
Enhancing siRNA efficacy in vivo with extended nucleic acid backbones

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Supporting Information

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■ General remarks

The NMR spectra were recorded using Bruker 500 MHz spectrometer. ^1H , ^{13}C , ^{19}F and ^{31}P NMR spectra were recorded at 500 MHz (^1H NMR, 500 MHz; ^{13}C -NMR, 126 MHz; ^{31}P -NMR, 202 MHz, and 470 MHz for ^{19}F) and analyzed by using MestReNova. The chemical shifts were measured from tetramethylsilane (0 ppm), CDCl_3 (7.26 ppm), $\text{DMSO-}d_6$ (2.49 ppm) and $\text{CD}_3\text{CN-}d_3$ (1.93 ppm) for ^1H -NMR spectra, CDCl_3 (77.0 ppm), $\text{DMSO-}d_6$ (39.7 ppm) and $\text{CD}_3\text{CN-}d_3$ (1.30 ppm) for ^{13}C -NMR spectra, and 85% H_3PO_4 for ^{31}P -NMR spectra as external standards. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis for the monomers and dimer nucleos(t)ides were performed on a Thermo Scientific Orbitrap Velos Pro mass spectrometer in the positive ion mode. The mass analysis of oligonucleotides was conducted by LC-MS on an Agilent 6530 accurate-mass Q-TOF LC/MS (Agilent technologies, Santa Clara, CA). Thin-layer chromatography (TLC) analysis was conducted using silica gel-coated aluminium-backed TLC plates (0.20 mm thickness) containing F-254 UV indicator (Silicycle Inc., Canada). Column chromatography was performed with silica column (Flash Column Silica-CS-Agela; 12-330 g; 40-60 μm), using the CombiFlash Rf200 (Teledyne Isco, Inc.) Companion Chromatograph. The synthesis of modified oligonucleotides was performed using MerMaid-12 DNA/RNA synthesizer (Bio automation, USA). Analytical anion-exchange HPLC and reverse phase HPLC were performed on Agilent 1260 Infinity Analytical SFC System combined with an Agilent 1100 series quaternary pump with a degasser. Purified oligonucleotides were desalted by Sephadex G-25 (GE Healthcare).

Synthesis of compound 2a.^{1,2} Anhydrous solution of 3'-*O*-TBDMS-2'-*O*-methyl-uridine (2.94 g, 7.89 mmol) in CH₃CN (80 mL) was added 2-iodoxybenzoic acid (IBX) (5.53 g, 19.7 mmol) and stirred for 2 h at 85 °C. After cooling the mixture in an ice bath, the precipitate in the solution was filtered off through celite. The collected filtrate was evaporated, and co-evaporated with anhydrous CH₃CN three times under an argon atmosphere, and obtained compound **1a** as a white foam was used without further purification. In a separate flask, anhydrous THF (80 mL) solution containing methyltriphenylphosphonium bromide (8.47 g, 23.7 mmol) was added *tert*-BuOK (2.57 g, 22.9 mmol) at 0 °C and stirred for 30 min at 0 °C. To this solution, anhydrous THF solution (80 mL) of compound **1a** was added dropwise (10 min) at 0 °C and stirred overnight at rt. After evaporating excess THF, the obtained mixture was dissolved in excess ethyl acetate, washed by aq. sat. NH₄Cl, dried over MgSO₄, filtered, and evaporated. The obtained material was dissolved into a minimum amount of CH₂Cl₂ and added dropwise to excess diethyl ether solution under vigorous stirring over ice bath. Precipitate in solution was filtered off through celite and filtrate was evaporated. The obtained crude material was purified by silica gel column chromatography (hexane/ethyl acetate, 9:1 to 1:2) yielding compound **2a** as a white foam (2.19 g, 75 % in 2 steps). ¹H NMR (500 MHz, CDCl₃) δ 9.55 (br-s, 1H), 7.38 (d, 1H, *J* = 8.2 Hz), 5.89 (ddd, 1H, *J* = 17.1, 10.6, 6.6 Hz), 5.82 (d, 1H, *J* = 2.0 Hz), 5.77 (dd, 1H, *J* = 8.1, 1.5 Hz), 5.44 (dt, 1H, *J* = 17.2, 1.2 Hz), 5.34 (dt, 1H, *J* = 10.5, 1.1 Hz), 4.43-4.40 (m, 1H), 3.90 (dd, 1H, *J* = 7.7, 5.1 Hz), 3.71 (dd, 1H, *J* = 5.0, 2.0 Hz), 3.55 (s, 3H), 0.89 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.4, 150.0, 139.7, 134.4, 119.2, 102.4, 89.7, 84.0, 83.5, 74.5, 58.7, 25.7, 18.2, -4.6, -4.7; HRMS (ESI) calcd. for C₁₇H₂₉N₂O₅Si⁺ [M + H]⁺ *m/z* 369.1840, found *m/z* 369.1838.

*Synthesis of compound 3a.*³ Anhydrous solution of compound **2a** (7.29 g, 19.8 mmol) in THF (158.3 mL) was added 0.5 M 9-BBN/THF solution (237.4 mL, 118.7 mmol) dropwise for 10 min at 0°C. After stirring the mixture at rt overnight, the solution was cooled over ice bath and added methanol (65.4 mL) and stirred until bubbling cease down. Then under vigorous stirring, H₂O (98.4 mL) was added dropwise for 10 min to avoid precipitation of an intermediate compound. At 0°C, NaBO₃·4H₂O (15.7 g, 102.0 mmol) was added in one portion and stirred at rt overnight. After evaporation of excess THF, obtained crude mixture was dissolved into excess ethyl acetate, and washed repeatedly by sat. aq. NH₄Cl solution (5-6 times), then organic layer was evaporated. To complete the reaction, obtained material was dissolved in THF (450 mL) and H₂O (450 mL), and then NaBO₃·4H₂O (15.7 g, 102.0 mmol) was added in one portion at rt and stirred overnight at rt. After evaporating of excess THF, the mixture was added ethyl acetate, then extracted. Obtained organic layer was repeatedly washed by aq. sat. NH₄Cl, dried over MgSO₄, filtered, and evaporated. Obtained crude material was purified by silica gel column chromatography (hexane/ethyl acetate, 7:3 to 0:10) yielding compound **3a** as a white foam (4.73 g, 62 % in 2 steps). ¹H NMR (500 MHz, CDCl₃) δ 9.21(br-s, 1H), 7.35 (d, 1H, *J* = 8.1 Hz), 5.78-5.76 (m, 2H), 4.14-4.10 (m, 1H), 3.92-3.79 (m, 4H), 3.75 (dd, 1H, *J* = 5.2, 2.3 Hz), 3.52 (s, 3H), 2.06-2.00 (m, 1H), 1.90-1.82 (m, 1H), 0.91 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 163.1, 149.9, 140.0, 102.6, 90.1, 83.0, 82.0, 74.5, 60.3, 58.4, 35.5, 25.7, 18.1, -4.6, -4.9; HRMS (ESI) calcd. for C₁₇H₃₁N₂O₅Si⁺ [M + H]⁺ *m/z* 387.1946, found *m/z* 387.1944.

Synthesis of compound 5a. Anhydrous solution of compound **3a** (9.46 g, 24.5 mmol) in pyridine (240 mL) was added DMTr-Cl (9.95 g, 29.4 mmol) and stirred at rt for 2h. After quenching the reaction mixture by MeOH (20 mL), excess pyridine was evaporated, then obtained material was dissolved into excess ethyl acetate. The organic solution was washed by aq. sat. NaHCO₃, dried over MgSO₄, filtered, evaporated, then co-evaporated with toluene to remove pyridine residues. This crude mixture containing compound **4a** was dissolved into THF (330 mL), added 1.0 M TBAF-THF solution (36.7 mL, 36.7 mmol), then stirred for 1 h at rt. After evaporation excess THF and co-evaporation with CH₂Cl₂, the crude material was purified by silica gel column chromatography yielding compound **5a** (13.15 g, 93 % in 2 steps). ¹H NMR (500 MHz, CDCl₃) δ 9.41 (br-s, 1H), 7.43-7.21 (m, 2H), 7.32-7.14 (m, 8H), 6.83-6.82 (m, 1H), 5.80 (d, 1H, *J* = 1.8 Hz), 5.69 (d, 1H, *J* = 8.2 Hz), 4.02-3.98 (m, 1H), 3.85 (dd, 1H, *J* = 6.7, 6.7 Hz), 3.79 (s, 6H), 3.72 (dd, 1H, *J* = 5.5, 1.9 Hz), 3.58 (s, 3H), 3.34-3.25 (m, 2H), 2.91 (br-s, 1H), 2.11-2.04 (m, 1H), 1.95-1.89 (m, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 163.2, 163.1, 158.4, 149.9, 114.8, 139.1, 136.1, 136.0, 129.92, 129.90, 128.0, 127.8, 126.8, 113.1, 102.5, 88.1, 86.6, 83.5, 81.3, 73.2, 60.1, 58.8, 55.2, 53.4, 33.4; HRMS (ESI) calcd. for C₃₂H₃₄N₂O₈Na [M + Na]⁺ m/z 597.2203, found m/z 597.2153.

Synthesis of compound 6a. Compound **5a** (9.57 g, 16.65 mmol) was rendered anhydrous by repeated co-evaporation with anhydrous CH₃CN and then dissolved into anhydrous CH₂Cl₂ (150 mL). To this solution *N,N*-diisopropylethylamine (7.6 mL, 62.4 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (4.85 mL, 25.0 mmol) were added at 0 °C.⁴ After stirring for 0.5 h at rt, the reaction mixture was added CH₂Cl₂ (200 mL) then aq. sat. NaHCO₃ (350 mL). The organic layer was repeatedly washed by aq. sat. NaHCO₃, dried over MgSO₄, filtered, then evaporated. The obtained crude material was purified by silica gel column chromatography (1%TEA-hexanes-ethyl acetate, from 80:20 to 30:70) yielding compound **6a** with an impurity of phosphorylating reagent residues. To remove the impurity, obtained material was dissolved in Et₂O-ethylacetate (1:1, v/v, 400 mL), then repeatedly washed by aq. sat. NaHCO₃ yielding compound **6a** as a white solid (11.12 g, 86 %); ¹H NMR (500 MHz, CDCl₃) δ 8.88 (br-s, 1H), 7.44-7.41 (m, 2H), 7.33-7.19 (m, 7H), 7.14-7.09 (m, 1H), 6.84-6.80 (m, 4H), 5.83 (d, *J* = 3.7 Hz), 5.66 (d, 0.5H, *J* = 8.2 Hz), 5.65 (d, 0.5H, *J* = 8.1 Hz), 4.28-4.21 (m, 1H), 4.14-4.10 (m, 1H), 4.06-4.02 (m, 0.5H), 3.93-3.75 (m, 7.5H), 3.71-3.59 (m, 2.5H), 3.49 (s, 1.5H), 3.47 (s, 1.5H), 3.30-3.23 (m, 2H), 2.64 (t, 1H, *J* = 6.3 Hz), 2.56 (t, 1H, *J* = 6.2 Hz), 2.18-2.04 (m, 1H), 1.94-1.82 (m, 1H), 1.27-1.18 (m, 12H); ³¹P NMR (202 MHz, CDCl₃) δ 150.0, 149.9; HRMS (ESI) calcd. for C₄₁H₅₂N₄O₉P [M + H]⁺ m/z 775.3486, found m/z 775.3414.

Synthesis of compound 2b. Anhydrous solution of 3'-*O*-TBDMS-2'-deoxy-2'-fluoro-uridine⁵ (10.8 g, 30.0 mmol) in CH₃CN (300 mL) was added IBX (21.0 g, 75.0 mmol) and stirred for 2 h at 85 °C.¹⁻² After cooling the mixture over an ice bath, the precipitate in the solution was filtered off through celite. Collected filtrate was evaporated, co-evaporated with anhydrous CH₃CN three times under argon atmosphere, and obtained compound **1b** as a white foam was used without further purification. In a separate flask, anhydrous THF (250 mL) solution containing *tert*-BuOK (7.30 g, 65.1 mmol) was added methyltriphenylphosphonium bromide (24.0 g, 68.1 mmol) was added in one portion at 0 °C and stirred for 1 h at 0 °C. To this solution, anhydrous THF solution (150 mL) of compound **1b** was added dropwise (10

min) at 0 °C and stirred overnight at rt. After evaporating excess THF, the obtained mixture was dissolved in excess ethyl acetate, washed by aq. sat. NH₄Cl, dried over MgSO₄, filtered, and evaporated. The obtained material was dissolved into minimum amount of CH₂Cl₂ and added dropwise to excess diethyl ether solution under vigorous stirring over an ice bath. Precipitate in the solution was filtered off through celite and filtrate were evaporated. Obtained crude material was purified by silica gel column chromatography (hexane/ethyl acetate, 8:2 to 6:4) yielding compound **2b** as a white foam (7.12 g, 67 % in 2 steps). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.4 (br-s, 1H), 7.65 (d, 1H, *J* = 8.1 Hz), 5.93 (ddd, 1H, *J* = 17.5, 10.4, 7.5 Hz), 5.82 (dd, 1H, *J*_{HF} = 22.2 Hz, *J*_{HH} = 1.3 Hz), 5.65 (d, 1H, *J* = 8.1 Hz), 5.42-5.38 (m, 1H), 5.33-5.31 (m, 1H), 5.15 (ddd, 1H, *J*_{HF} = 53.4 Hz, *J*_{HH} = 4.6, 1.2 Hz), 4.27 (ddd, 1H, *J*_{HF} = 20.6 Hz, *J*_{HH} = 8.4, 4.9 Hz), 4.18 (dd, 1H, *J* = 7.7, 7.7 Hz), 0.88 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 170.8, 163.7, 150.7, 142.5, 135.3, 120.2, 102.4, 92.9 (d, *J*_{CF} = 186.2 Hz), 90.2 (d, *J*_{CF} = 36.4 Hz), 83.4, 73.8 (d, *J*_{CF} = 15.5 Hz), 60.2, 26.0, 21.2, 18.2, 14.6, -4.4, -4.5; ¹⁹F NMR (470 MHz, DMSO-*d*₆) δ -198.3 (ddd, *J* = 53.8, 20.8, 20.8 Hz). HRMS (ESI) calcd. for C₁₆H₂₆FN₂O₄Si [M + H]⁺ *m/z* 357.1640, found *m/z* 357.1649.

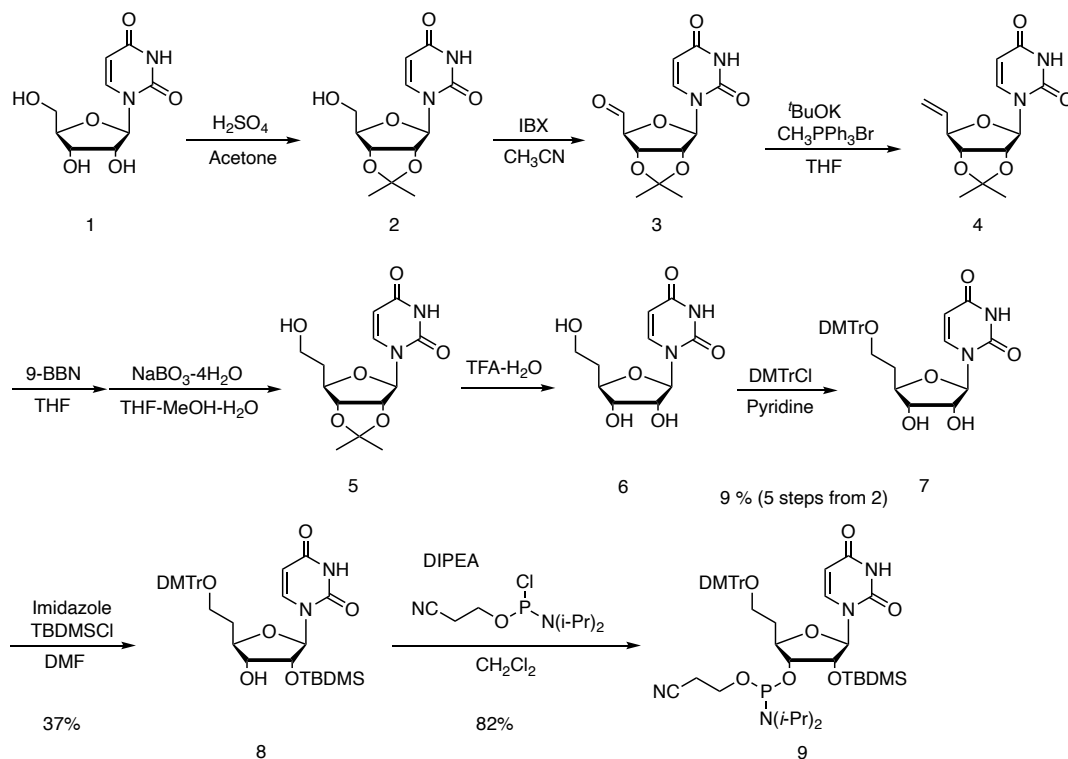
*Synthesis of compound 4b.*³ Anhydrous solution of compound **2b** (10.15 g, 28.5 mmol) in THF (228 mL) was added 0.5 M 9-BBN/THF solution (342 mL, 171 mmol) dropwise for 20 min at 0°C. After stirring the mixture at rt overnight, the solution was cooled over an ice bath and added methanol (131 mL) and stirred until bubbling cease down. Then under vigorous stirring, H₂O (197 mL) was added dropwise for 15 min to avoid precipitation of an intermediate compound. At 0°C, NaBO₃·4H₂O (21.9 g, 142.5 mmol) was added in one portion and stirred at rt overnight. After evaporation of excess THF, obtained crude mixture was dissolved into excess ethyl acetate, washed repeatedly by sat. aq. NH₄Cl solution, then organic layer was evaporated. To complete the reaction, obtained material was dissolved in THF (450 mL) and H₂O (450 mL), then NaBO₃·4H₂O (21.9 g, 142.5 mmol) was added in one portion at rt and stirred overnight at rt. After evaporating of excess THF, the mixture was added ethyl acetate, then extracted. Obtained organic layer was repeatedly washed by aq. sat. NH₄Cl (5-6 times), dried over MgSO₄, filtered, and evaporated. Obtained crude material was purified by silica gel column chromatography (CH₂Cl₂/methanol, 100:0 to 93:7) yielding compound **3b** as a syrup (2.44 g with reagent impurity); HRMS (ESI) calcd. for C₁₆H₂₈FN₂O₅Si⁺ [M + H]⁺ *m/z* 375.1746, found *m/z* 375.1746. This compound **3b** with reagent impurity was rendered anhydrous by repeated co-evaporation with anhydrous pyridine under argon atmosphere, then dissolved in anhydrous pyridine (64 mL). To this solution, DMTr-Cl (2.64 g, 7.79 mmol) was added and stirred at rt for 1 h. After the reaction was quenched by addition of methanol (5 mL), reaction mixture was diluted with ethyl acetate (300 mL) and washed repeated by aq. sat. NaHCO₃, dried over MgSO₄, filtered, evaporated, then co-evaporated with toluene three times to remove remaining pyridine. Obtained crude material was purified by silica gel chromatography (hexane-ethyl acetate from 2:8 to 4:6) yielding compound **4b** as a white solid (2.25 g, 12% in 3 steps). ¹H NMR (500 MHz, CD₃CN) δ 9.16 (br-s, 1H), 7.43-7.42 (m, 2H), 7.31-7.28 (m, 8H), 6.86-6.85 (m, 4H), 5.75 (dd, 1H, *J*_{HF} = 20.0 Hz, *J*_{HH} = 1.9 Hz), 5.59 (d, 1H, *J* = 8.1 Hz), 4.96 (ddd, *J*_{HF} = 53.3 Hz, *J*_{HH} = 4.6, 1.8 Hz), 4.06-3.98 (m, 2H), 3.76 (s, 6H), 3.19 (dd, 2H, *J* = 7.4, 5.6 Hz), 2.09-2.02 (m, 1H), 1.89-1.82 (m, 1H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ¹³C NMR (126 MHz, CD₃CN) δ 163.9, 159.6, 151.1, 146.4, 141.9, 137.31, 137.26, 130.92, 130.89, 128.9, 128.8, 127.8, 114.0, 102.9, 93.7 (d, *J*_{CF} = 188.0 Hz), 90.6 (d, *J* = 36.4 Hz), 87.0, 80.7, 74.6 (d, *J*

= 15.4 Hz), 60.9, 55.9, 33.8, 26.1, 18.7, -4.5, -4.8; ^{19}F NMR (470 MHz, CD_3CN) δ -201.4 (ddd, J = 53.7, 19.1, 19.1 Hz); HRMS (ESI) calcd. for $\text{C}_{37}\text{H}_{45}\text{FN}_2\text{O}_7\text{Na}$ $[\text{M} + \text{Na}]^+$ m/z 699.2872, found m/z 699.2866.

