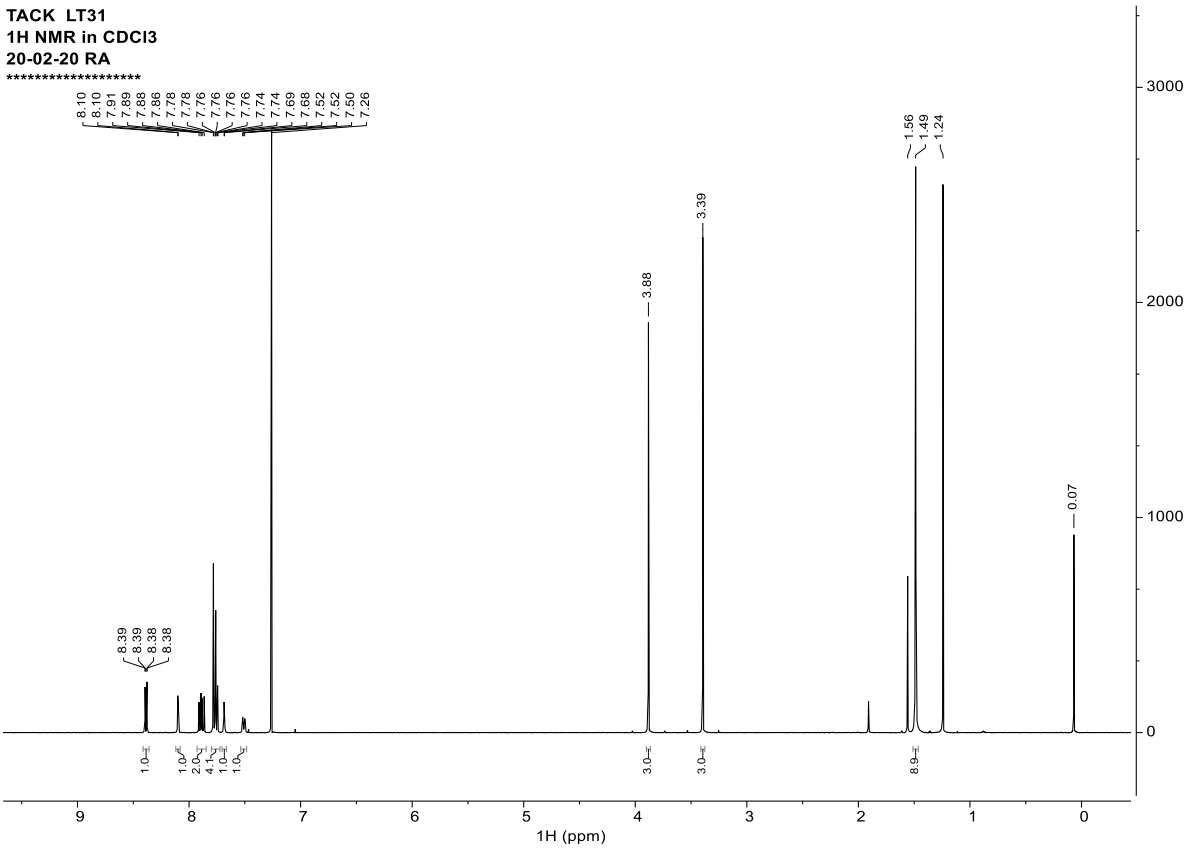


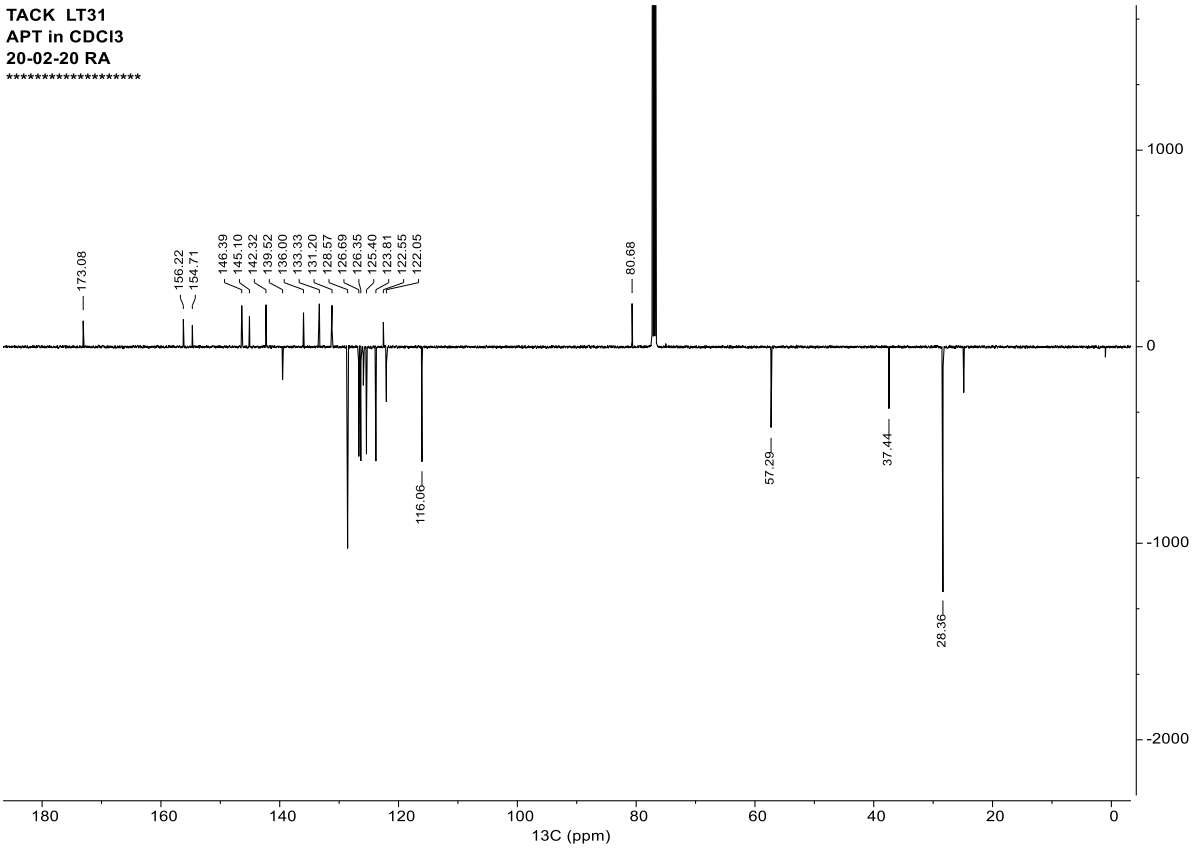
LT31

^{1}H NMR (500.0 MHz, $CDCl_3$): 1.49 (s, 9H, $(CH_3)_3C$); 3.39 (s, 3H, CH_3N); 3.88 (s, 3H, CH_3O); 7.51 (dd, 1H, $J_{3,4}$ = 8.7, $J_{3,1}$ = 2.2, H-3-naphth); 7.68 (d, 1H, $J_{1,3}$ = 2.2, H-1-naphth); 7.74 – 7.78 (m, 3H, H-6,8-chromene, H-7-naphth); 7.78 (s, 1H, H-2-chromene); 7.87 (d, 1H, $J_{4,3}$ = 8.7, H-4-naphth); 7.90 (d, 1H, $J_{8,7}$ = 8.9, H-8-naphth); 8.10 (d, 1H, $J_{5,7}$ = 2.1, H-5-naphth); 8.39 (d, 1H, $J_{5,6}$ = 8.1, $J_{5,8}$ = 0.7, H-5-chromene).