Synthesis of compound 5b. Compound **4b** (2.24 g, 3.30 mmol) was dissolved into THF (36.0 mL), then added 1.0 M TBAF-THF solution (4.0 mL, 4.0 mmol), then stirred for 1 h at rt. After evaporation excess THF and co-evaporation with CH_2Cl_2 , the crude material was purified by silica gel column chromatography [CH_2Cl_2 (1% TEA)-methanol from 100:0 to 95:5] yielding compound **5b** (1.51 g, 81%). ^1H NMR (500 MHz, CD_3CN) δ 9.20 (br-s, 1H), 7.45-7.43 (m, 2H), 7.32-7.13 (m, 8H), 6.87-6.85 (m, 4H), 5.77 (dd, 1H, J_{HF} = 20.1 Hz, J_{HH} = 1.5 Hz), 5.60 (d, 1H, J = 8.1 Hz), 4.98 (ddd, J_{HF} = 51.0 Hz, J_{HH} = 4.6, 1.6 Hz), 4.04-3.92 (m, 2H), 3.76 (s, 6H), 3.23-3.17 (m, 2H), 2.20 (br-s, 1H), 2.11-2.06 (m, 1H), 1.91-1.87 (m, 1H); ^{13}C NMR (126 MHz, CD_3CN) δ 164.1, 159.7, 151.2, 146.4, 141.7, 139.0, 137.33, 137.29, 131.00, 130.97, 129.3, 129.0, 128.9, 127.8, 126.3, 114.1, 103.0, 94.7 (d, J = 184.4 Hz), 90.3 (d, J = 35.4 Hz), 87.2, 80.5, 73.9 (d, J = 16.4 Hz), 61.0, 56.0, 33.9; ^{19}F NMR (470 MHz, CD_3CN) δ -201.8 (ddd, J = 53.7, 20.8, 20.8 Hz). HRMS (ESI) calcd. for $\text{C}_{31}\text{H}_{31}\text{FN}_2\text{O}_7\text{Na}$ $[\text{M} + \text{Na}]^+$ m/z 585.2013, found m/z 585.1999.

*Synthesis of compound 6b.*⁴ Compound **5b** (1.5 g, 2.67 mmol) was rendered anhydrous by repeated co-evaporation with anhydrous CH_3CN and then dissolved into anhydrous CH_2Cl_2 (30 mL). To this solution *N,N*-diisopropylethylamine (1.76 mL, 10.1 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.90 mL, 4.01 mmol) were added at 0 °C. After stirring for 1 h at rt, the reaction mixture was added CH_2Cl_2 (70 mL) then aq. sat. NaHCO_3 (100 mL). Organic layer was repeatedly washed by aq. sat. NaHCO_3 , dried over MgSO_4 , filtered, then evaporated. Obtained crude material was purified by silica gel column chromatography (1%TEA-hexanes-ethyl acetate, from 80:20 to 20:80) yielding compound **6b** with an impurity of phosphitylating reagent residues. To remove the impurity, obtained material was dissolved in Et_2O (100 mL), then repeatedly washed by aq. sat. NaHCO_3 yielding compound **6b** as a white solid (1.47 g, 64 %); ^1H NMR (500 MHz, CD_3CN) δ 9.10 (br-s, 1H), 7.44-7.41 (m, 2H), 7.32-7.27 (m, 7H), 7.24-7.20 (m, 1H), 6.87-6.84 (m, 4H), 5.76 (dd, 0.55H, J_{HF} = 20.8 Hz, J_{HH} = 1.5 Hz), 5.74 (dd, 0.45H, J_{HF} = 20.5 Hz, J_{HH} = 1.7 Hz), 5.57 (d, 1H, J = 8.1 Hz), 4.26-4.08 (m, 2H), 3.87-3.73 (m, 8H), 3.68-3.61 (m, 2H), 3.22-3.18 (m, 2H), 2.67-2.63 (m, 2H), 2.22-2.10 (m, 1H), 1.90-1.85 (m, 1H), 1.20-1.17 (m, 12H); ^{31}P NMR (202 MHz, CD_3CN) δ 150.4 (d, J = 9.0 Hz), 149.9 (d, J = 10.0 Hz); ^{19}F NMR (470 MHz, CD_3CN) δ -198.63 to 198.83 (m, 0.57F), -199.04 to -199.25 (m, 0.43F). HRMS (ESI) calcd. for $\text{C}_{40}\text{H}_{49}\text{FN}_4\text{O}_8\text{P}$ $[\text{M} + \text{H}]^+$ m/z 763.3267, found m/z 763.3255.

Scheme S1. Synthesis of 2'-riboUr-exNA phosphoramidite



*Synthesis of compound 6.*⁶⁻⁸ Anhydrous solution of compound **2** (15.4 g, 54.1 mmol) in CH₃CN (520 mL) was added IBX (30.3 g, 108.2 mmol) and stirred for 2 h at 85 °C. After cooling the mixture in an ice bath, the precipitate in the solution was filtered off through celite. Collected filtrate was evaporated, co-evaporated with anhydrous CH₃CN three times under argon atmosphere, and obtained compound **3** as a white foam was used without further purification. In a separate flask, anhydrous THF (500 mL) solution containing *tert*-BuOK (13.2 g, 117.4 mmol) was added methyltriphenylphosphonium bromide (43.3 g, 121.2 mmol) was added in one portion at 0 °C and stirred for 1 h at 0 °C. To this solution, anhydrous THF solution (150 mL) of compound **3** was added dropwise (10 min) at 0 °C and stirred for 4 h at rt. After evaporating excess THF, the obtained mixture was dissolved in excess ethyl acetate, washed by aq. sat. NH₄Cl, dried over MgSO₄, filtered, and evaporated. Obtained material was dissolved into minimum amount of CH₂Cl₂ and added dropwise to excess diethyl ether solution under vigorously stirring at 0 °C. Precipitate in solution was filtered off through celite and filtrate was evaporated. Obtained crude material was purified by silica gel column chromatography (hexane/ethyl acetate, 8:2 to 3:7) yielding compound **4** with impurity of triphenylphosphineoxide. 3/4 of this crude material was rendered anhydrous by repeated co-evaporation with anhydrous CH₃CN, and then dissolved in anhydrous THF (200 mL). To this solution, 0.5 M 9-BBN/THF (300 mL, 150.0 mmol) was added dropwisely for 10 min at 0 °C, then stirred overnight at rt. After confirming disappearance of starting material by TLC, the solution was cooled over an ice bath, then added methanol (200 mL) dropwisely for 10 min. After bubbling is cease down, H₂O (300 mL) was added dropwise then NaBO₃·4H₂O (19.2 g, 125.0 mmol) was added in one portion. The solution was stirred overnight at rt. After evaporation of excess THF, obtained crude mixture was dissolved into excess ethyl acetate, washed repeatedly by sat. aq. NH₄Cl solution, then organic layer was evaporated. To complete the reaction, obtained

material was dissolved in THF (400 mL) and H₂O (400 mL), and then NaBO₃·4H₂O (19.2 g, 125.0 mmol) was added in one portion at rt and stirred overnight at rt. After evaporating of excess THF, the mixture was added ethyl acetate, then extracted. Obtained organic layer was repeatedly washed by aq. sat. NH₄Cl, dried over MgSO₄, filtered, and evaporated. Obtained crude material was purified by silica gel column chromatography (CH₂Cl₂-methanol, 100: 0 to 93:7) yielding compound **5**^{9,10} with impurity of reagent residues. This obtained material was added TFA solution [TFA (85 mL) and H₂O (9.2 mL)] and stirred at 0 °C for 1 h. After evaporation, co-evaporation with toluene four times, crude material was purified by silica gel column chromatography (CH₂Cl₂-MeOH from 100:0 to 90:10) yielding compound **6** (760 mg, 12% in 4 steps). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.4 (br-s, 1H), 7.58 (d, 1H, *J* = 5.0 Hz), 5.71 (d, 1H, *J* = 5.0 Hz), 5.64 (dd, 1H, *J* = 8.0, 2.2 Hz), 5.34 (d, 1H, *J* = 5.2 Hz), 5.09 (d, 1H, *J* = 4.7 Hz), 4.51 (br-s, 1H), 4.06, (dd, 1H, *J* = 9.8, 4.9 Hz), 3.80-3.78 (m, 1H), 3.53-3.45 (m, 2H), 1.84-1.70 (m, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 163.5, 151.1, 141.6, 102.5, 89.0, 80.9, 73.5, 73.2, 58.0, 46.2, 36.8, 9.1; HRMS (ESI) calcd. for C₁₀H₁₄N₂O₆Na [M + Na]⁺ *m/z* 281.0744, found *m/z* 281.0730.

Synthesis of compound 7. The compound **6** (760 mg, 2.94 mmol) was added anhydrous pyridine (30 mL) and then added DMTr-Cl (1.3 g, 3.82 mmol). After stirring for 2 h, reaction mixture was first extracted with CH₂Cl₂ an aq. sat. NaHCO₃, and organic layer was dried over MgSO₄, filtered, evaporated, co-evaporated to remove pyridine. The obtained crude material was used for the next reaction without further purification. HRMS (ESI) calcd. for C₃₁H₃₂N₂O₈Na [M + Na]⁺ *m/z* 583.2051, found *m/z* 583.2025.

Synthesis of compound 8. An anhydrous solution of compound **7** (2.35 g, 4.19 mmol) in pyridine (21 mL) was added imidazole (576.1 mg, 8.46 mmol) and TBDMS-Cl (1.10 g, 7.33 mmol), and then stirred for 2h at rt. To this reaction mixture was added CH₂Cl₂ (150 mL) was then added aq. sat. NaHCO₃ (150 mL). The organic layer was repeatedly washed by aq. sat. NaHCO₃, dried over MgSO₄, filtered, evaporated, then co-evaporated with toluene to remove pyridine residue. Obtained crude material containing compound **8**, 3'-*O*-TBDMS protected compound, 2'-3'-*O*-bis-TBDMS protected compound was separated by silica gel column chromatography [CH₂Cl₂ (1% TEA)-Acetone from 100:0 to 85:15] yielding pure compound **8** (780 mg, 28%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.4 (br-s, 1H), 7.53-7.52 (m, 2H), 7.39-7.22 (m, 8H), 6.89-6.88 (m, 4H), 5.72 (d, 1H, *J* = 5.0 Hz), 5.62 (d, 1H, *J* = 8.1, 2.0 Hz), 5.00 (d, 1H, *J* = 6.0 Hz), 4.19 (dd, 1H, *J* = 5.1, 5.1 Hz), 3.92 (ddd, 1H, *J* = 8.8, 8.8, 4.5 Hz), 3.78-3.73 (m, 7H), 3.07-3.03 (m, 2H), 2.05-1.83 (m, 2H), 0.83 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 162.9, 158.0, 150.5, 145.1, 140.7, 135.8, 130.1, 129.4, 128.7, 128.3, 128.1, 127.1, 125.8, 113.6, 102.5, 88.8, 86.0, 81.5, 74.9, 73.4, 60.6, 55.5, 33.8, 26.1, 18.4, -4.8, -5.2; HRMS (ESI) calcd. for C₃₇H₄₆N₂O₈Na [M + Na]⁺ *m/z* 697.2916, found *m/z* 697.2867.

Synthesis of compound 9. Compound **8** (780 g, 1.16 mmol) was rendered anhydrous by repeated co-evaporation with anhydrous CH₃CN and then dissolved into anhydrous CH₂Cl₂ (12 mL). To this solution *N,N*-diisopropylethylamine (0.53 mL, 4.34 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.34 mL, 1.73 mmol)⁴ were added at 0 °C. After stirring for 4 h at rt, the reaction mixture was added CH₂Cl₂ (90 mL) then aq. sat. NaHCO₃ (100 mL). Organic layer was repeatedly washed by aq. sat. NaHCO₃, dried over MgSO₄, filtered, then evaporated. Obtained crude material was purified by silica gel column chromatography (1%TEA-hexanes-ethyl acetate, from 80:20 to 50:50) yielding compound **9** (825.9 mg, 82%). ¹H NMR (500 MHz, CDCl₃) δ 8.06 (br-s, 1H), 7.47-7.45 (m, 2H), 7.33-7.15 (m, 7H), 6.83-6.80 (m, 4H), 5.71 (t, 1H, *J* = 4.1 Hz), 5.66 (d, 1H, *J* = 8.1 Hz), 4.39-4.36 (m, 0.45H), 4.32-4.29 (m, 0.55H), 4.26 (t, 0.55H, *J* = 4.3 Hz), 4.18 (t, 0.45H, *J* = 4.1 Hz), 3.99-3.76 (m, 8.5H), 3.69-3.59 (m, 2.5H), 3.29-3.24 (m, 1H), 2.61 (t, 1H, *J* = 6.2Hz), 2.54 (t, 1H, *J* = 6.2 Hz), 2.25-2.19 (m, 0.45H), 2.11-2.04 (m, 0.55H), 1.90-1.82 (m, 1H), 1.20-1.16 (m, 12H), 0.89 (s, 9H), 0.10-0.08 (m, 6H); ³¹P NMR (202 MHz, CDCl₃) δ 149.6, 149.1; HRMS (ESI) calcd. for C₄₆H₆₄N₄O₉PSi [M + H]⁺ *m/z* 875.4175, found *m/z* 875.4200.

Table S1. List of exNA-modified siRNA duplexes used for in-vitro screening.

Duplex#	Strands (AS/SS)	Sequence (5' -> 3') ^a
D1	ex-AS1	P(ex-mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D2	ex-AS2	P(mU)#(ex-fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D2-b ^b	ex-AS2-b	P(mU)#(ex-fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	SS2	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D3	ex-AS3	P(mU)#(fU)#(ex-mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	SS2	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D4	ex-AS4	P(mU)#(fU)#(mU)(ex-fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	SS2	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D5	ex-AS5	P(mU)#(fU)#(mA)(fA)(ex-mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D6	ex-AS6	P(mU)#(fC)#(mC)(fA)(mC)(ex-fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D7	ex-AS7	P(mU)#(fU)#(mA)(fA)(mU)(fC)(ex-mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D8	ex-AS8	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(ex-fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D9	ex-AS9	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(ex-mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D9-b ^b	ex-AS9-b	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(ex-mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	SS2	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D10	ex-AS10	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(ex-fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D11	ex-AS11	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(ex-mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D11-b ^b	ex-AS11-b	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(ex-mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol

^a "#": Phosphorothioate bond, "m": 2'-O-methyl, "f": 2'-Fluoro, "P": 5'-Phosphate, (ex-mU): 2'-O-Methyl-exNA uridine, (ex-fU): 2'-Fluoro-exNA uridine, TegChol:

Tetraethylene glycol-linked cholesterol conjugate. ^bsiRNA used for in-vitro siRNA potency changes in different sequence context (Figure S9)

Table S1. List of exNA-modified siRNA duplexes used for in-vitro screening.

Duplex#	Strands (AS/SS)	Sequence (5' -> 3') ^a
D12	ex-AS12	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(ex-fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D13	ex-AS13	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(ex-mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D14	ex-AS14	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(ex-fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D15	ex-AS15	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(ex-mU)#(fU)#(mU)#(fA)#(mG)#(fU)
	SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D16	ex-AS16	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(ex-fU)#(mU)#(fA)#(mG)#(fU)
	SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D17	ex-AS17	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(ex-mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D18	ex-AS18	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(ex-fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D19	ex-AS19	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(ex-mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D20	ex-AS20	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(ex-fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D20-b ^b	ex-AS20-b	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)#(ex-fU)
	SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D21	ex-AS21	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(mU)#(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D22	ex-AS22	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(ex-mU) #(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D23	ex-AS23	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(ex-fU)#(ex-mU) #(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol

^a "#": Phosphorothioate bond, "m": 2'-O-methyl, "f": 2'-Fluoro, "P": 5'-Phosphate, (ex-mU): 2'-O-Methyl-exNA uridine, (ex-fU): 2'-Fluoro-exNA uridine, TegChol:

Tetraethylene glycol-linked cholesterol conjugate. ^bsiRNA used for in-vitro siRNA potency changes in different sequence context (Figure S9).

Table S1 (continued). List of exNA-modified siRNA duplexes used for in-vitro screening.

Duplex#	Strands (AS/SS)	Sequence (5' -> 3') ^a
D16	ex-AS16	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(ex-fU)#(mU)#(fA)#(mG)#(fU)
	SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D17	ex-AS17	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(ex-mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D18	ex-AS18	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(ex-fU)#(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D19	ex-AS19	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(ex-mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D20	ex-AS20	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(ex-fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D20-b ^b	ex-AS20-b	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)#(ex_fU)
	SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D21	ex-AS21	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(mU)#(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D22	ex-AS22	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(ex-mU) #(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D23	ex-AS23	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(ex-fU)#(ex-mU) #(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D24	ex-AS24	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(ex-mU)#(ex-fU)#(ex-mU) #(ex-fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D25	AS1	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)
	ex-SS1	(ex-fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D26	AS2	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	ex-SS2	(fC)#(ex-mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D26-b ^b	AS5	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)#(fU)
	ex-SS2-b	(fA)#(ex-mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol

^a "#": Phosphorothioate bond, "m": 2'-O-methyl, "f": 2'-Fluoro, "P": 5'-Phosphate, (ex-mU):2'-O-Methyl-exNA uridine, (ex-fU): 2'-Fluoro-exNA uridine, TegChol:

Tetraethylene glycol-linked cholesterol conjugate. ^bsiRNA used for in-vitro siRNA potency changes in different sequence context (Figure S9).

Table S1 (continued). List of exNA-modified siRNA duplexes used for in-vitro screening.

Duplex#	Strands (AS/SS)	Sequence (5' -> 3') ^a
D27	AS3	P(mU)#(fC)#(mC)(fG)(mG)(fU)(mC)(fA)(mC)(fA)(mA)(fC)(mA)#(fU)#(mU)#(fG)#(mG)#(fU)
	ex-SS3	(fA)#(mA)#(ex-fU)(mG)(fU)(mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol
D28	AS4	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	ex-SS4	(fC)#(mA)#(fG)(ex-mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)(mA)#(fA)-TegChol
D29	AS3	P(mU)#(fC)#(mC)(fG)(mG)(fU)(mC)(fA)(mC)(fA)(mA)(fC)(mA)#(fU)#(mU)#(fG)#(mU)#(fG)#(mG)#(fU)
	ex-SS5	(fA)#(mA)#(fU)(mG)(ex-fU)(mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol
D30	AS3	P(mU)#(fC)#(mC)(fG)(mG)(fU)(mC)(fA)(mC)(fA)(mA)(fC)(mA)#(fU)#(mU)#(fG)#(mU)#(fG)#(mG)#(fU)
	ex-SS6	(fA)#(mA)#(fU)(mG)(fU)(ex-mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol
D31	AS5	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)#(fU)
	ex-SS7	(fA)#(mU)#(fG)(mU)(fG)(mC)(ex-fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D32	AS2	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	ex-SS8	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(ex-mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D33	AS2	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	ex-SS9	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(ex-fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D33-b ^b	AS1	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)(mA)#(fU)
	Ex-SS9-b	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(ex-fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D34	AS2	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	ex-SS10	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(ex-mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D34-b ^b	AS5	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)#(fU)
	ex-SS10-b	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(ex-mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D35	AS6	P(mU)#(fA)#(mU)(fC)(mA)(fG)(mC)(fU)(mU)(fU)(mU)(fC)(mC)#(fA)#(mG)#(fG)#(mG)#(fU)#(mC)#(fG)
	ex-SS11	(fC)#(mU)#(fG)(mG)(fA)(mA)(fA)(mA)(fG)(mC)(ex-fU)(mG)(fA)#(mU)#(fA)-TegChol
D36	AS4	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	ex-SS12	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(ex-mU)(fU)#(mA)#(fA)-TegChol
D37	AS4	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	ex-SS13	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(ex-fU)#(mA)#(fA)-TegChol
D38	AS6	P(mU)#(fA)#(mU)(fC)(mA)(fG)(mC)(fU)(mU)(fU)(mU)(fC)(mC)#(fA)#(mG)#(fG)#(mG)#(fU)#(mC)#(fG)
	ex-SS14	(fC)#(mU)#(fG)(mG)(fA)(mA)(fA)(mA)(fG)(mC)(fU)(mG)(fA)#(ex-mU)#(fA)-TegChol
D39	AS7	P(mA)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	ex-SS15	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(ex_fU)-TegChol

^a "#": Phosphorothioate bond, "m": 2'-O-methyl, "F": 2'-Fluoro, "P": 5'-Phosphate, (ex-mU):2'-O-Methyl-exNA uridine, (ex-fU): 2'-Fluoro-exNA uridine, TegChol:

Tetraethylene glycol-linked cholesterol conjugate. ^bsiRNA used for in-vitro siRNA potency changes in different sequence context (Figure S9).

Table S2. List of control siRNA duplexes used for in-vitro screening.

Duplex#	Strands (AS/SS)	Sequence (5' -> 3') ^a
D40	AS4	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D41	AS8	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(mU)#(fU)
	SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol
D42	AS2	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)
	SS2	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol
D43	AS1	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)(mA)#(fU)
	SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol
D44	AS5	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)#(fU)
	SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol
D45	AS6	P(mU)#(fA)#(mU)(fC)(mA)(fG)(mC)(fU)(mU)(fU)(mU)(fC)(mC)#(fA)#(mG)#(fG)#(mG)#(fU)#(mC)#(fG)
	SS5	(fC)#(mU)#(fG)(mG)(fA)(mA)(fA)(mA)(fG)(mC)(fU)(mG)(fA)#(mU)#(fA)-TegChol
D46	AS3	P(mU)#(fC)#(mC)(fG)(mG)(fU)(mC)(fA)(mC)(fA)(mA)(fC)(mA)#(fU)#(mU)#(fG)#(mU)#(fG)#(mG)#(fU)
	SS6	(fA)#(mA)#(fU)(mG)(fU)(mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol
D47	AS7	P(mA)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)
	SS7	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fU)-TegChol

^a "#": Phosphorothioate bond, "m": 2'-O-methyl, "f": 2'-Fluoro, "P": 5'-Phosphate, "TegChol": Tetraethylene glycol-linked cholesterol conjugate.

Table S3. IC50 (M^a) of each exNA modified siRNAs and potency change.

siRNA#	D1	D2	D3	D4	D5
IC50	1.619E-07	1.703E-07	3.825E-07	2.034E-07	1.864E-07
IC50 of corresponding control	7.662E-08	3.735E-08	7.416E-08	7.416E-08	7.662E-08
% Potency Change	-111.302532	-355.957162	-415.776699	-174.2718447	-143.2785174
siRNA#	D6*	D7	D8*	D9	D10
IC50	8.65E-11	2.235E-07	1.346E-10	0.000000097	0.000000111
IC50 of corresponding control	7.086E-11	7.662E-08	7.086E-11	7.662E-08	7.662E-08
% Potency Change	-22.07169066	-191.6992952	-89.95201806	-26.59879927	-44.87079092
siRNA#	D11	D12*	D13*	D14	D15
IC50	7.196E-08	2.09E-10	3.541E-10	0.000000175	3.119E-07
IC50 of corresponding control	7.662E-08	7.086E-11	9.675E-11	7.662E-08	1.411E-07
% Potency Change	6.081962934	-194.9477844	-265.994832	-128.3998956	-121.0489015
siRNA#	D16	D17	D18*	D19	D20*
IC50	2.782E-07	8.911E-08	1.924E-10	8.779E-08	1.589E-10
IC50 of corresponding control	1.411E-07	7.662E-08	9.675E-11	7.662E-08	9.675E-11
% Potency Change	-97.16513111	-16.30122683	-98.8630491	-14.57843905	-64.2377261
siRNA#	D21	D22	D23	D24	D25*
IC50	3.365E-08	3.713E-08	3.884E-08	7.226E-08	1.192E-10
IC50 of corresponding control	9.633E-08	9.633E-08	9.633E-08	9.633E-08	8.502E-11
% Potency Change	65.06799543	61.45541368	59.68026575	24.98702377	-40.20230534
siRNA#	D26	D27*	D28	D29*	D30*
IC50	1.206E-07	8.65E-11	1.229E-07	1.346E-10	2.09E-10
IC50 of corresponding control	7.416E-08	7.019E-11	7.662E-08	7.019E-11	7.019E-11
% Potency Change	-62.62135922	-23.23692834	-60.40198382	-91.76520872	-197.7632141
siRNA#	D31	D32	D33	D34	D35
IC50	1.186E-07	1.668E-07	8.044E-08	9.471E-08	3.102E-07
IC50 of corresponding control	1.411E-07	7.416E-08	7.416E-08	7.416E-08	2.972E-07
% Efficacy Change	15.94613749	-124.9190939	-8.468176915	-27.71035599	-4.374158816
siRNA#	D36	D37	D38	D39	
IC50	4.829E-08	4.945E-08	2.133E-07	3.917E-08	
IC50 of corresponding control	7.662E-08	7.662E-08	2.972E-07	3.266E-08	
% Efficacy Change	36.97468024	35.46071522	28.23014805	-19.93263931	

^a Molar concentration (mol/L)

Table S4. Sequence of siRNAs used for mice study.

Duplex#	Target	Antisense strand (5'->3') ^a	Sense strand (5'->3') ^a
D48 ^b (NTC ^c)	N/A	VP(mU)#(fA)#(mA)(fU)(mC)(fG)(mU)(fA)(mU)(fU)(mU)(fG) (mU)(fC)(mA)(fA)(mU)#(fC)#(mA)#(ex_mU)#(ex_fU)	(mU)#(fU)#(mG)(fA)(mC)(fA)(mA)(fA)(mU)(fA)(mC) (fG)(mA)(fU)#(mU)#(fA)(dT)(dT)-DCA
D49	Htt	VP(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA) (mC)(fU)(mG)(fA)(mU)#(fA)#(mU)#(mU)#(fU)	(mU)#(fC)#(mA)(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG) (fA)(mU)(fU)#(mA)#(fA)(dT)(dT)-DCA
D50	Htt	VP(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA) (mC)(fU)(mG)(fA)(mU)#(fA)#(mU)#(ex_mU)#(ex_fU)	(mU)#(fC)#(mA)(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG) (fA)(mU)(fU)#(mA)#(fA)(dT)(dT)-DCA
D51 ^b (NTC)	N/A	VP(mU)#(fA)#(mA)(fU)(fC)(fG)(mU)(fA)(mU)(fU)(mU)(fG)(mU) (fC)(mA)(fA)(mU)(mC)(mA)#(mU)#(fU)	(mU)#(mU)#(mG)(fA)(mC)(fA)(mA)(fA)(mU)(fA) (mC)(mG)(mA)(fU)#(mU)#(mA)(dT)(dT)-DCA
D52	Mstn	VP(mU)#(fU)#(mA)(fU)(fU)(fA)(mU)(fU)(mU)(fG)(mU)(fU)(mC) (fU)#(mU)#(fU)#(mG)#(mC)#(mC)#(mU)#(fU)	(mA)#(mA)#(mA)(fG)(mA)(fA)(mC)(fA)(mA)(fA)(mU) (mA)(mA)(fU)#(mA)#(mA)(dT)(dT)-DCA
D53	Mstn	VP(mU)#(fU)#(mA)(fU)(fU)(fA)(mU)(fU)(mU)(fG)(mU)(fU)(mC) (fU)#(mU)#(fU)#(mG)#(mC)#(mC)#(ex-mU)#(ex-fU)	(mA)#(mA)#(mA)(fG)(mA)(fA)(mC)(fA)(mA)(fA)(mU) (mA)(mA)(fU)#(mA)#(mA)(dT)(dT)-DCA
D54 ^b (NTC)	N/A	VP(mU)#(fA)#(mA)(mU)(mC)(fG)(mU)(mA)(mU)(mU)(mU) (mG)(mU)(fC)#(mA)#(fA)#(mU)#(mC)#(mA)#(fU)#(mA)	(mU)#(mU)#(mG)(mA)(mC)(fA)(fA)(fA)(mU)(fA) (mC)(mG)(mA)(mU)#(mU)#(mA)-divalent
D55	ApoE	VP(mU)#(fU)#(mG)(mG)(mA)(fU)(mA)(mU)(mG)(mG)(mA) (mU)(mG)(fU)(mU)(fG)(mU)(mU)(mU)#(mU)#(mU)	(mC)#(mA)#(mA)(mC)(mA)(mU)(mC)(fC)(mA)(fU) (fA)(fU)(mC)(mC)#(mA)#(mA)-divalent
D56	ApoE	VP(mU)#(fU)#(mG)(mG)(mA)(fU)(mA)(mU)(mG)(mG)(mA) (mU)(mG)(fU)(mU)(fG)(mU)(mU)(mU)#(ex-mU)#(ex-mU)	(mC)#(mA)#(mA)(mC)(mA)(mU)(mC)(fC)(mA)(fU) (fA)(fU)(mC)(mC)#(mA)#(mA)-divalent
D57 ^b (NTC)	N/A	VP(mU)#(fA)#(mA)(fU)(fC)(fG)(mU)(fA)(mU)(fU)(mU)(fG)(mU) (fC)(mA)(fA)(mU)(mC)(mA)#(mU)#(fU)	(mU)#(mU)#(mG)(fA)(mC)(mA)(mA)(fA)(mU)(fA) (mC)(fG)(mA)(fU)#(mU)#(mA)-divalent
D58	Htt	VP(mU)#(fU)#(mA)(fA)(fU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC) (fU)(mG)(fA)(mU)(mA)(mU)#(mU)#(fU)	(mU)#(mC)#(mA)(fG)(mU)(mA)(mA)(fA)(mG)(fA) (mG)(fA)(mU)(fU)#(mA)#(mA)-divalent
D59	Htt	VP(mU)#(fU)#(mA)(fA)(fU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC) (fU)(mG)(fA)(mU)(mA)(mU)#(ex-mU)#(ex-fU)	(mU)#(mC)#(mA)(fG)(mU)(mA)(mA)(fA)(mG)(fA) (mG)(fA)(mU)(fU)#(mA)#(mA)-divalent
Structure of siRNAs			

^a "VP": 5' -(E) Vinylphosphonate, "#": phosphorothioate, "m": 2'-O-Methyl, "f": 2'-Fluoro, "DCA": Docosanoic acid conjugate, "divalent": Two sense strands covalently connected as previously reported¹¹. ^bD48, D51, D54, and D57 are NTC for D49 (and D50), D52 (and D53), D55 (and D56), and D58 (and D59), respectively. ^c Non-targeting control.

Table S5. The sequence of oligonucleotides used for thermal melting analysis

ON #	Sequence (5' -> 3') ^a	Calcd mass [M-H] ⁻	Found mass
ON1	(rC)(rG)(rC)(rA)(rU)(rU)(rA)(rG)(rC)(rC)(rG)	3465.1	3467.0
ON2	(rC)(rG)(rC)(rA)(rU)(rxU)(rA)(rG)(rC)(rC)(rG)	3479.6	3478.5
ON3	(rU)(rC)(rU)(rC)(rU)(rU)(rU)(rC)(rU)(rC)(rU)	3300.9	3301.4
ON4	(rU)(rC)(rU)(rC)(rU)(rxU)(rU)(rC)(rU)(rC)(rU)	3315.0	3315.4
ON5	(rU)(rC)(rU)(rC)(rxU)(rxU)(rxU)(rC)(rU)(rC)(rU)	3343.0	3343.5
ON6	(rxU)(rC)(rxU)(rC)(rxU)(rU)(rxU)(rC)(rxU)(rC)(rxU)	3385.1	3385.5
cRNA1	(rC)(rG)(rG)(rC)(rU)(rA)(rA)(rU)(rG)(rC)(rG)	3505.2	3504.5
cRNA2	(rC)(rG)(rG)(rC)(rU)(rG)(rA)(rU)(rG)(rC)(rG)	3481.1	3480.5
cRNA3	(rC)(rG)(rG)(rC)(rU)(rU)(rA)(rU)(rG)(rC)(rG)	3482.1	3481.5
cRNA4	(rC)(rG)(rG)(rC)(rU)(rC)(rA)(rU)(rG)(rC)(rG)	3521.2	3520.4
cRNA5	(rA)(rG)(rA)(rG)(rA)(rA)(rA)(rG)(rA)(rG)(rA)	3622.3	3622.6
cDNA1	(dC)(dG)(dG)(dC)(dT)(dA)(dA)(dT)(dG)(dC)(dG)	3356.2	3356.6
cDNA2	(dC)(dG)(dG)(dC)(dT)(dG)(dA)(dT)(dG)(dC)(dG)	3372.2	3372.6
cDNA3	(dC)(dG)(dG)(dC)(dT)(dT)(dA)(dT)(dG)(dC)(dG)	3347.2	3347.6
cDNA4	(dC)(dG)(dG)(dC)(dT)(dC)(dA)(dT)(dG)(dC)(dG)	3332.2	3332.6

^a (rN) : 2'-ribonucleosides, (dN): 2'-deoxynucleosides, (rxU): exNA-uridine (2'-OH).