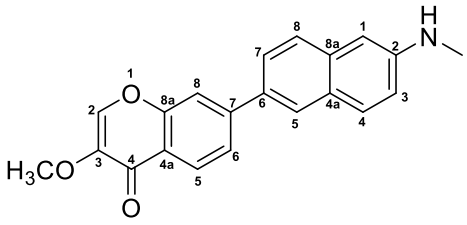
^{13}C NMR (125.7 MHz, $CDCl_3$): 28.36 (($\mathbf{C}H_3)_3C$); 37.44 (CH_3N); 57.29 (CH_3O); 80.68 ($(CH_3)_3\mathbf{C}$); 116.06 (CH-8-chromene); 122.05 (CH-1-naphth); 122.55 (C-4a-chromene); 123.81 (CH-6-chromene); 125.40 (CH-7-naphth); 125.92 (CH-3-naphth); 126.35 (CH-5-naphth); 126.69 (CH-5-

chromene); 128.57 (CH-4,8-naphth); 131.20 (C-4a-naphth); 133.33 (C-8a-naphth); 136.00 (C-6-naphth); 139.52 (CH-2-chromene); 142.32 (C-2-naphth); 145.10 (C-3-chromene); 146.39 (C-7-chromene); 154.71 (NCOO); 156.22 (C-8a-chromene); 173.08 (C-4-chromene).





Compound 12

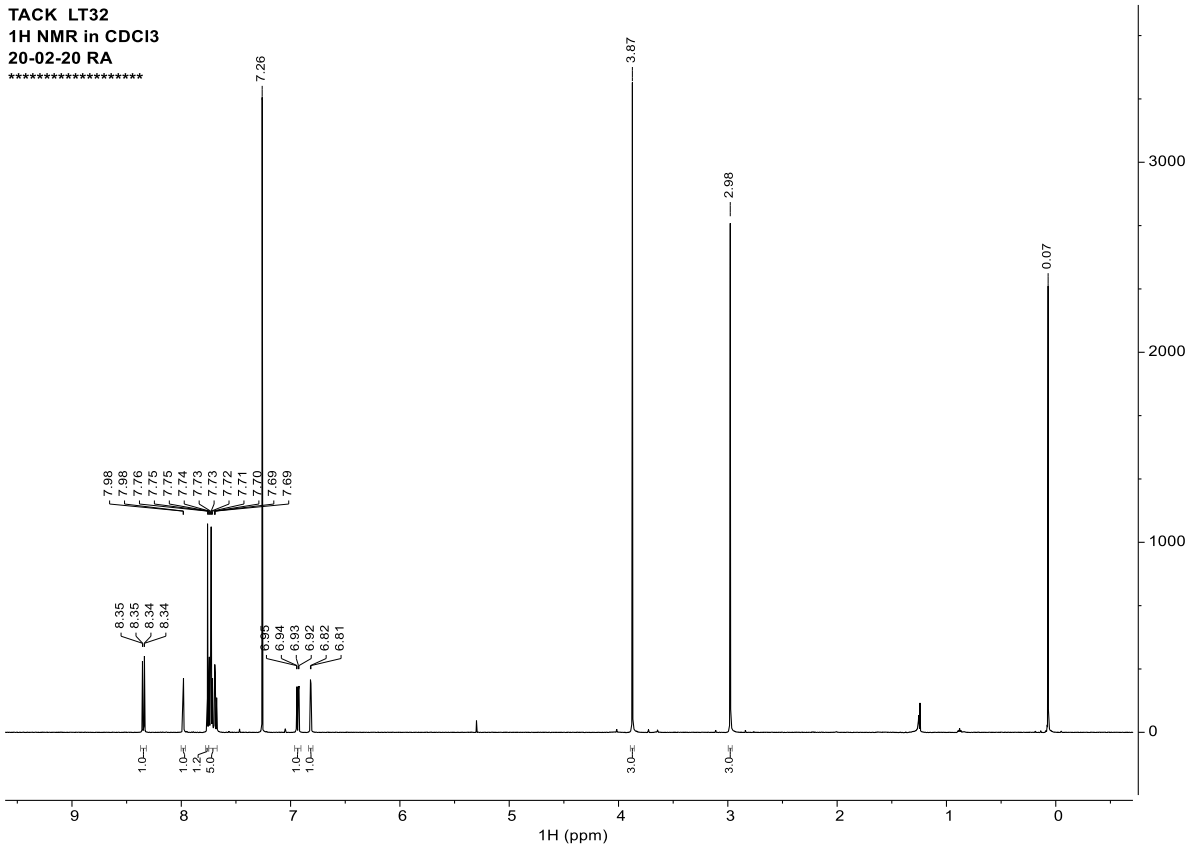


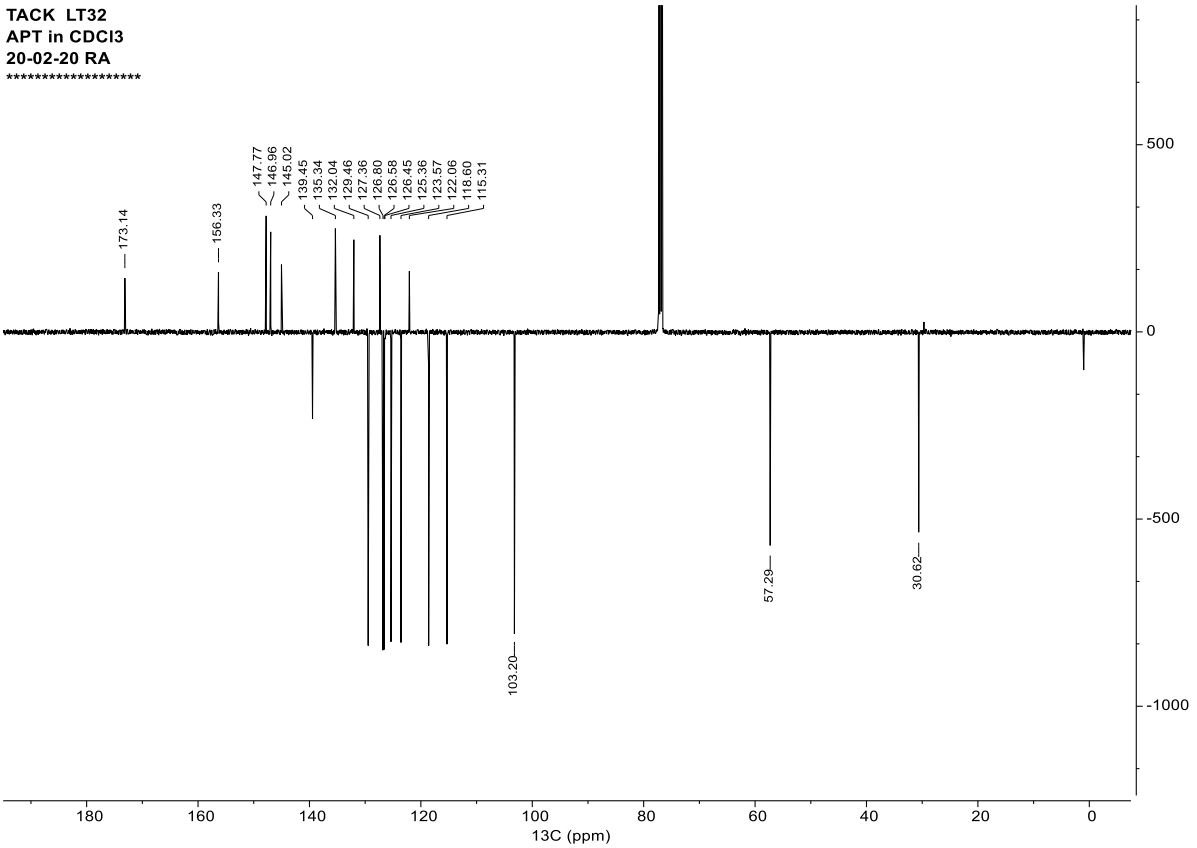
LT32

^{1}H NMR (500.0 MHz, $CDCl_3$): 2.98 (s, 3H, CH_3N); 3.87 (s, 3H, CH_3O); 6.82 (d, 1H, $J_{1,3}$ = 2.4, H-1-naphth); 6.93 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,1}$ = 2.4, H-3-naphth); 7.67 – 7.75 (m, 5H, H-6,8-chromene, H-4,7,8-naphth); 7.76 (s, 1H, H-2-chromene); 7.98 (d, 1H, $J_{5,7}$ = 2.1, H-5-naphth); 8.35 (d, 1H, $J_{5,6}$ = 8.0, $J_{5,8}$ = 0.9, H-5-chromene).