Table S6. Oligonucleotide used for 3'-exonuclease resistance assay

ON#	Sequence (5'->3') ^a	Calcd mass [M-H] ⁻	Found mass
ON7	(dT) ₁₈ (mU)(mU)	6053.9	6053.0
ON8	(dT) ₁₈ (ex-mU)(ex-mU)	6113.9	6113.0
ON9	(dT) ₁₈ #(mU)#(mU)	6086.1	6085.9
ON10	(dT) ₁₈ #(ex-mU)#(ex-mU)	6114.1	6114.0
ON11	(dT) ₁₈ #(mU)#(ex-mU)	6100.1	6100.0
ON12	(dT) ₁₈ #(mU)#(ex-fU)	6088.0	6087.9

^a (dT) : 2'-deoxythymidine, "mU": 2'-OMe-uridine, "ex-mU": 2'-OMe-exNA-uridine, "ex-fU": 2'-F-exNA-uridine "#": phosphorothioate

Table S7. Oligonucleotide used for 5'-exonuclease resistance assay

ON#	Sequence (5'->3') ^a	Calcd mass [M-H] ⁻	Found mass
ON13	(mU)(dT)(dT) ₁₈	6037.9	6036.9
ON14	(mU)#(dT)(dT) ₁₈	6054.0	6053.9
ON15	(ex-mU)(dT)(dT) ₁₈	6052.0	6050.9
ON16	(mU)(mU)(dT) ₁₈	6053.9	6053.9
ON17	(mU)#(mU)(dT) ₁₈	6070.0	6070.0
ON18	(mU)(ex-mU)(dT) ₁₈	6067.9	6067.9

^a (dT) : 2'-deoxythymidine, "mU": 2'-OMe-uridine, "ex-mU": 2'-OMe-exNA-uridine, "#": phosphorothioate

Table S8. Mass analysis of exNA modified antisense strands used in exNA walk study

Name	Sequence (5'->3') ^a	Mass calcd [M-H] ⁻	Mass found
ex-AS1	P(ex-mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS2	P(mU)#(ex-fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS2-b	P(mU)#(ex-fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)	6759.6	6758.5
ex-AS3	P(mU)#(fU)#(ex-mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)	6758.6	6758.8
ex-AS4	P(mU)#(fU)#(mU)(ex-fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)	6758.6	6758.8
ex-AS5	P(mU)#(fU)#(mA)(fA)(ex-mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS6	P(mU)#(fC)#(mC)(fA)(mC)(ex-fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)(mA)#(fU)(mA)#(fU)	6608.4	6608.7
ex-AS7	P(mU)#(fU)#(mA)(fA)(mU)(fC)(ex-mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6632.7
ex-AS8	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(ex-fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)(mA)#(fU)(mA)#(fU)	6608.4	6608.7
ex-AS9	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(ex-mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS9-b	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(ex-mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)#(mA)#(fA)	6759.6	6758.5
ex-AS10	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(ex-fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS11	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(ex-mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	663.7
ex-AS11-b	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(ex-mU)(fU)(mU)#(fC)#(mA)#(fC)(mA)#(fU)(mA)#(fU)	6609.4	6608.4
ex-AS12	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(ex-fU)(mU)#(fC)#(mA)#(fC)(mA)#(fU)(mA)#(fU)	6608.4	6608.7
ex-AS13	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(ex-mU)#(fC)#(mA)#(fC)(mA)#(fU)(mA)#(fU)	6608.4	6608.7
ex-AS14	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(ex-fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS15	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(ex-mU)#(fU)(mU)#(fA)#(mG)#(fU)	6749.6	6749.8
ex-AS16	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(ex-fU)(mU)#(fA)#(mG)#(fU)	6749.6	6749.8
ex-AS17	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(ex-mU)#(fA)#(mU)#(fA)	6633.4	6633.7
ex-AS18	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)(mA)#(ex-fU)(mA)#(fU)	6608.4	6608.7
ex-AS19	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(ex-mU)#(fA)	6633.4	6633.7
ex-AS20	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)(mA)#(fU)(mA)#(ex-fU)	6608.4	6608.7
ex-AS20-b	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)(mU)#(fA)#(mG)#(ex-fU)	6750.6	6749.5
ex-AS21	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)(mU)#(ex-fU)	6587.4	6587.6
ex-AS22	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(ex-mU) #(ex-fU)	6601.4	6601.6
ex-AS23	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(ex-fU) #(ex-mU)#(ex-fU)	6615.4	6615.7
ex-AS24	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(ex-mU)#(ex-fU) #(ex-mU)#(ex-fU)	6629.4	6629.7

^aP: 5'-phosphate, "m": 2'-OME, "f": 2'-fluoro, "ex-mU": 2'-OME-exNA-uridine, "ex-fU": 2'-F-exNA-uridine, #: phosphorothioate

Table S9. Mass analysis of exNA modified sense strands used in exNA walk study.

Name	Sequence (5'->3') ^a	Mass calcd [M-H] ⁻	Mass found
ex-SS1	(ex-fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol	5795.3	5795.2
ex-SS2	(fC)#(ex-mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol	5716.3	5716.2
ex-SS2-b	(fA)#(ex-mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol	5701.2	5700.9
ex-SS3	(fA)#(mA)#(ex-fU)(mG)(fU)(mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol	5764.3	5764.1
ex-SS4	(fC)#(mA)#(fG)(ex-mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol	5779.4	5778.2
ex-SS5	(fA)#(mA)#(fU)(mG)(ex-fU)(mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol	5764.3	5763.2
ex-SS6	(fA)#(mA)#(fU)(mG)(fU)(ex-mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)#(fA)-TegChol	5764.3	5764.2
ex-SS7	(fA)#(mU)#(fG)(mU)(fG)(mC)(ex-fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol	5701.2	5701.1
ex-SS8	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(ex-mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol	5716.3	5716.2
ex-SS9	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(ex-fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol	5716.3	5716.2
ex-SS9-b	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(ex-fU)(mA)(fG)(mU)(fG)#(mG)#(fA)-TegChol	5795.3	5795.0
ex-SS10	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(ex-mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol	5716.3	5716.1
ex-SS10-b	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(ex-mU)(fA)(mG)(fG)#(mC)#(fA)-TegChol	5701.2	5701.0
ex-SS11	(fC)#(mU)#(fG)(mG)(fA)(mA)(fA)(mA)(fG)(mC)(ex-fU)(mG)(fA)#(mU)#(fA)-TegChol	5771.3	5771.2
ex-SS12	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(ex-mU)(fU)#(mA)#(fA)-TegChol	5779.4	5779.2
ex-SS13	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(ex-fU)#(mA)#(fA)-TegChol	5779.3	5779.2
ex-SS14	(fC)#(mU)#(fG)(mG)(fA)(mA)(fA)(mA)(fG)(mC)(fU)(mG)(fA)#(ex-mU)#(fA)-TegChol	5771.3	5771.2
ex-SS15	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(ex-fU)-TegChol	5756.3	5756.2

^a “m”: 2'-OMe, “f”: 2'-fluoro, “ex-mU”: 2'-OMe-exNA-uridine, “ex-fU”: 2'-F-exNA-uridine, #: phosphorothioate, “TegChol”: Tetraethylene glycol-linked cholesterol conjugate.

Table S10. Mass analysis of control antisense and sense strands used in exNA walk study

Name	Sequence (5'->3') ^a	Mass calcd [M-H] ⁻	Mass found
AS1	P(mU)#(fC)#(mC)(fA)(mC)(fU)(mA)(fU)(mG)(fU)(mU)(fU)(mU)#(fC)#(mA)#(fC)#(mA)#(fU)#(mA)#(fU)	6595.4	6594.7
AS2	P(mU)#(fU)#(mU)(fU)(mU)(fA)(mA)(fA)(mU)(fC)(mC)(fU)(mG)#(fA)#(mG)#(fA)#(mA)#(fG)(mA)#(fA)	6745.6	6744.7
AS3	P(mU)#(fC)#(mC)(fG)(mG)(fU)(mC)(fA)(mC)(fA)(mA)(fC)(mA)#(fU)#(mU)#(fG)#(mU)#(fG)#(mG)(fU)	6728.6	6727.7
AS4	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6620.4	6619.7
AS5	P(mU)#(fG)#(mC)(fC)(mU)(fA)(mA)(fG)(mA)(fG)(mC)(fA)(mC)#(fA)#(mU)#(fU)#(mU)#(fA)#(mG)(fU)	6736.4	6735.7
AS6	P(mU)#(fA)#(mU)(fC)(mA)(fG)(mC)(fU)(mU)(fU)(mU)(fC)(mC)#(fA)#(mG)#(fG)#(mG)(fU)#(mC)(fG)	6705.6	6704.7
AS7	P(mA)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fA)#(mU)#(fA)	6643.5	6642.7
AS8	P(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)#(fU)#(mG)#(fA)#(mU)#(fU)#(mU)#(fU)	6574.4	6573.6
SS1	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fA)-TegChol	5765.3	5765.2
SS2	(fC)#(mU)#(fC)(mA)(fG)(mG)(fA)(mU)(fU)(mU)(fA)(mA)(fA)#(mA)#(fA)-TegChol	5702.3	5702.2
SS3	(fU)#(mG)#(fA)(mA)(fA)(mA)(fC)(mA)(fU)(mA)(fG)(mU)(fG)#(mG)(fA)-TegChol	5781.3	5781.2
SS4	(fA)#(mU)#(fG)(mU)(fG)(mC)(fU)(mC)(fU)(mU)(fA)(mG)(fG)#(mC)(fA)-TegChol	5687.2	5686.1
SS5	(fC)#(mU)#(fG)(mG)(fA)(mA)(fA)(mA)(fG)(mC)(fU)(mG)(fA)#(mU)#(fA)-TegChol	5757.3	5757.2
SS6	(fA)#(mA)#(fU)(mG)(fU)(mU)(fG)(mU)(fG)(mA)(fC)(mC)(fG)#(mG)(fA)-TegChol	5750.3	5750.1
SS7	(fC)#(mA)#(fG)(mU)(fA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA)#(fU)-TegChol	5742.3	5742.2

^a"m": 2'-OMe, "f": 2'-fluoro, "ex-mU": 2'-OMe-exNA-uridine, "ex-fU": 2'-F-exNA-uridine, #: phosphorothioate, "TegChol": Tetraethylene glycol-linked cholesterol conjugate.

Table S11. Mass analysis of oligonucleotides used for in vivo study.

Name	Sequence (5'->3') ^a	Calcd mass [M-H] ⁻	Found mass
D48-AS (NTC)	VP(mU)#(fA)#(mA)(fU)(mC)(fG)(mU)(fA)(mU)(fU)(mU)(fG)(mU)(fC)(mA) (fA)(mU)#(fC)#(mA)#(ex-mU)#(ex-fU)	6954.5	6954.9
D49-AS	VP(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)(fU)(mG) (fA)(mU)#(fA)#(mU)#(mU)#(fU)	6864.4	6864.8
D50-AS	VP(mU)#(fU)#(mA)(fA)(mU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)(fU)(mG) (fA)(mU)#(fA)#(mU)#(ex-mU)#(ex-fU)	6892.4	6892.8
D52-AS	VP(mU)#(fU)#(mA)(fU)(fU)(fA)(mU)(fU)(mU)(fG)(mU)(fU)(mC)(fU)#(mU)#(fU)#(mG)#(mC) #(mC)#(mU)#(fU)	6882.5	6882.9
D53-AS	VP(mU)#(fU)#(mA)(fU)(fU)(fA)(mU)(fU)(mU)(fG)(mU)(fU)(mC)(fU)#(mU)#(fU)#(mG)#(mC) #(mC)#(ex-mU)#(ex-fU)	6910.5	6910.8
<i>Mstn</i> -DCA- sense strand	(mA)#(mA)#(mA)(fG)(mA)(fA)(mC)(fA)(mA)(fA)(mU)(mA)(mA)(fU)#(mA)#(mA)(dT)(dT)-DCA	6520.0	6520.4
D55-AS	VP(mU)#(fU)#(mG)(mG)(mA)(fU)(mA)(mU)(mG)(mG)(mA)(mU)(mG)(fU) (mU)(fG)(mU)(mU)(mU)#(mU)#(mU)	7056.6	7056.9
D56-AS	VP(mU)#(fU)#(mG)(mG)(mA)(fU)(mA)(mU)(mG)(mG)(mA)(mU)(mG)(fU) (mU)(fG)(mU)(mU)(mU)#(ex-mU)#(ex-mU)	7084.6	7084.9
<i>ApoE</i> -di- sense strand	(mC)#(mA)#(mA)(mC)(mA)(mU)(mC)(fC)(mA)(fU)(fA)(fU)(mC)(mC)#(mA)#(mA)-Divalent	10857.3	10857.6
D58-AS	VP(mU)#(fU)#(mA)(fA)(fU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)(fU)(mG) (fA)(mU)(mA)(mU)#(mU)#(fU)	6832.2	6832.8
D59-AS	VP(mU)#(fU)#(mA)(fA)(fU)(fC)(mU)(fC)(mU)(fU)(mU)(fA)(mC)(fU)(mG) (fA)(mU)(mA)(mU)#(ex-mU)#(ex-fU)	6860.3	6860.9
<i>Htt</i> -di- sense strand	(mU)#(mC)#(mA)(fG)(mU)(mA)(mA)(fA)(mG)(fA)(mG)(fA)(mU)(fU)#(mA) #(mA)-Divalent	11123.4	11123.8

^a"m": 2'-OMe, "f": 2'-fluoro, "ex-mU": 2'-OMe-exNA-uridine, "ex-fU": 2'-F-exNA-uridine, #: phosphorothioate, "Divalent": two sense strands covalently connected as previously reported¹¹.

ON#	Sequence	T_m (°C)	ΔT_m^a (°C)
ON3	5'- r(UCUCUUUCUCU) -3'	44	
ON4	5'- r(UCUC U rxU U CUCU) -3'	40	-4
ON5	5'- r(UCUC rxU rxU rxU CUCU) -3'	30	-14
ON6	5'- r(rxU C rxU C rxU U rxU C rxU C rxU) -3'	25	-19
cRNA5	3'- r(AGAGAAAGAGA) -5'		

Fig S1. Thermostability of single or multiply rxU modified RNA duplex. T_m measurements were carried out in buffer containing 10 mM sodium phosphate (pH 7.0), 100 mM NaCl, 0.1 mM EDTA, and 1 μ M duplex. ΔT_m^a is the difference in T_m value between control ON3/cRNA5 duplex versus ON4 (or ON5 and ON6)/cRNA5 duplexes.

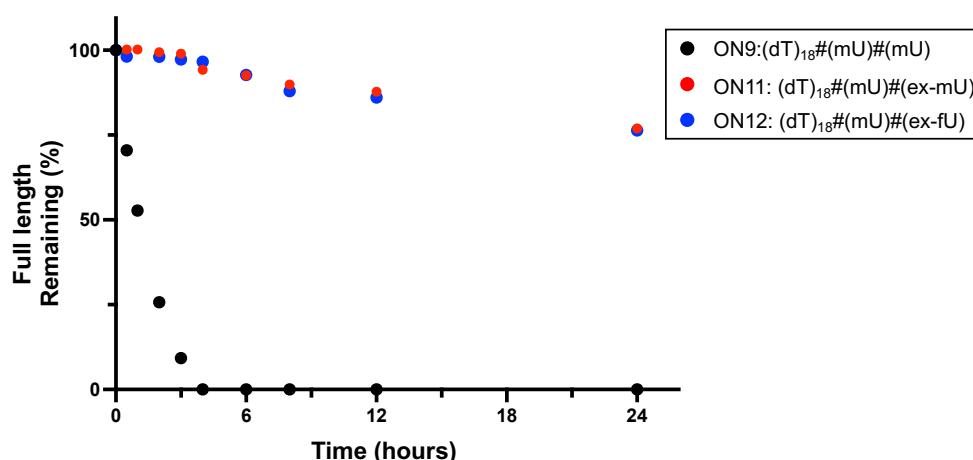


Fig S2. Comparison of 3'-exonuclease resistance of ex-mU and ex-fU. 16.7 μ M oligonucleotide was incubated in a 50 mM Tris-HCl (pH 8) buffer containing 10 mM $MgCl_2$ and 0.37 U/mL Snake Venom Phosphodiesterase I (Sigma Aldrich, Cas#: 9025-82-5, Source#:SLCQ2867). Residual oligonucleotide length was monitored over time and quantified by HPLC. (dT): 2'-deoxy-thymidine, (mU): 2'-OMe-uridine, (ex-mU): 2'-OMe-exNA-uridine. (ex-fU): 2'-F-exNA-uridine, “#” indicates phosphorothioate linkage, otherwise phosphodiester linkage.

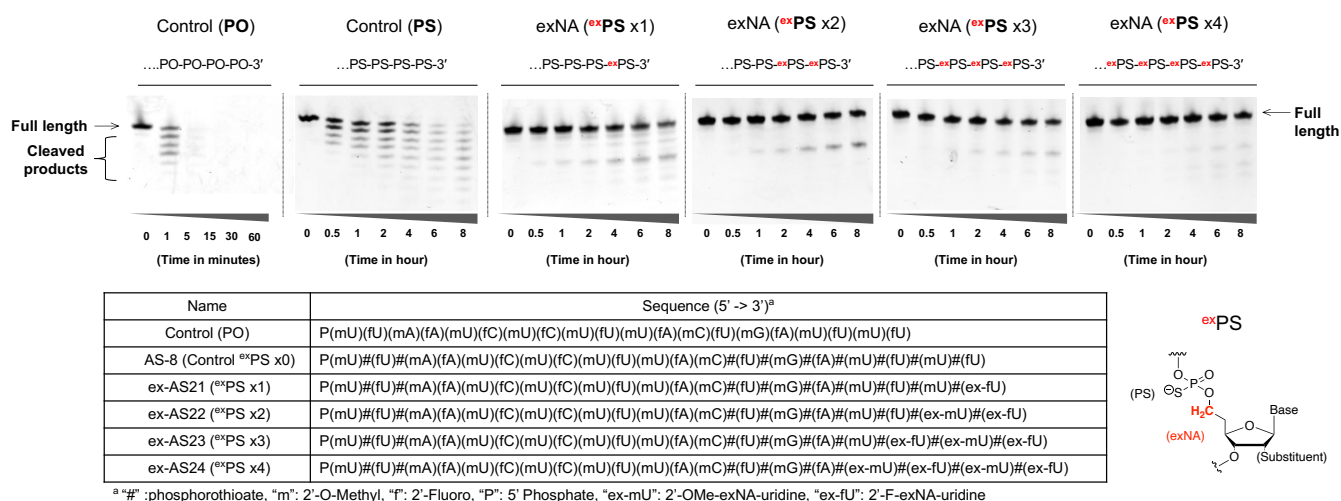


Fig S3. Comparison of 3'-exonuclease resistance among AS-8, ex-AS21 through ex-AS24. 17.5 μ M oligonucleotide was incubated in a 10 mM Tris-HCl (pH 8) buffer containing 2 mM $MgCl_2$ and 20 mU/mL Snake Venom Phosphodiesterase I (Sigma Aldrich, Lot#: SLBW3442). Aliquot at each time point was dissolved into 95% formamide containing 5% EDTA, and analyzed by denature gel (representative gels were shown from duplicated experiments).

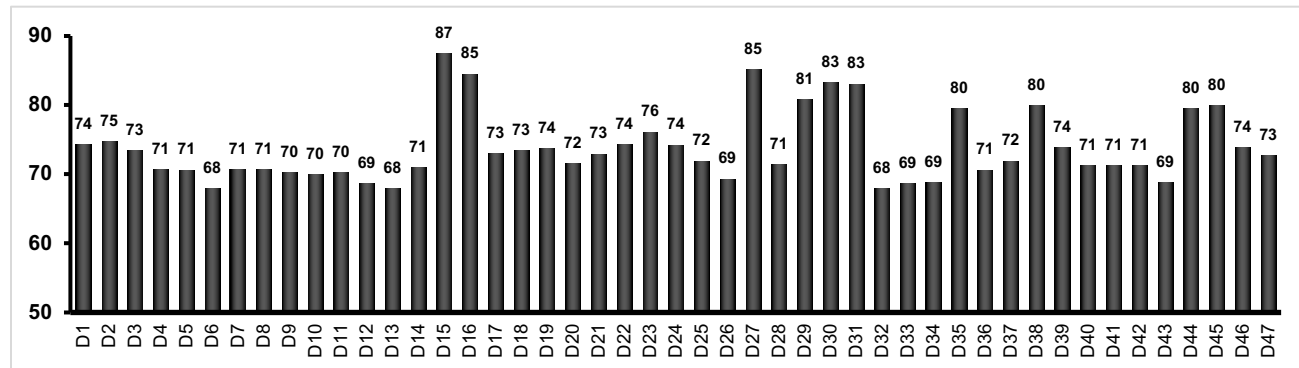


Fig S4. T_m Values of each siRNA duplexes (D1-47) used in in-vitro exNA walk. Melting temperature analysis was conducted in a buffer 10mM Sodium phosphate buffer (pH 7.2) containing 100 mM NaCl, 0.1 mM EDTA and 1 μ M guide/sense strand. T_m values were average values determined in triplicate experiments.

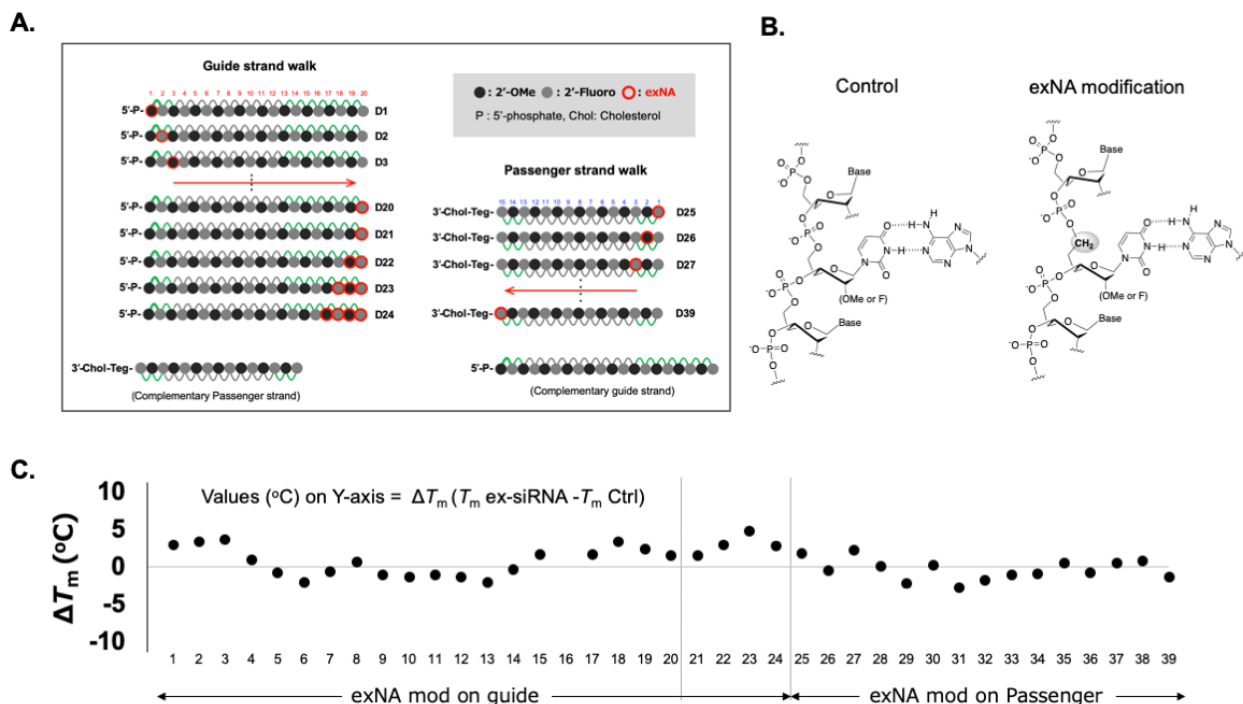


Fig S5. Delta- T_m of each siRNA duplexes. (A) Schematic of exNA walk and corresponding Duplex numbers. (B) Structural difference between duplex with canonical backbone and with exNA backbone. (C) delta- T_m values for each exNA modification positions on guide and passenger strand. Calculation of delta- T_m was conducted based on T_m values shown in **Fig. S4**.

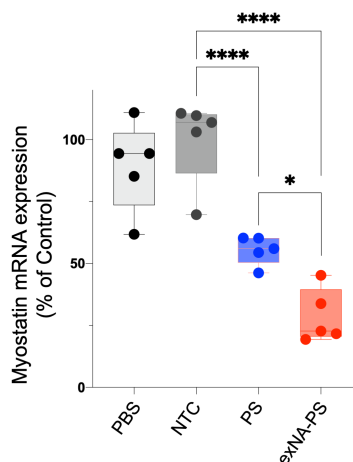


Fig S6. exNA-PS modification enhances Myostatin-targeting siRNA efficacy. DCA conjugated siRNAs (**D51**, **52**, and **53**) were administered to FVB mice (female, 7-8 weeks old) at 20 mg/kg dosage twice (3 days apart, s.c.). Mice was sacrificed at 1 week post injection, and muscle quadriceps was collected. Myostatin mRNA level was quantified by QuantiGene. NTC: Non-targeting control (**D51**), PS: non-exNA PS modified siRNA (**D52**, or PS), exNA-PS: exNA-PS modified siRNA (**D53**, or exNA-PS). For sequences, see **Table S4**.

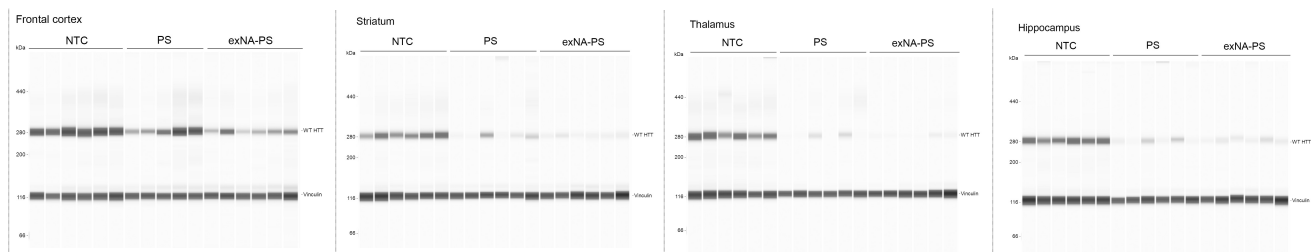


Fig S7. Raw capillary-based immunoassay images (ProteinSimple) of **D57** (NTC), **D58** (or PS) and **D59** (or exNA-PS) compounds in WT FVB/NJ mice. Divalent siRNAs with and without the introduction of the exNA modification were injected at 1.25nmol dose ICV at 8 weeks. The level of WT huntingtin protein levels normalized to vinculin loading control were evaluated one month following ICV injections (n=5,6).

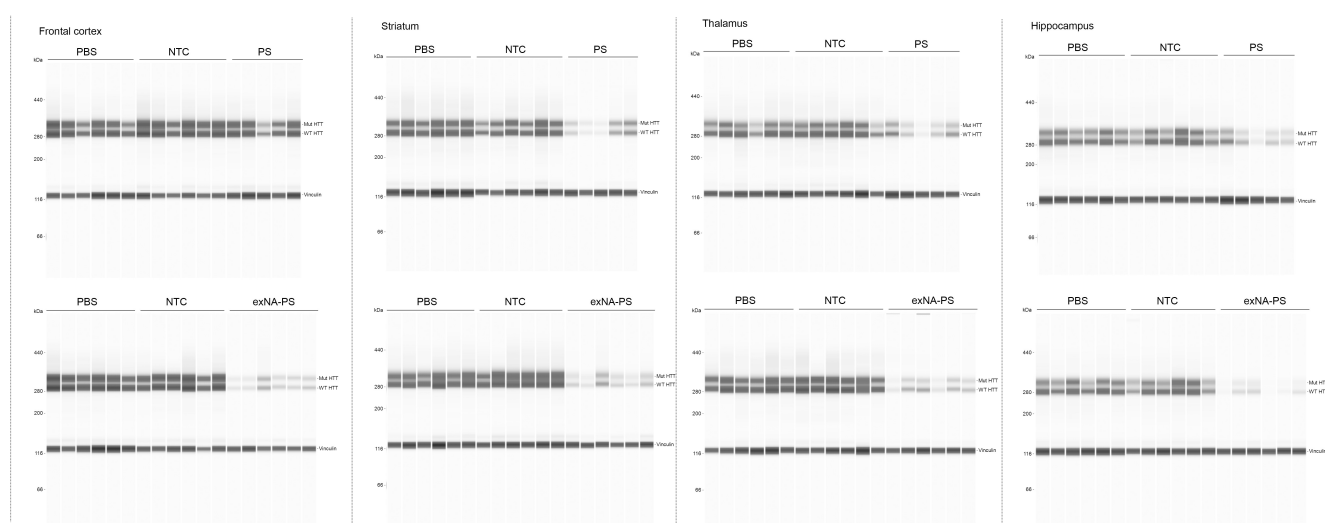


Fig S8. Raw capillary-based immunoassay images (ProteinSimple) of PBS, **D57** (NTC), **D58** (or PS) and **D59** (or exNA-PS) compounds in YAC128-HD mice. Divalent siRNAs with and without the introduction of the exNA modification were injected at 20nmol dose ICV at 8 weeks. The level of WT huntingtin protein levels normalized to vinculin loading control were evaluated six months following ICV injections (n=6).

siRNA#	D2-b	D9-b	D11-b*	D20-b	D26-b
IC50 (M ^a)	710E-09	313E-09	248E-12	20E-09	89E-09
IC50 of corresponding control	32E-09	32E-09	107E-12	45E-09	45E-09
% Potency Change	-2119	-878	-132	+56	-97
siRNA#	D33-b*	D34-b			
IC50	117E-12	36E-09			
IC50 of corresponding control	107E-12	45E-09			
% Potency Change	-9.3	+20			

^a Molar concentration (mol/L)

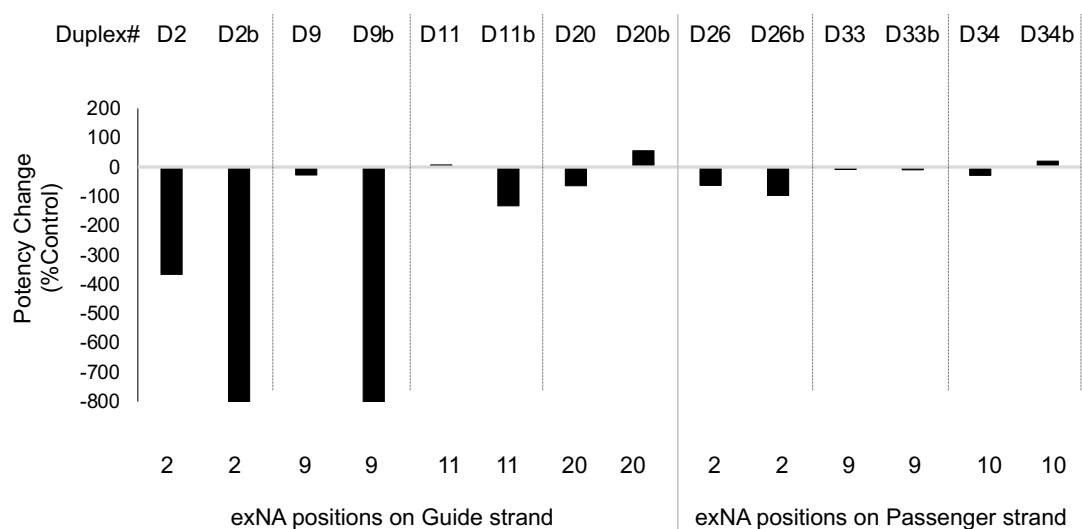
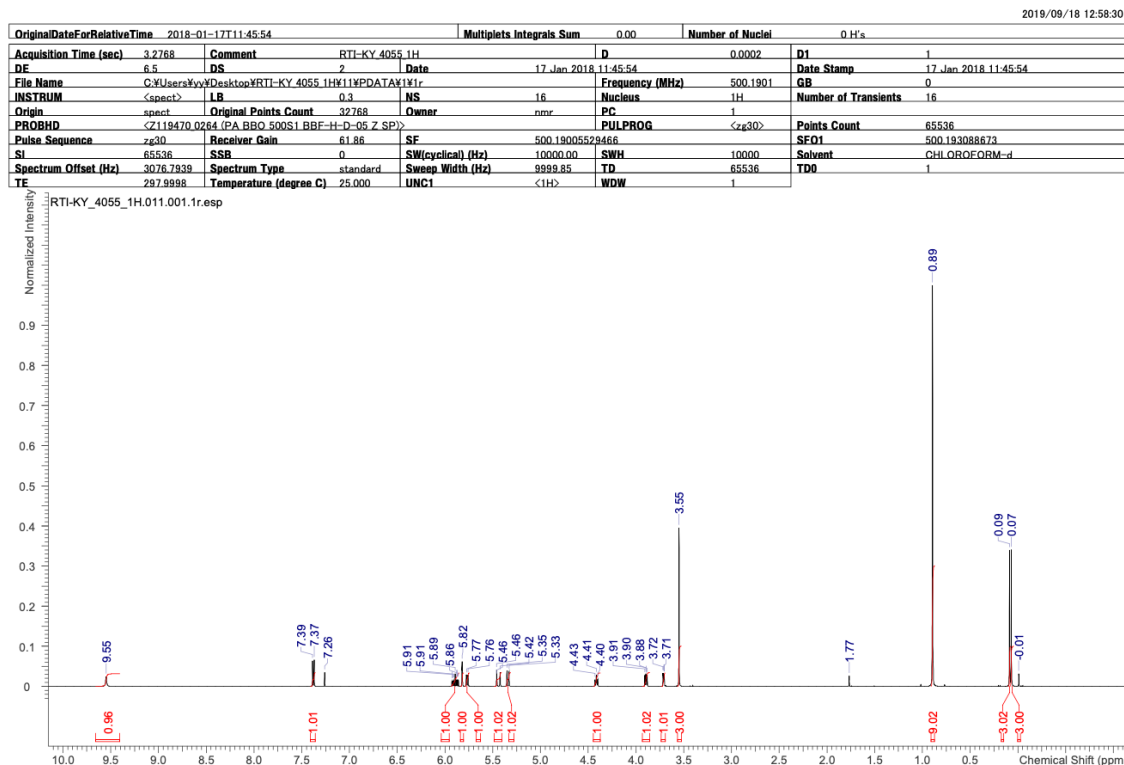


Fig S9. siRNA potency changes in different sequence context.

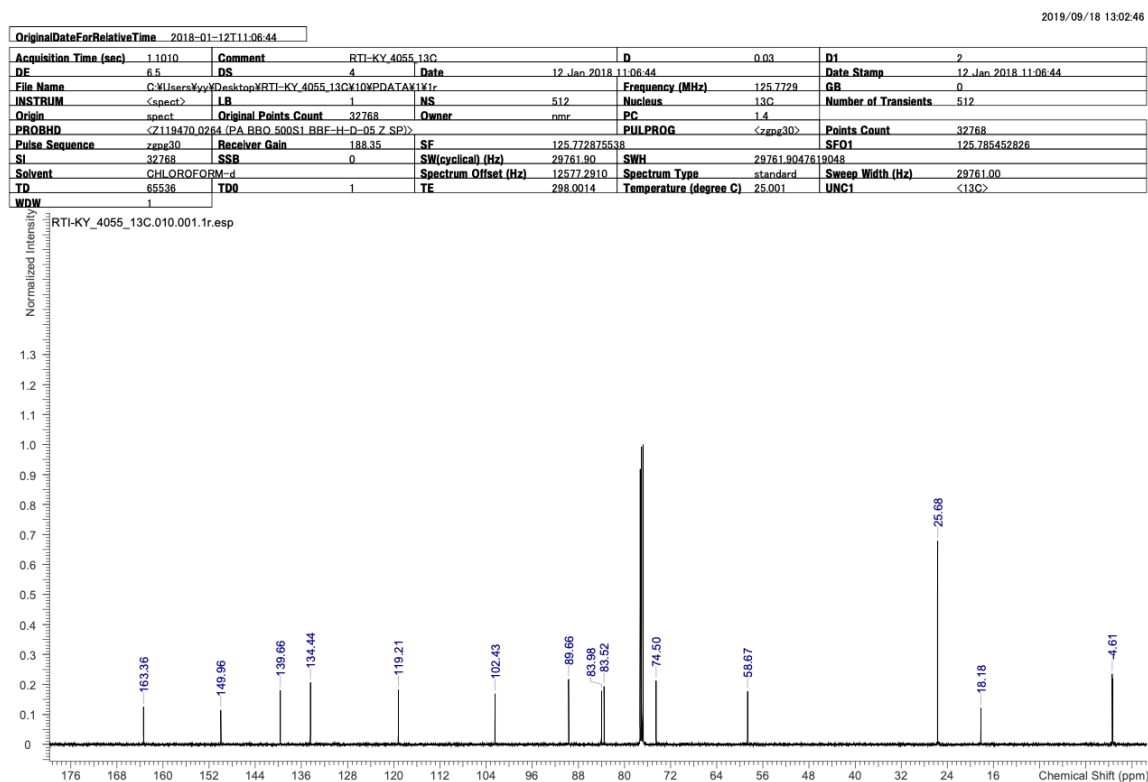
Reference:

1. Pradere, U. et al. Synthesis of 5'-Methylene-Phosphonate Furanonucleoside Prodrugs: Application to D-2'-Deoxy-2'- α -fluoro-2'- β -C-methyl Nucleosides. *Org. Lett.* **14**, 4426-4429 (2012).
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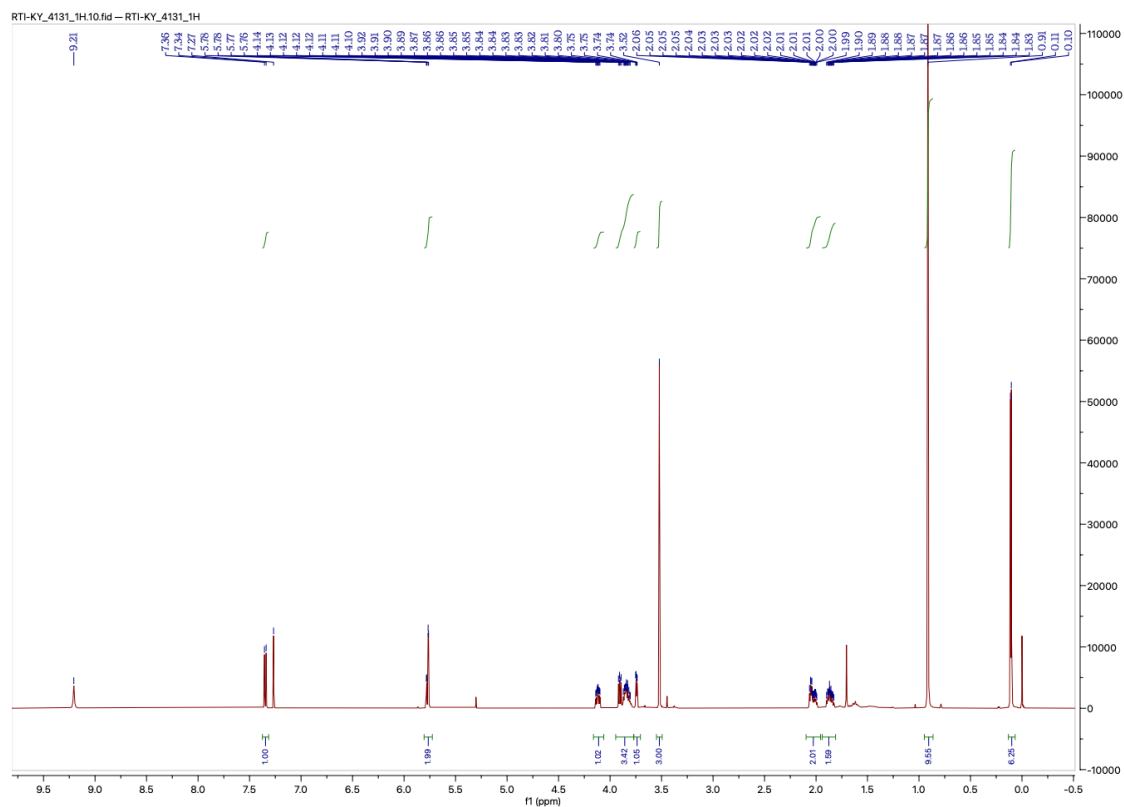
Compound **2a**, ^1H -NMR (500 MHz, CDCl_3)



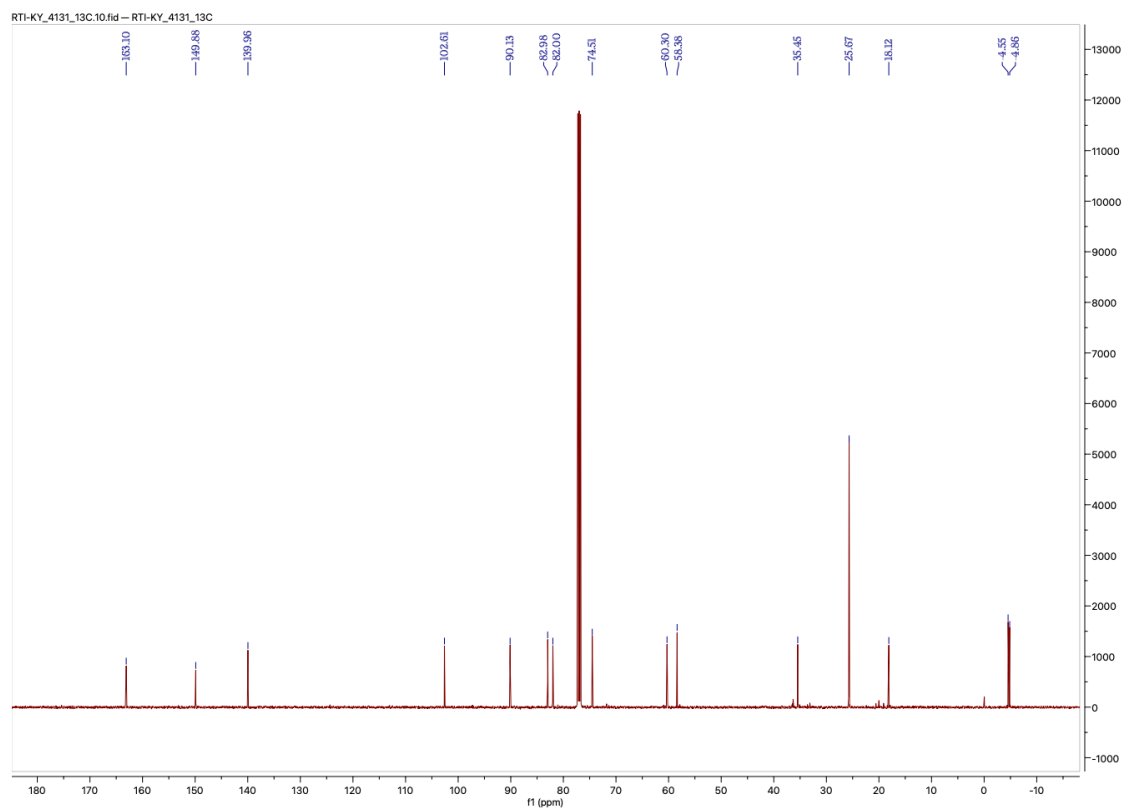
Compound **2a**, ^{13}C -NMR (126 MHz, CDCl_3)



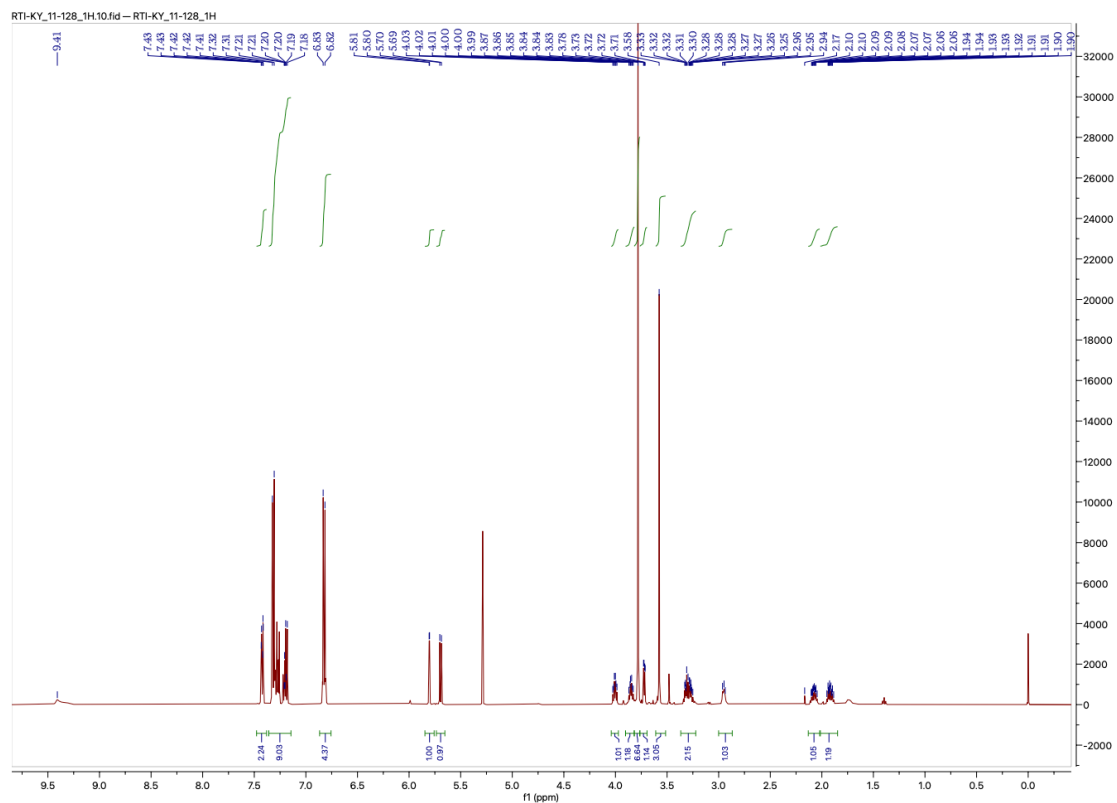
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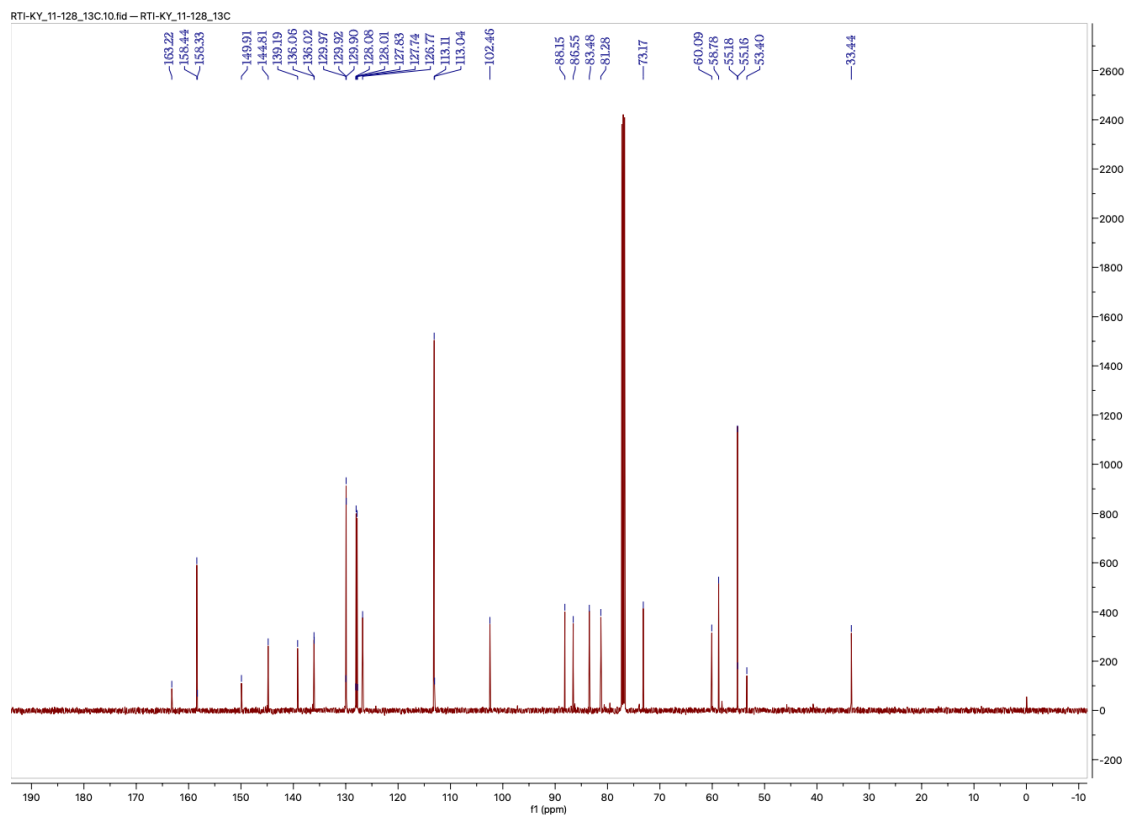
Compound **3a**, ^{13}C -NMR (126 MHz, CDCl_3)



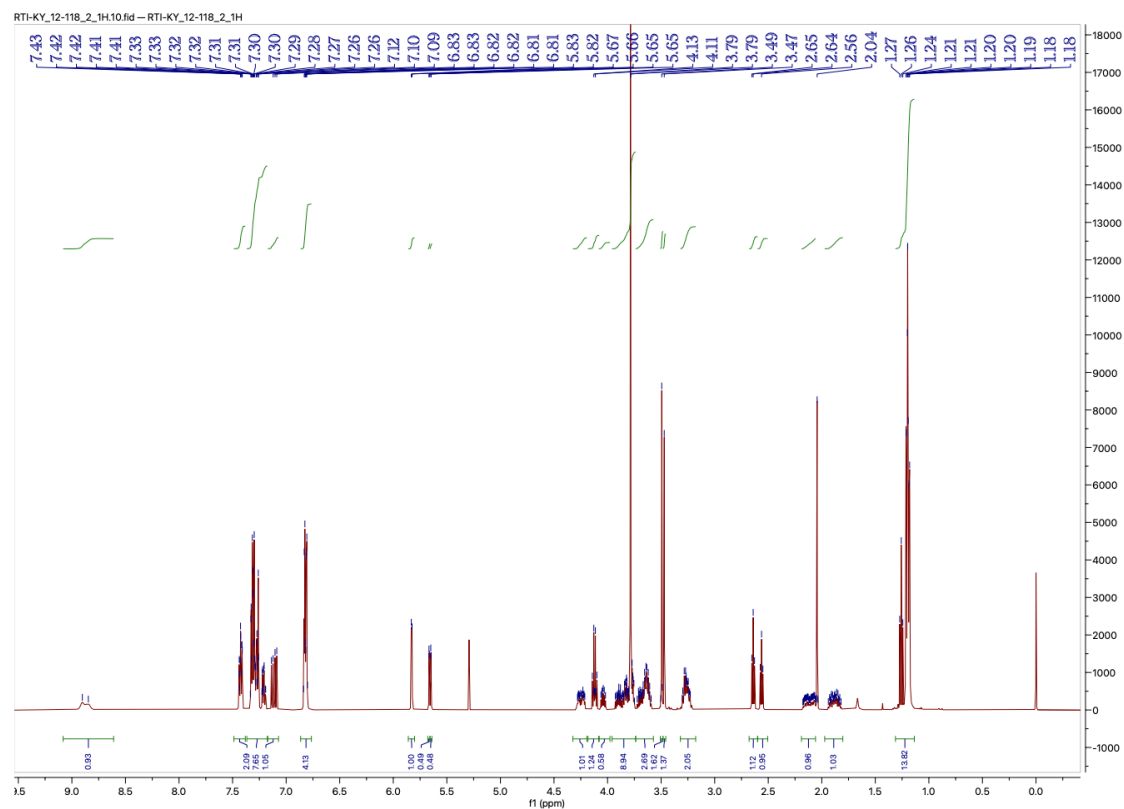
Compound **5a**, ^1H -NMR (500 MHz, CDCl_3)



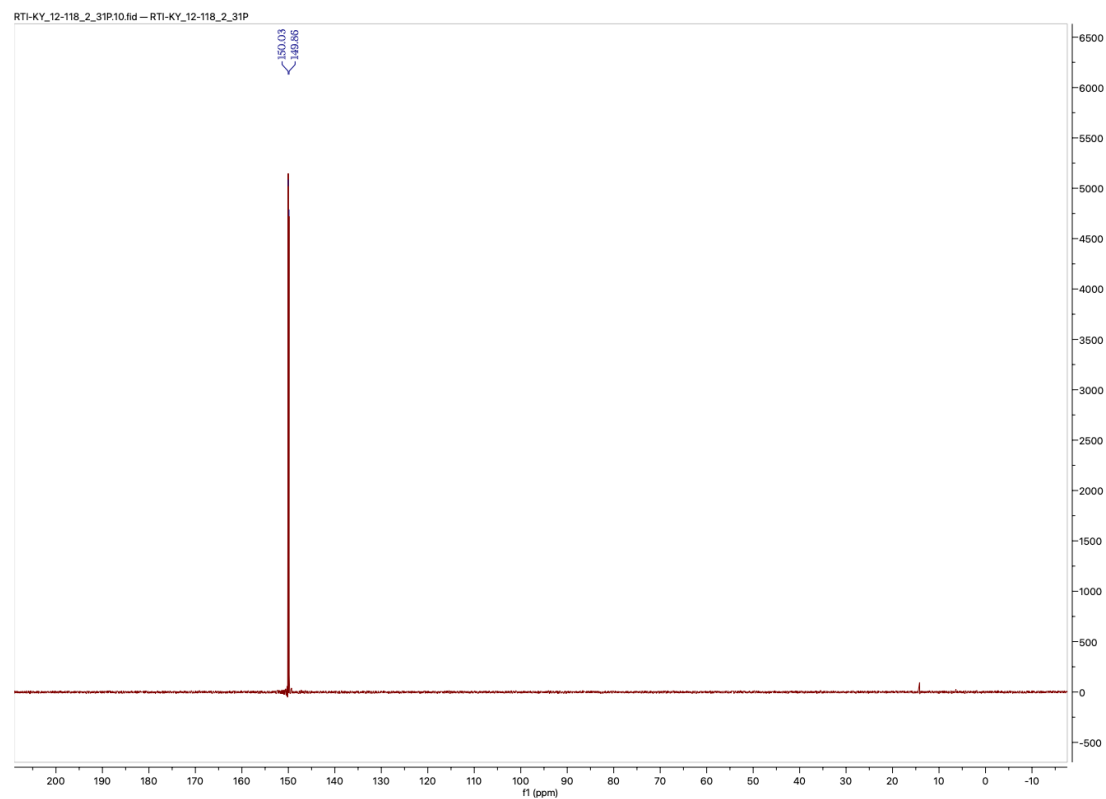
Compound **5a**, ^{13}C -NMR (126 MHz, CDCl_3)



Compound **6a**, ^1H -NMR (500 MHz, CDCl_3)



Compound **6a**, ^{31}P -NMR (202 MHz, CDCl_3)