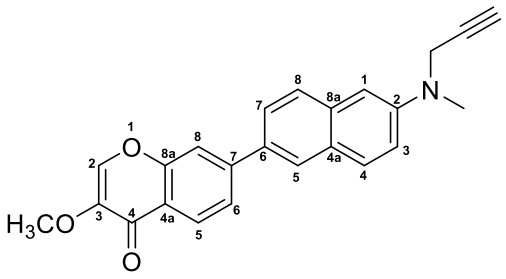
^{13}C NMR (125.7 MHz, $CDCl_3$): 30.62 (CH_3N); 57.29 (CH_3O); 103.20 (CH-1-naphth); 115.31 (CH-8-chromene); 118.60 (CH-3-naphth); 122.06 (C-4a-chromene); 123.57 (CH-6-chromene); 125.36 (CH-7-naphth); 126.45 (CH-5-chromene); 126.58 (CH-5-naphth); 126.80 (CH-8-naphth);

127.36 (C-4a-naphth); 129.46 (CH-4-naphth); 132.04 (C-6-naphth); 135.34 (C-8a-naphth); 139.42 (CH-2-chromene); 145.02 (C-3-chromene); 146.96 (C-7-chromene); 147.77 (C-2-naphth); 156.33 (C-8a-chromene); 173.14 (C-4-chromene).