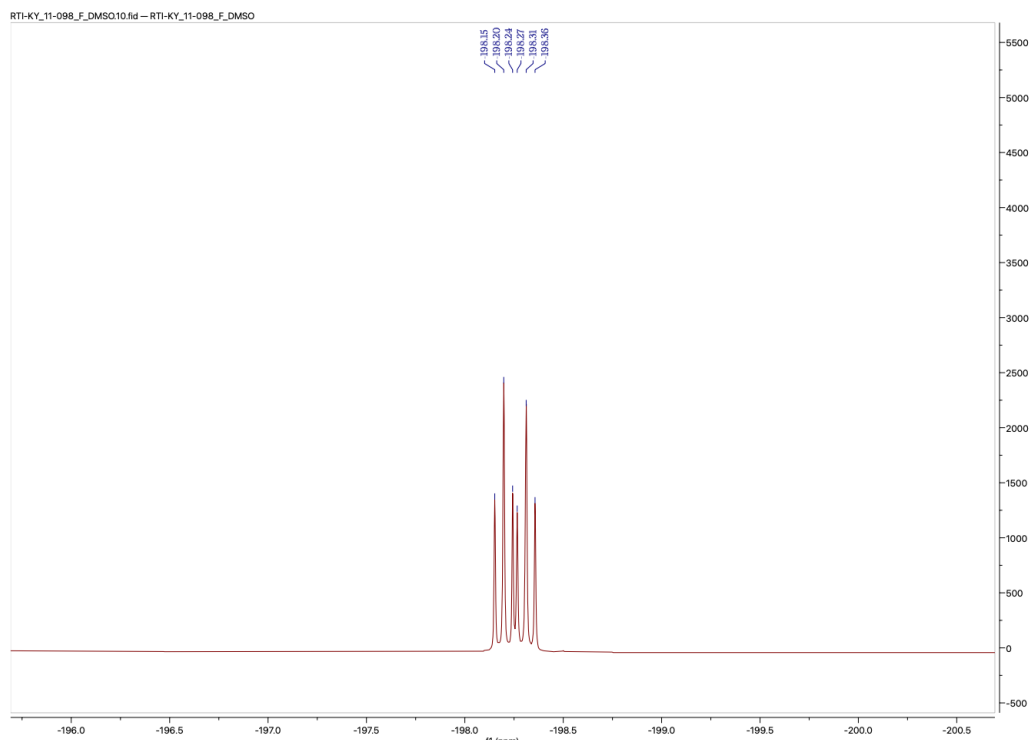


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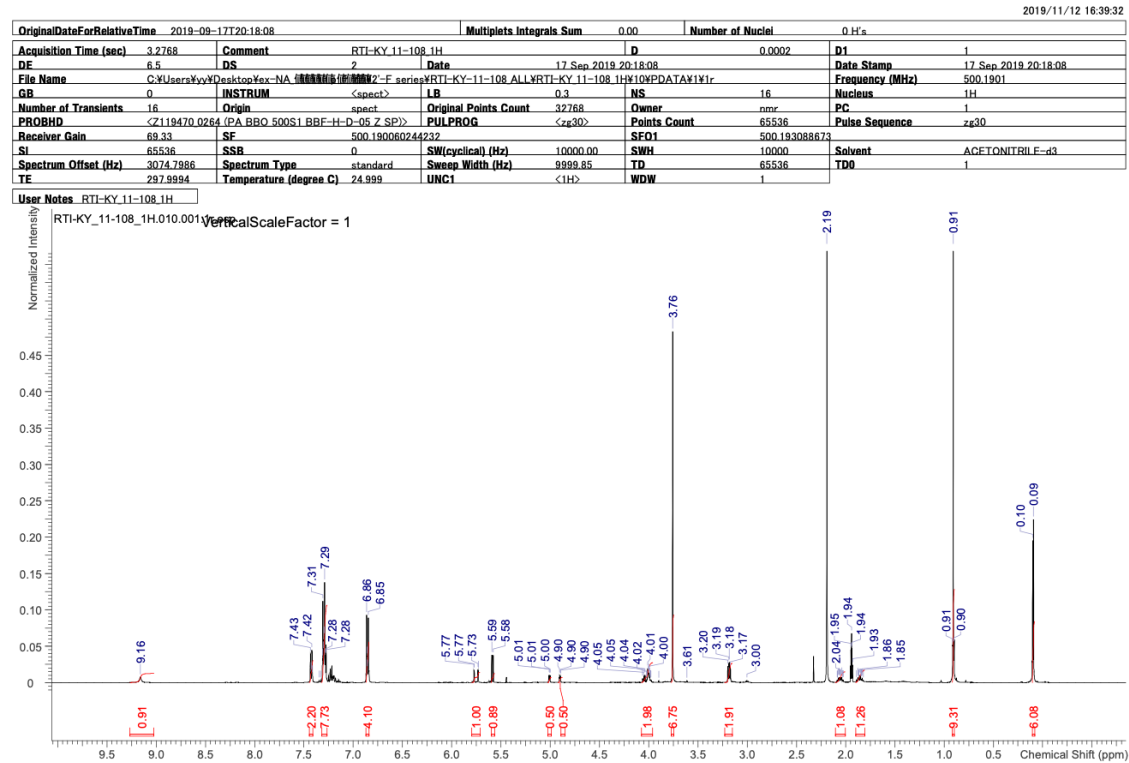
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150.64
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90.30
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83.39
78.87
78.74
60.22
26.01
22.21
18.25
14.56
4.39
4.45

f1 (ppm)

Compound **2b**, ^{19}F -NMR (DMSO- d_6 , 470 MHz)

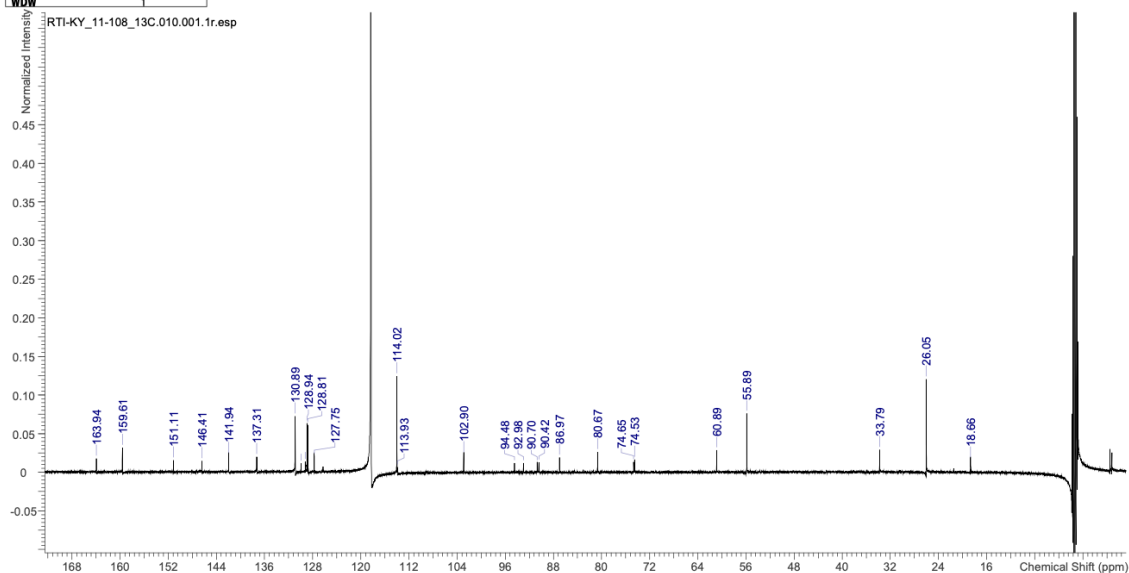


Compound **4b**, ^1H -NMR (CD_3CN , 500 MHz)

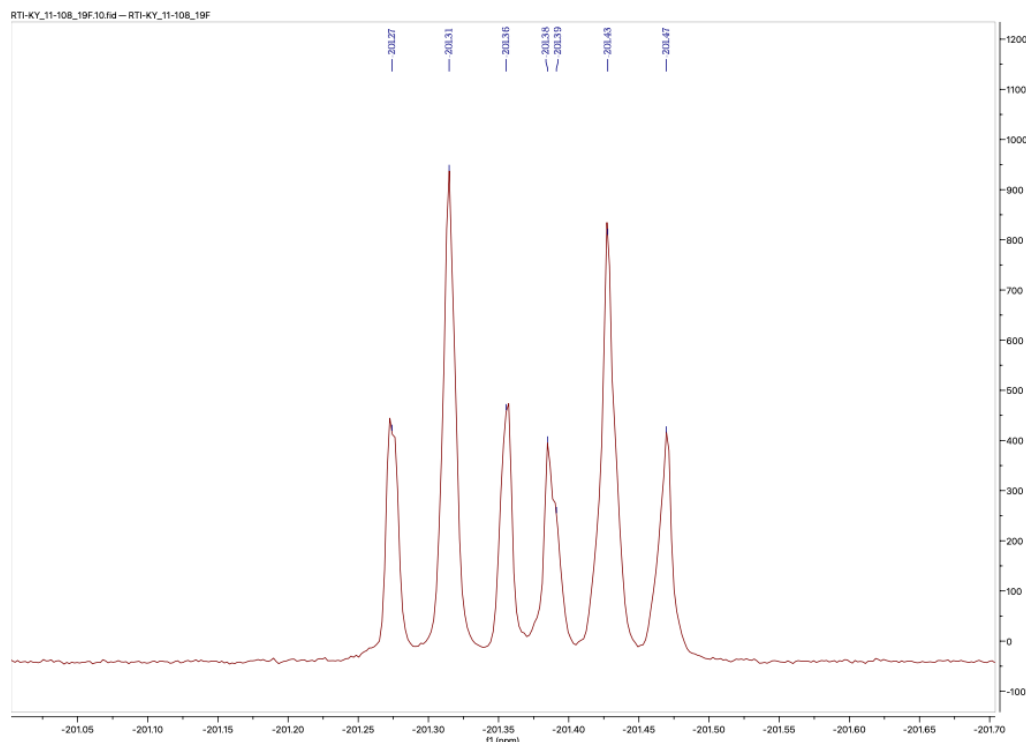


Compound **4b**, ^{13}C -NMR (CD_3CN , 126 MHz)