Compound 13

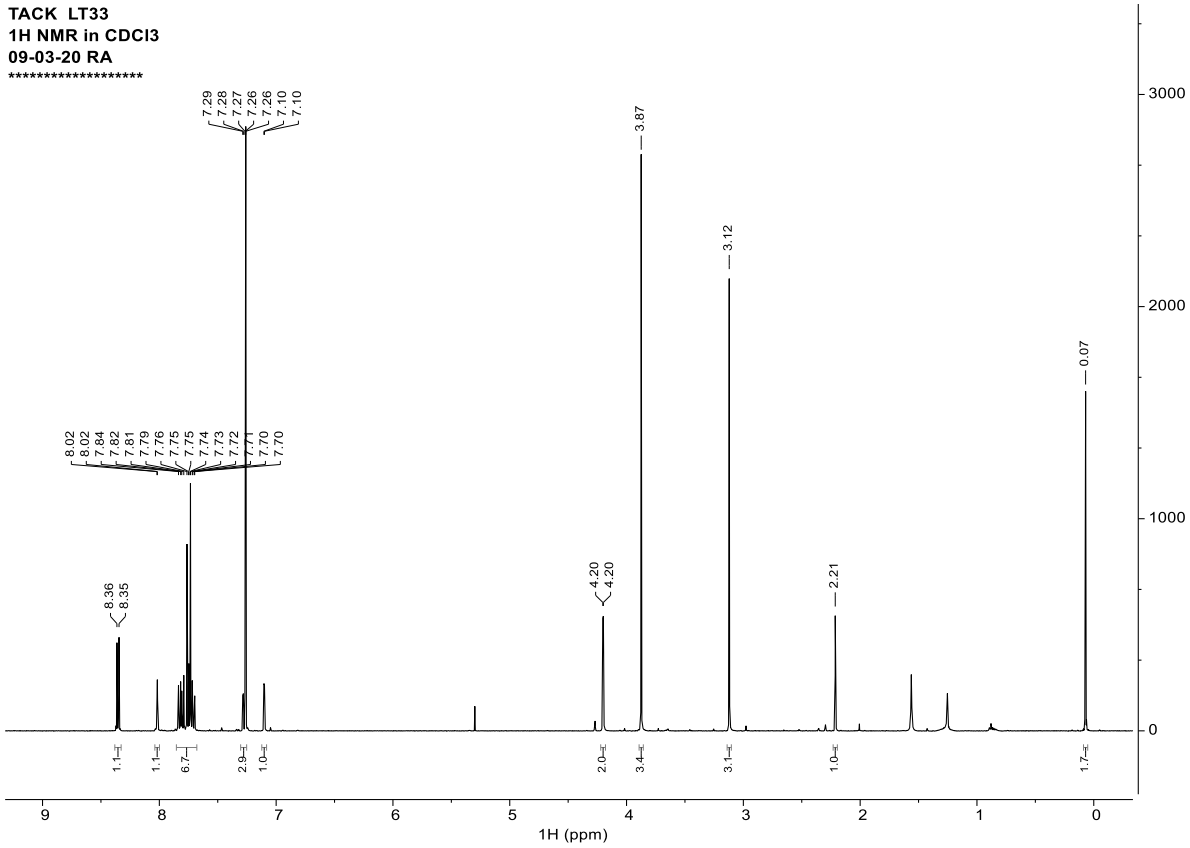


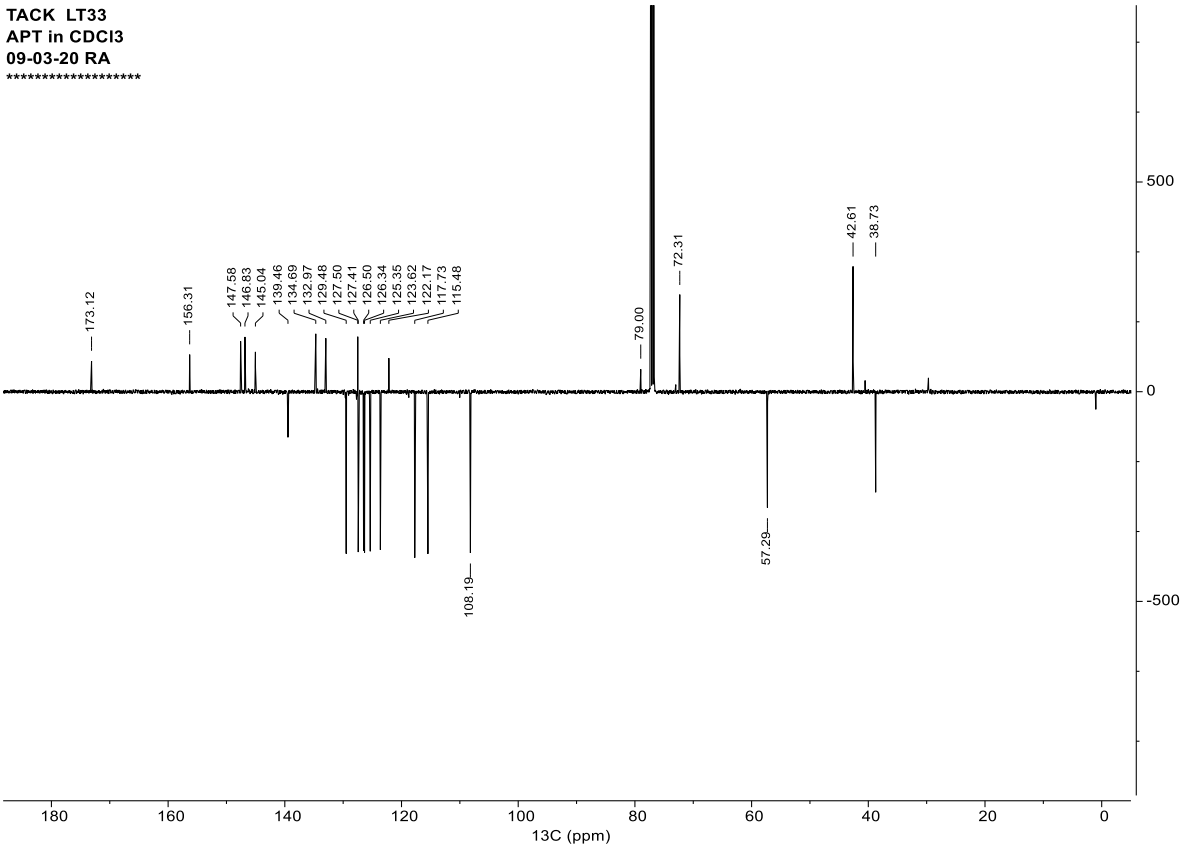
LT33

^{1}H NMR (500.0 MHz, $CDCl_3$): 2.22 (t, 1H, ^{4}J = 2.4, **H**C≡CCH_2); 3.12 (s, 3H, CH_3N); 3.87 (s, 3H, CH_3O); 4.20 (d, 2H, ^{4}J = 2.4, HC≡CC**H**$_2$); 7.10 (d, 1H, $J_{1,3}$ = 2.6, H-1-naphth); 7.27 (dd, 1H, $J_{3,4}$ = 9.0, $J_{3,1}$ = 2.6, H-3-naphth); 7.71 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.9, H-7-naphth); 7.73 – 7.75 (m, 2H, H-6,8-chromene); 7.76 (s, 1H, H-2-chromene); 7.80 (d, 1H, $J_{8,7}$ = 8.6, H-8-naphth); 7.83 (d, 1H, $J_{4,3}$ = 9.0, H-4-naphth); 8.02 (d, 1H, $J_{5,7}$ = 1.9, H-5-naphth); 8.35 (m, 1H, H-5-chromene).

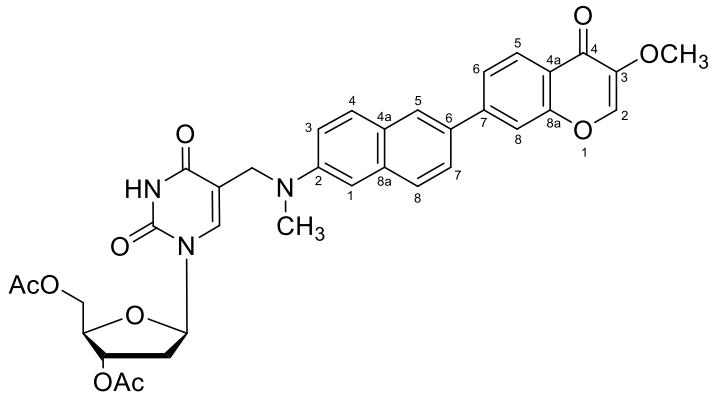
^{13}C NMR (125.7 MHz, $CDCl_3$): 38.73 (CH_3N); 42.61 (**C**H_2C≡CH); 57.29 (CH_3O); 72.31 (CH_2C≡**C**H); 79.00 (CH_2**C**≡CH); 108.19 (CH-1-naphth); 115.48 (CH-8-chromene); 117.73 (CH-3-naphth); 122.17 (C-4a-chromene); 123.62 (CH-6-chromene); 125.35 (CH-7-naphth); 126.34

(CH-5-naphth); 126.50 (CH-5-chromene); 127.41 (CH-8-naphth); 127.50 (C-4a-naphth); 129.48 (CH-4-naphth); 132.97 (C-6-naphth); 134.69 (C-8a-naphth); 139.46 (CH-2-chromene); 145.04 (C-3-chromene); 146.83 (C-7-chromene); 147.58 (C-2-naphth); 156.31 (C-8a-chromene); 173.12 (C-4-chromene).





Compound 15

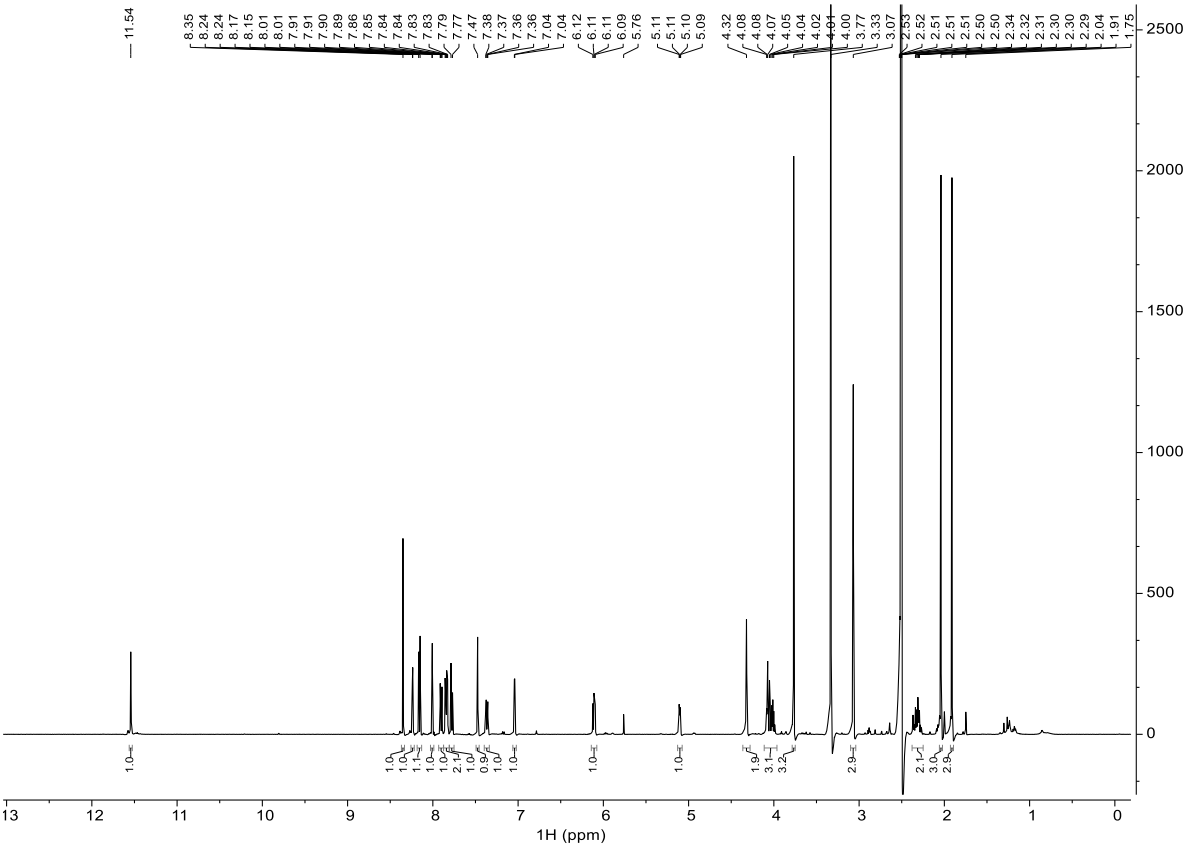


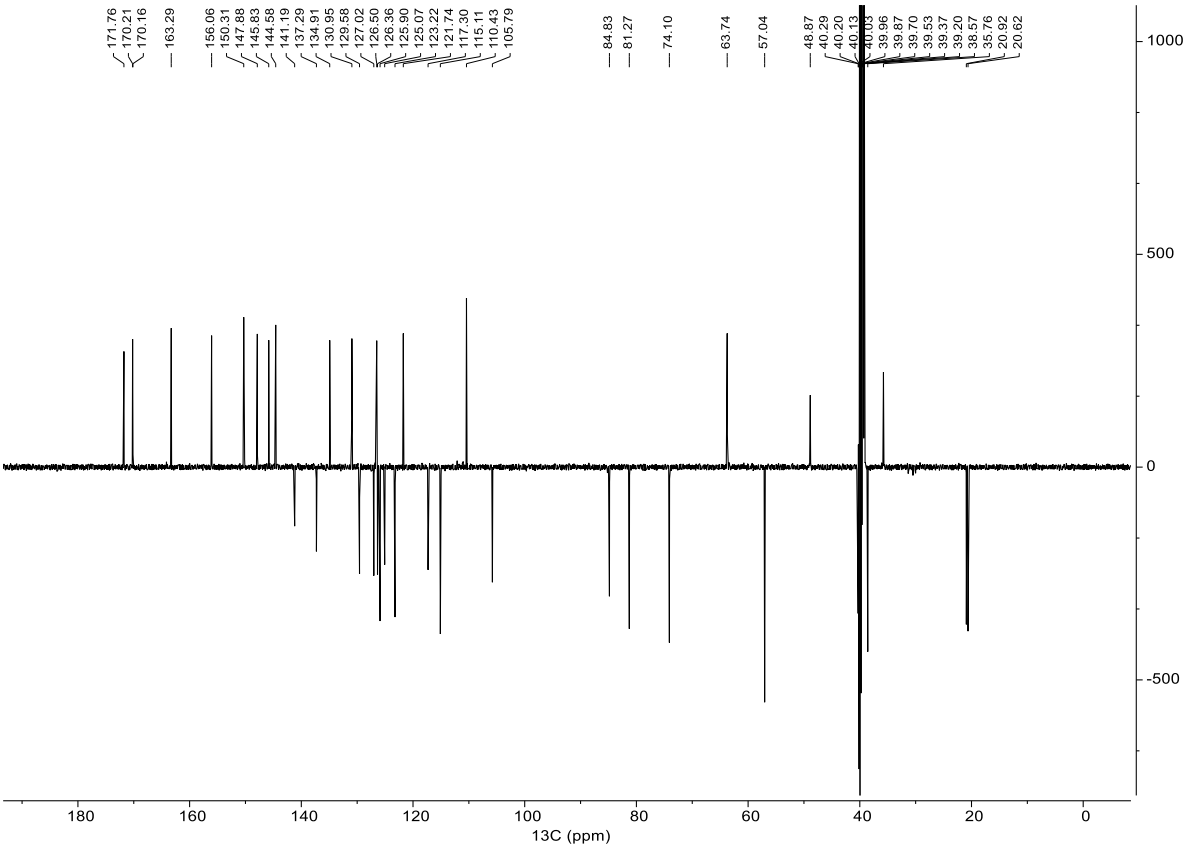
JM-631

^{1}H NMR (500.0 MHz, DMSO-d_6): 1.91, 2.04 (2 × s, 2 × 3H, CH_3CO); 2.29 (ddd, 1H, J_{gem} = 14.2, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 2.7, H-2′b); 2.34 (ddd, 1H, J_{gem} = 14.2, $J_{2'a,1'}$ = 8.1, $J_{2'a,3'}$ = 6.3, H-2′a); 3.07 (s, 3H, CH_3N); 3.77 (s, 3H, CH_3O); 3.97 – 4.11 (m, 3H, H-4′,5′); 4.32 (s, 2H, CH_2N); 5.11 (dt, 1H, $J_{3',2'}$ = 6.3, 2.7, $J_{3',4'}$ = 2.7, H-3′); 6.11 (dd, 1H, $J_{1',2'}$ = 8.1, 6.3, H-1′); 7.04 (d, 1H, $J_{1,3}$ = 2.6, H-1-

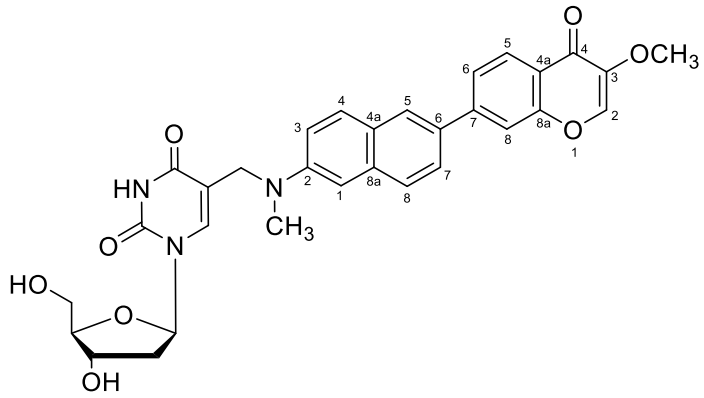
naphth); 7.37 (dd, 1H, $J_{3,4}$ = 9.2, $J_{3,1}$ = 2.6, H-3-naphth); 7.47 (s, 1H, H-6); 7.78 (d, 1H, $J_{8,7}$ = 8.9, H-8-naphth); 7.81 – 7.87 (m, 2H, H-4,7-naphth); 7.90 (dd, 1H, $J_{6,5}$ = 8.5, $J_{6,8}$ = 1.7, H-6-chromene); 8.01 (d, 1H, $J_{8,6}$ = 1.7, H-8-chromene); 8.16 (d, 2H, $J_{5,6}$ = 8.5, H-5-chromene); 8.24 (d, 1H, $J_{5,7}$ = 2.0, H-5-naphth); 8.35 (s, 1H, H-2-chromene); 11.54 (s, 1H, NH-3).

^{13}C NMR (125.7 MHz, DMSO-d_6): 20.62, 20.92 (**C**H_3CO); 35.76 (CH_2-2′); 38.57 (CH_3N); 48.87 (CH_2N); 57.04 (CH_3O); 63.74 (CH_2-5′); 74.10 (CH-3′); 81.27 (CH-4′); 84.83 (CH-1′); 105.79 (CH-1-naphth); 110.43 (C-5); 115.11 (CH-8-chromene); 117.30 (CH-3-naphth); 121.74 (C-4a-chromene); 123.22 (CH-6-chromene); 125.07 (CH-7-naphth); 125.90 (CH-5-chromene); 126.36 (CH-5-naphth); 126.50 (C-4a-naphth); 127.02 (CH-8-naphth); 129.58 (CH-4-naphth); 130.95 (C-6-naphth); 134.91 (C-8a-naphth); 137.29 (CH-6); 141.19 (CH-2-chromene); 144.58 (C-3-chromene); 145.83 (C-7-chromene); 147.88 (C-2-naphth); 150.31 (C-2); 156.06 (C-8a-chromene); 163.29 (C-4); 170.16, 170.21 (CH_3**C**O); 171.76 (C-4-chromene).





Compound 16

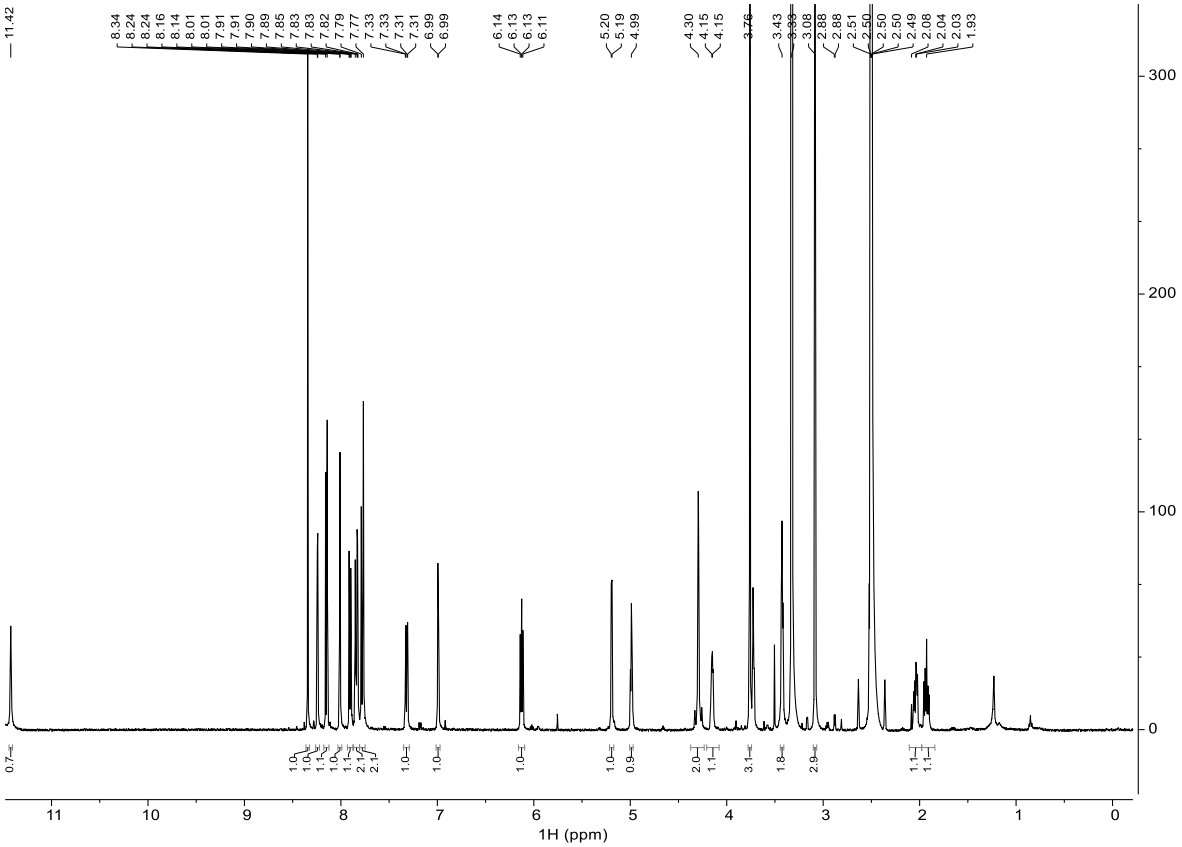


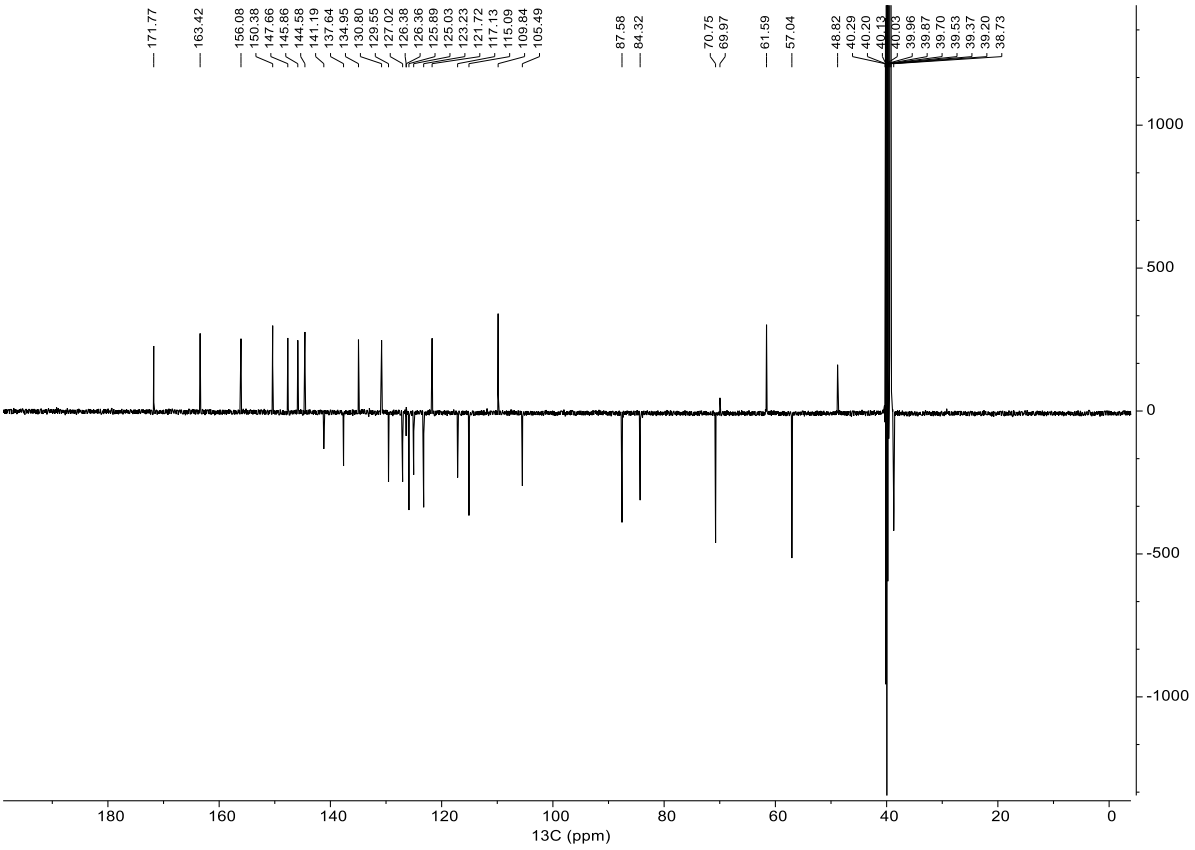
JM-632

^{1}H NMR (500.0 MHz, DMSO-d_6): 1.93 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.6, $J_{2'b,3'}$ = 5.8, H-2′b); 2.04 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.1, H-2′a); 3.08 (s, 3H, CH_3N); 3.43 (dd, 2H, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.9, H-5′); 3.72 (td, 1H, $J_{4',5'}$ = 3.9, $J_{4',3'}$ = 2.6, H-4′); 3.76 (s, 3H, CH_3O); 4.15 (dddd, 1H, $J_{3',2'}$ = 5.8, 3.1, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 2.6, H-3′); 4.28, 4.31 (2 × dd, 2 × 1H, J_{gem} = 17.3, ^{4}J = 1.0,

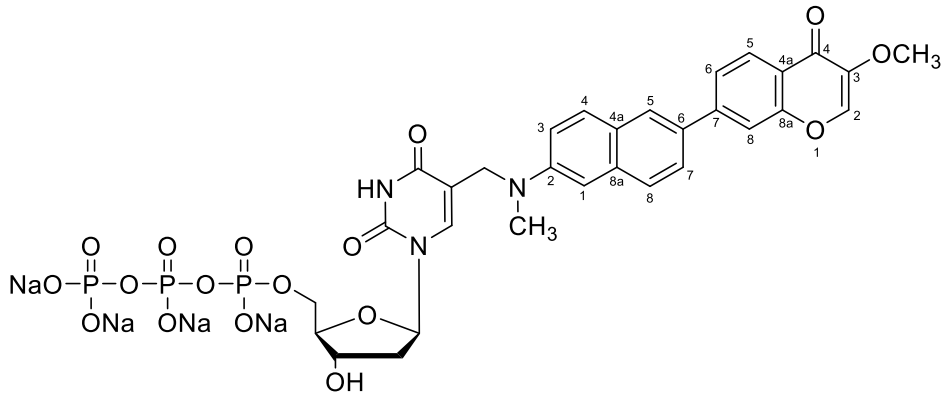
CH_2N); 4.99 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.19 (d, 1H, $J_{OH,3'}$ = 4.2, OH-3′); 6.13 (dd, 1H, $J_{1',2'}$ = 7.6, 6.1, H-1′); 6.99 (d, 1H, $J_{1,3}$ = 2.6, H-1-naphth); 7.32 (dd, 1H, $J_{3,4}$ = 9.2, $J_{3,1}$ = 2.6, H-3-naphth); 7.77 (t, 1H, ^4J = 1.0, H-6); 7.78 (d, 1H, $J_{8,7}$ = 8.8, H-8-naphth); 7.83 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.0, H-7-naphth); 7.84 (d, 1H, $J_{4,3}$ = 9.2, H-4-naphth); 7.90 (dd, 1H, $J_{6,5}$ = 8.5, $J_{6,8}$ = 1.7, H-6-chromene); 8.01 (d, 1H, $J_{8,6}$ = 1.7, H-8-chromene); 8.15 (d, 2H, $J_{5,6}$ = 8.5, H-5-chromene); 8.24 (d, 1H, $J_{5,7}$ = 2.0, H-5-naphth); 8.34 (s, 1H, H-2-chromene); 11.42 (s, 1H, NH-3).

^{13}C NMR (125.7 MHz, DMSO-d_6): 38.57 (CH_3N); 39.70 (CH_2-2′); 48.82 (CH_2N); 57.04 (CH_3O); 61.59 (CH_2-5′); 70.75 (CH-3′); 84.32 (CH-1′); 87.58 (CH-4′); 105.49 (CH-1-naphth); 109.84 (C-5); 115.09 (CH-8-chromene); 117.13 (CH-3-naphth); 121.72 (C-4a-chromene); 123.23 (CH-6-chromene); 125.03 (CH-7-naphth); 125.89 (CH-5-chromene); 126.36 (C-4a-naphth); 126.38 (CH-5-naphth); 127.02 (CH-8-naphth); 129.55 (CH-4-naphth); 130.80 (C-6-naphth); 134.95 (C-8a-naphth); 137.64 (CH-6); 141.19 (CH-2-chromene); 144.58 (C-3-chromene); 145.86 (C-7-chromene); 147.66 (C-2-naphth); 150.38 (C-2); 156.08 (C-8a-chromene); 163.42 (C-4); 171.77 (C-4-chromene).





Compound dT^{NC}TP



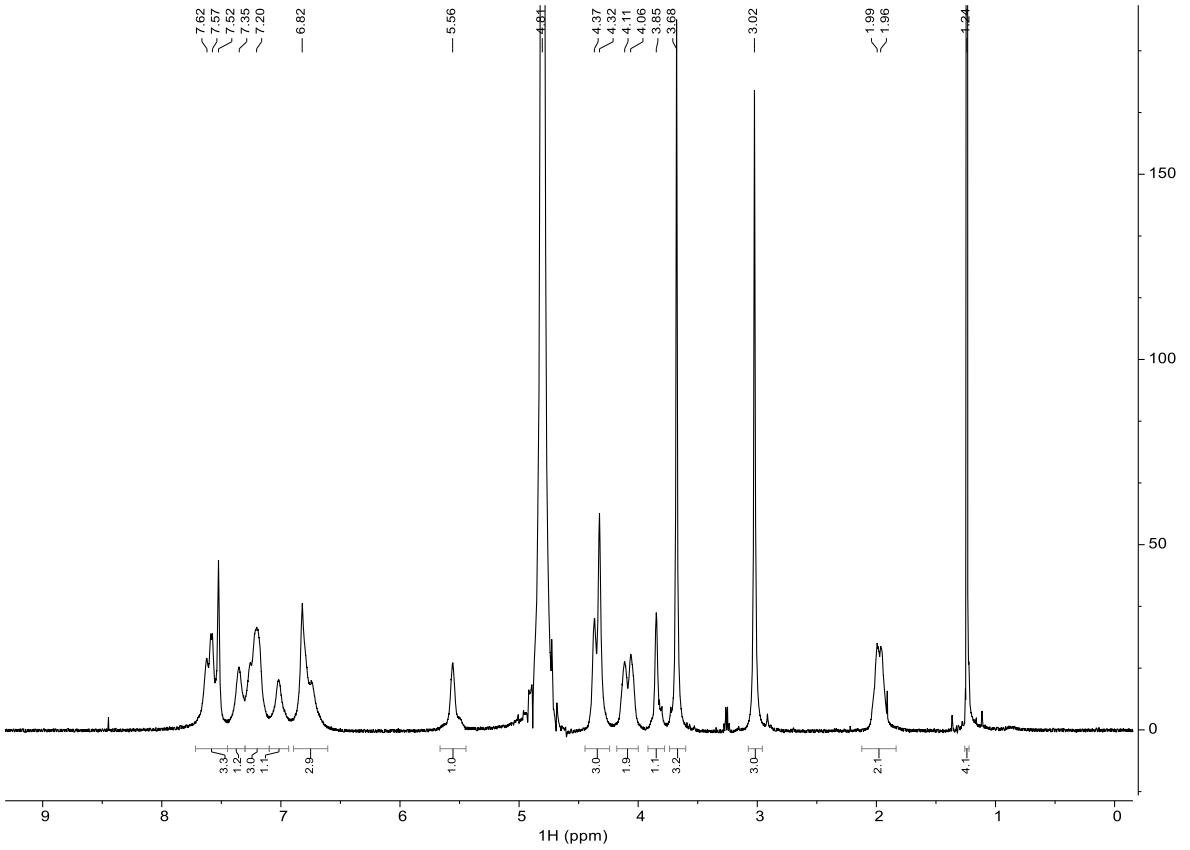
JM-633

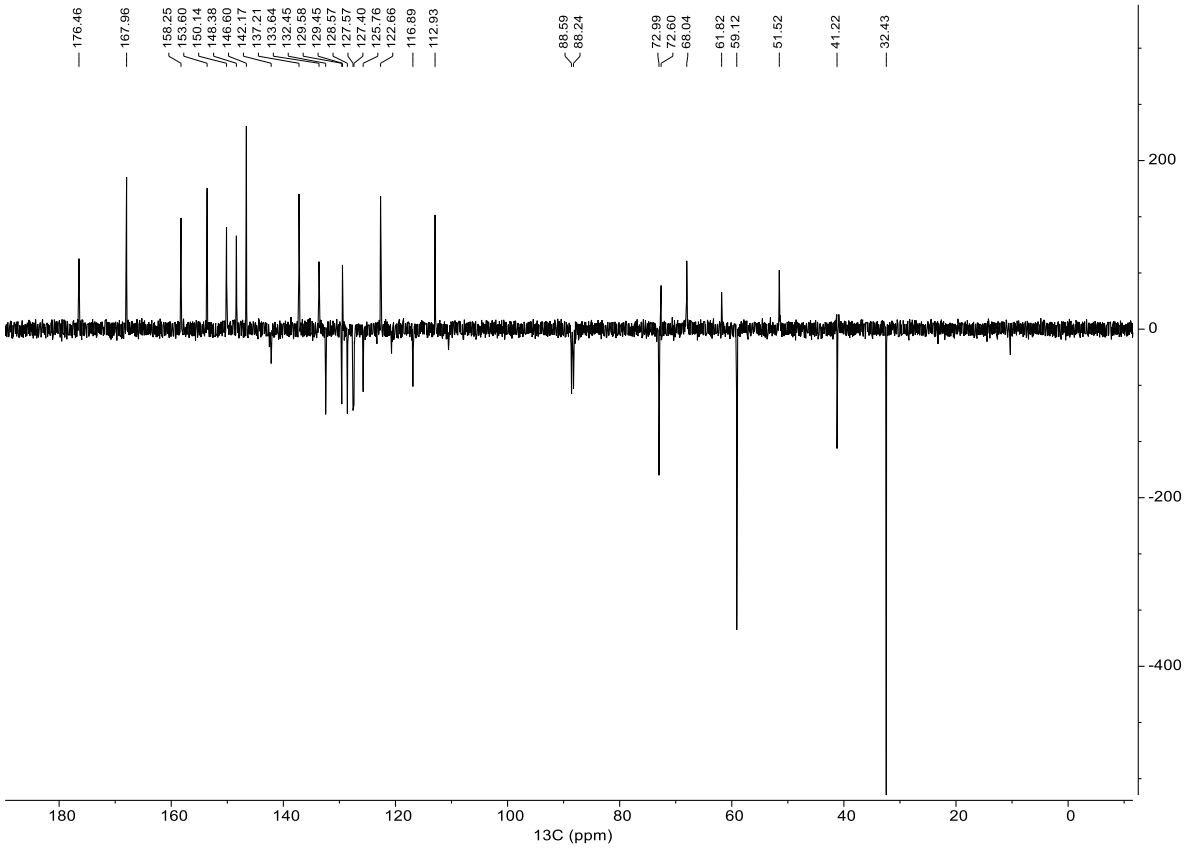
^{1}H NMR (500.0 MHz, D_2O, ref(*t*BuOH) = 1.24 ppm): 1.90 – 2.06 (bm, 2H, H-2′); 3.02 (bs, 3H, CH_3N); 3.68 (bs, 3H, CH_3O); 3.85 (bm, 1H, H-4′); 4.01 – 4.16 (bm, 2H, H-5′); 4.32 (bs, 2H, CH_2N); 4.37 (bm, 1H, H-3′); 5.56 (bm, 1H, H-1′); 6.66 – 6.89 (bm, 3H, H-1,7-naphth, H-8-chromene); 7.02 (bm, 1H, H-6-chromene); 7.11 – 7.32 (bm, 3H, H-3,5,8-naphth); 7.35 (bm, 1H,

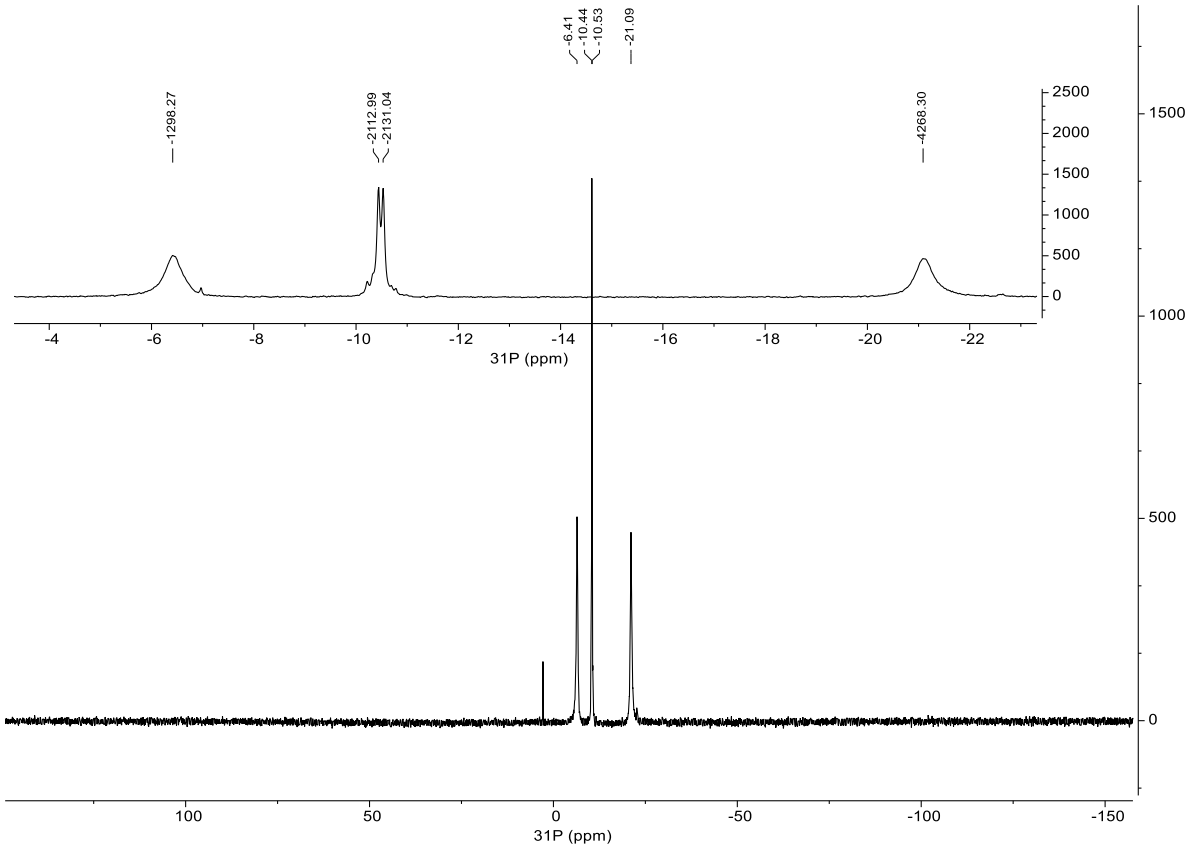
H-4-naphth); 7.52 (bs, 1H, H-6); 7.58 (bd, 1H, $J_{5,6}$ = 7.5, H-5-chromene); 7.62 (bs, 1H, H-2-chromene).

^{13}C NMR (125.7 MHz, D_2O, ref(*t*BuOH) = 32.43 ppm): 41.22 (CH_3N); 41.27 (CH_2-2′); 51.52 (CH_2N); 59.12 (CH_3O); 68.01 (d, $J_{C,P}$ = 5.7, CH_2-5′); 72.99 (CH-3′); 88.20 (bd, $J_{C,P}$ = 7.5, CH-4′); 88.59 (CH-1′); 110.55 (b, CH-1-naphth); 112.93 (C-5); 116.89 (CH-8-chromene); 120.70 (b, CH-3-naphth); 122.66 (C-4a-chromene); 125.76 (CH-6-chromene); 127.40 (CH-7-naphth); 127.57 (CH-5-chromene); 128.57 (CH-5-naphth); 129.45 (C-4a-naphth); 129.58 (CH-8-naphth); 132.45 (CH-4-naphth); 133.64 (C-6-naphth); 137.21 (C-8a-naphth); 142.17 (CH-6); 142.48 (b, CH-2-chromene); 146.60 (C-3-chromene); 148.38 (C-7-chromene); 150.14 (C-2-naphth); 153.60 (C-2); 158.25 (C-8a-chromene); 167.96 (C-4); 176.46 (C-4-chromene).

^{31}P{^{1}H} NMR (202.4 MHz, D_2O): -21.09 (bs, P_β); -10.48 (d, J = 18.0, P_α); -6.41 (bs, P_γ).


7.62
7.57
7.52
7.35
7.20
6.82
5.56
4.61
4.37
4.32
4.11
4.06
3.85
3.68
3.02
1.99
1.96
1.24
3.3
1.2
3.0
1.1
2.9
1.0
3.0
1.9
1.1
3.2
3.0
2.1
4.1
150
100
50
0
9
8
7
6
5
4
3
2
1
0
1H (ppm)


176.46
167.96
158.25
153.60
150.14
148.38
146.60
142.17
137.21
133.64
132.45
129.58
129.45
128.57
127.57
127.40
125.76
122.66
116.89
112.93
88.59
88.24
72.99
72.60
68.04
61.82
59.12
51.52
41.22
32.43
200
0
-200
-400
180
160
140
120
100
80
60
40
20
0
13C (ppm)


-6.41
-10.44
-10.53
-21.09
-1298.27
-2112.99
-2131.04
-4268.30
2500
2000
1500
1000
500
0
1500
1000
500
0
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
31P (ppm)
100
50
0
-50
-100
-150
31P (ppm)

References:

1. L. Giordano, V. V. Shvadchak, J. A. Fauerbach, E. A. Jares-Erijman, and T. M. Jovin, *J. Phys. Chem. Lett.,* 2012, **3**, 1011–1016.

2. L. H. Koole, M. H. P. Van Genderen, and H. M. Buck, *J. Am. Chem. Soc.*, 1987, **109**, 3916–3921.

3. A. M. Brouwer, *Pure Appl. Chem.,* 2011, **83**, 2213–2228.

4. C. Würth, M. Grabolle, J. Pauli, M. Spieles and U. Resch-Genger, *Nature Protocols,* 2013, **8**, 1535-1550.