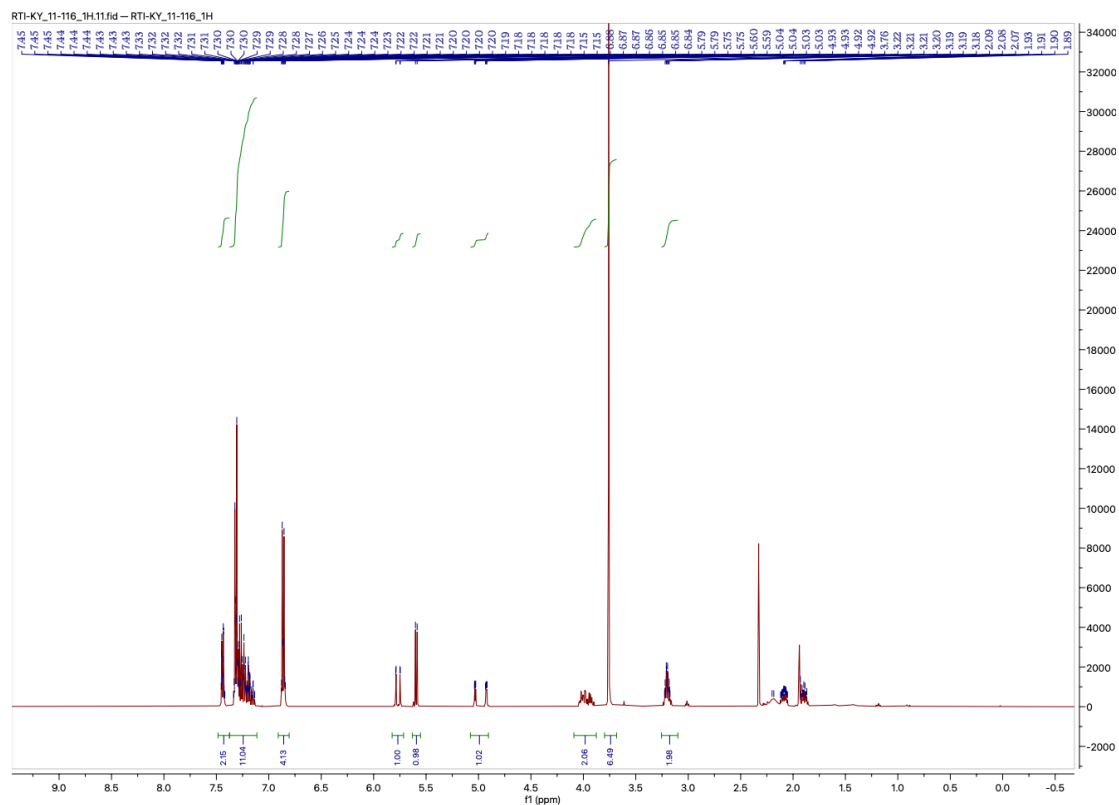
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Solvent	ACETONITRILE-d3	SW(cyclical) (Hz)	29761.90	Spectrum Type	standard
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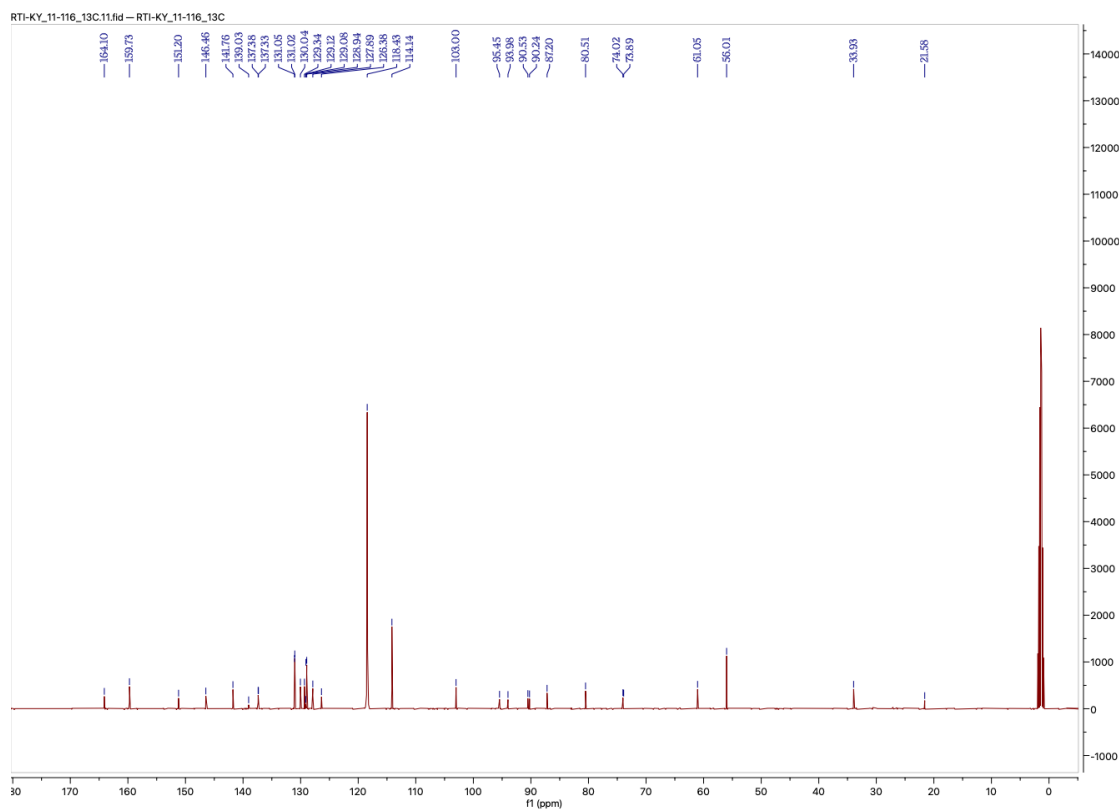
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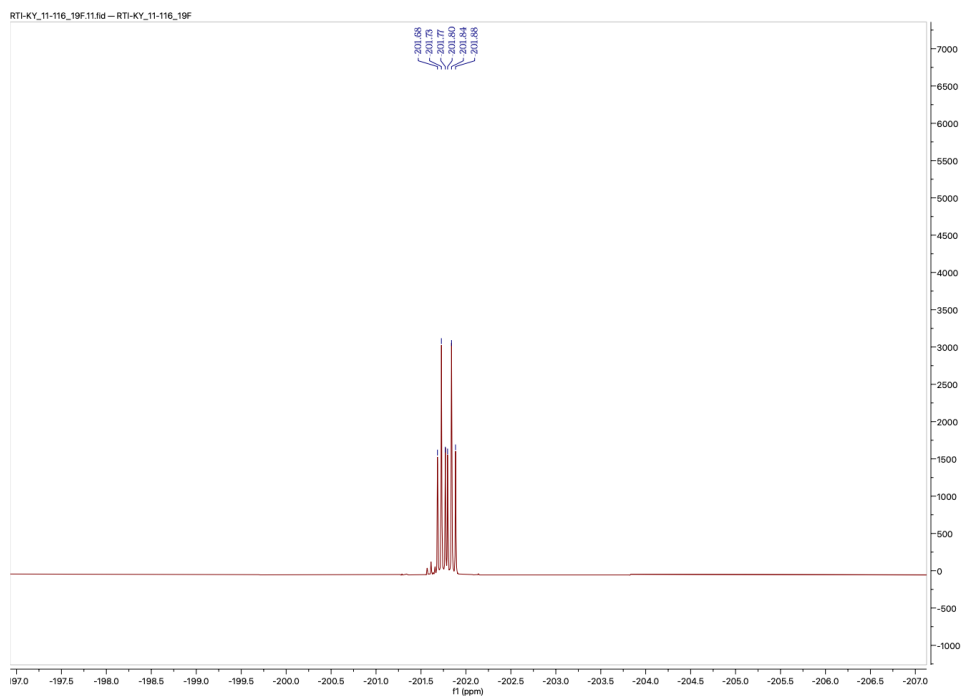
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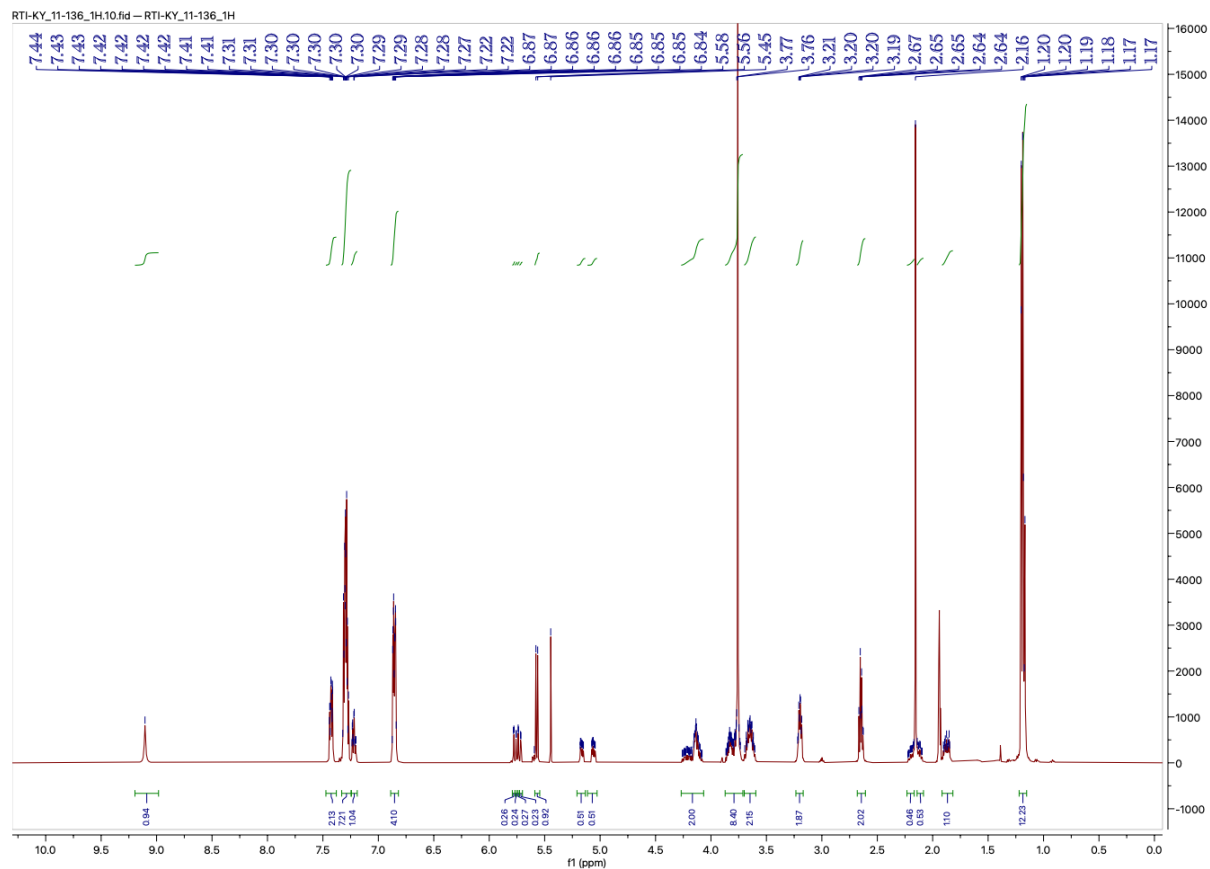
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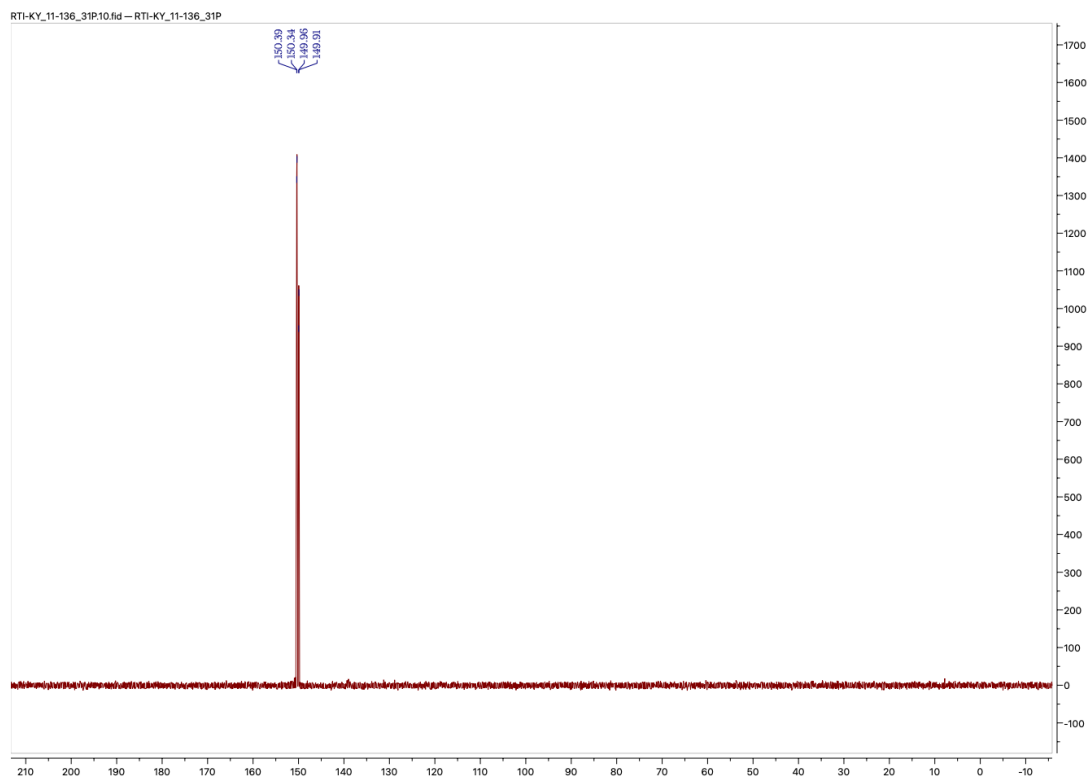
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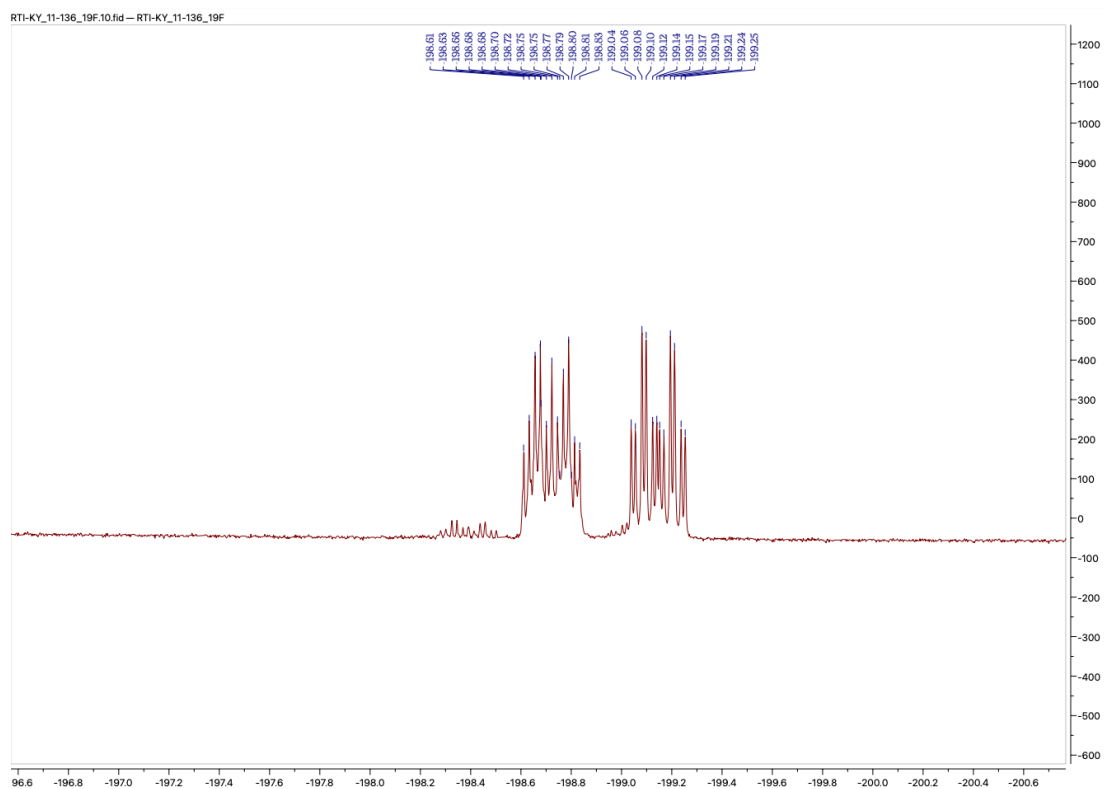
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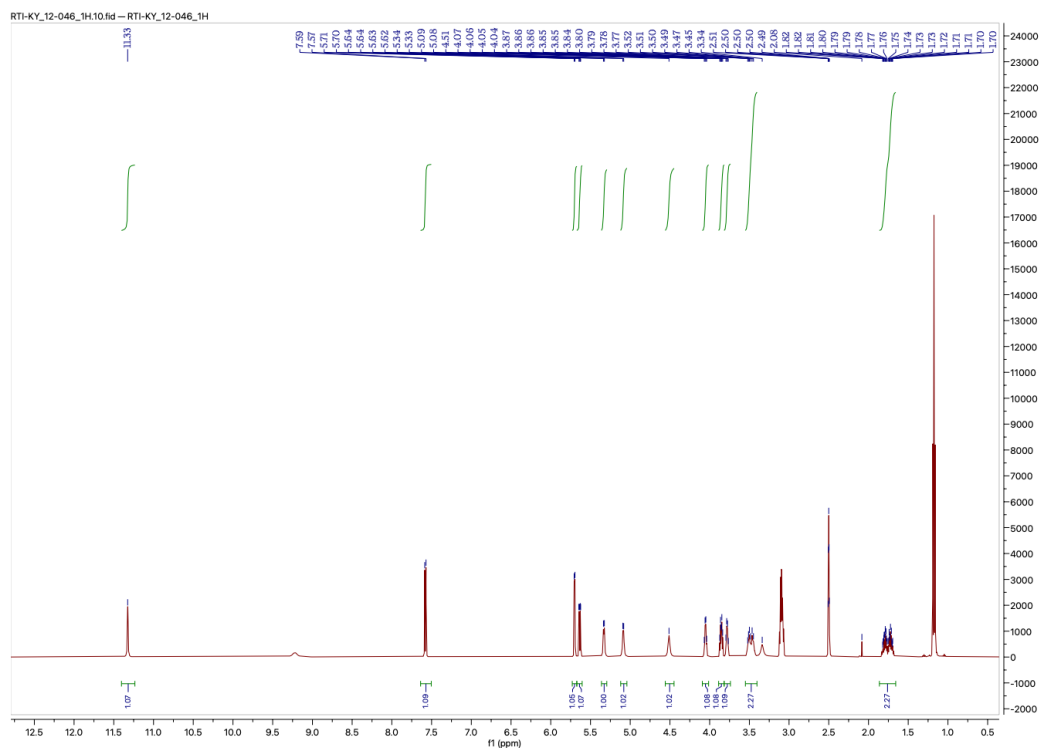
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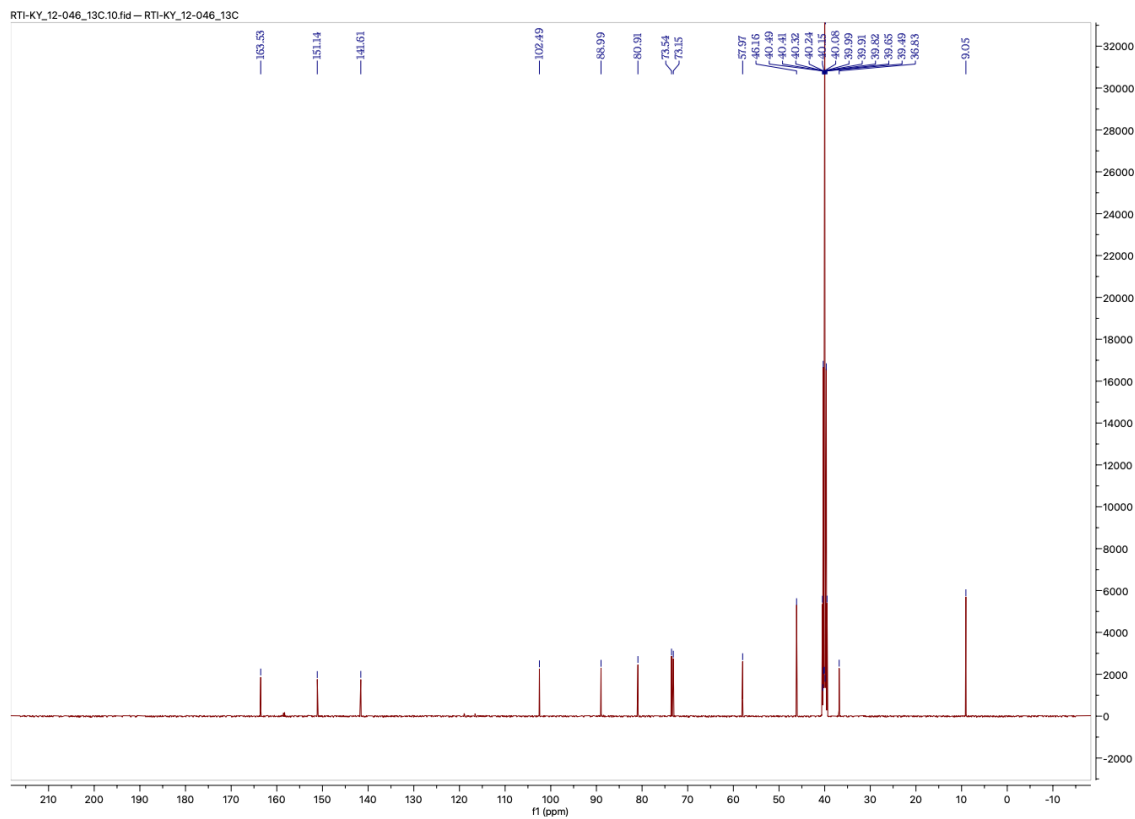
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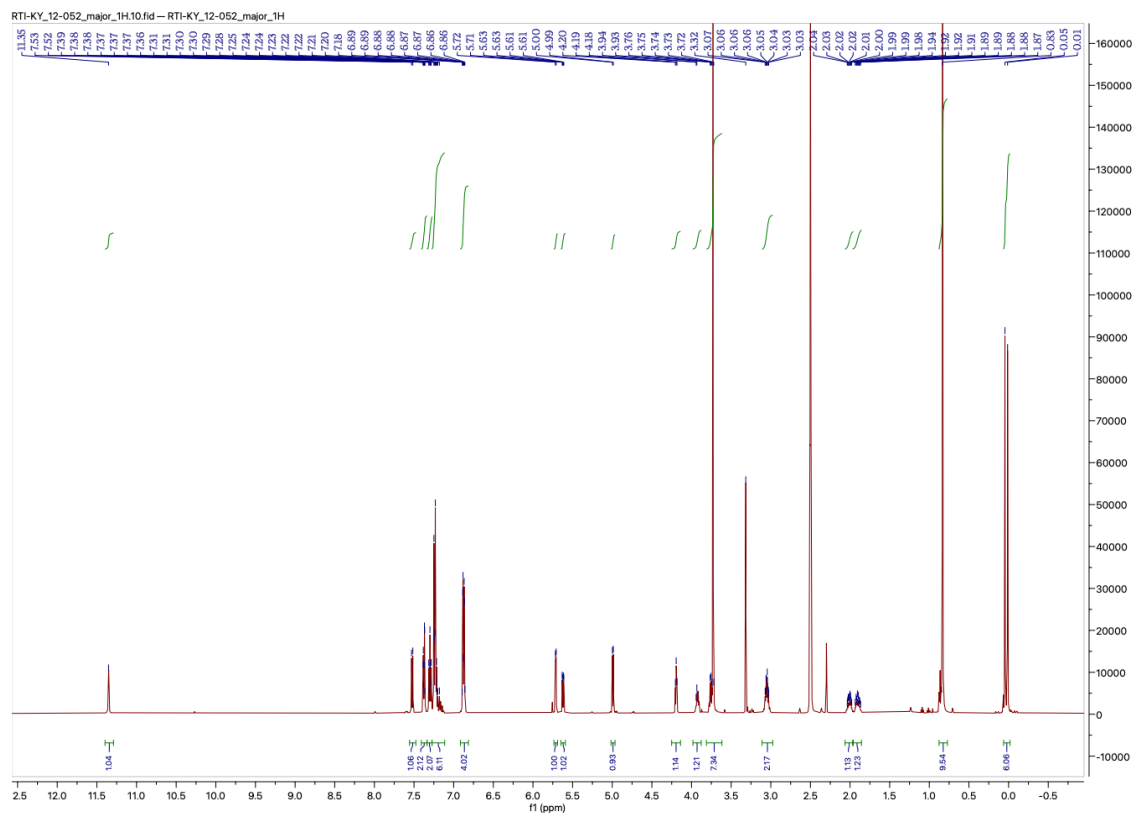
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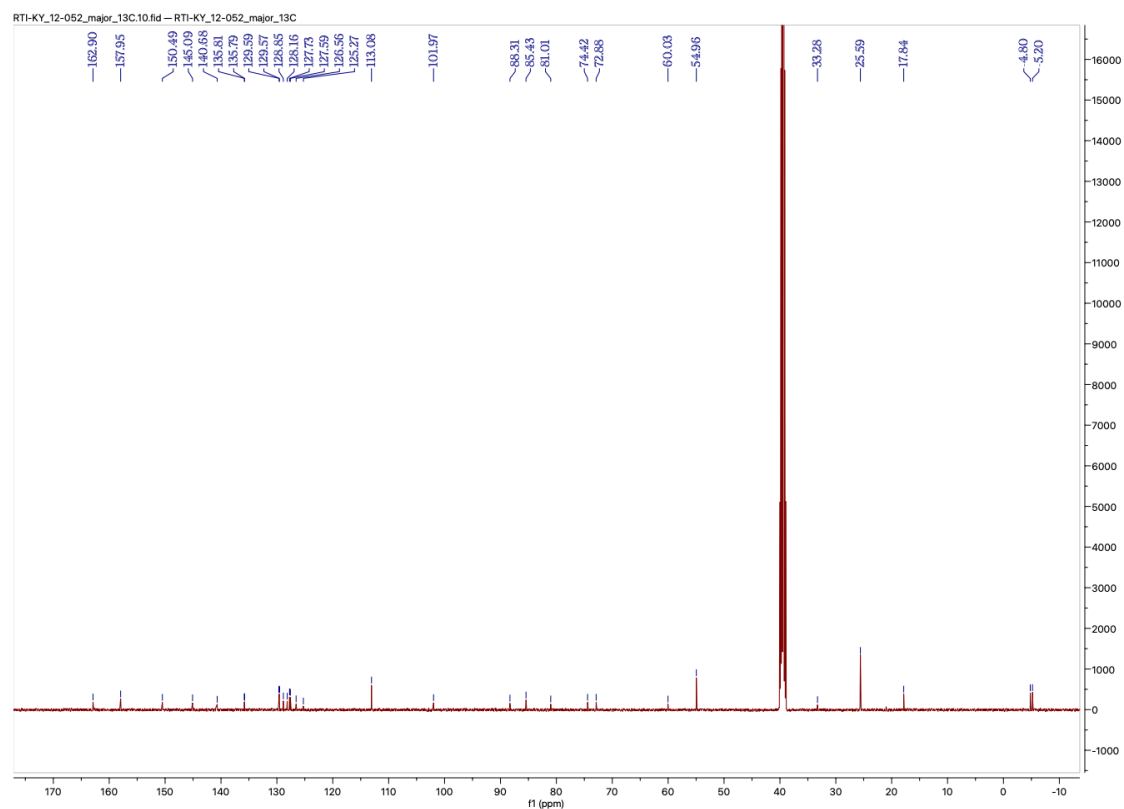
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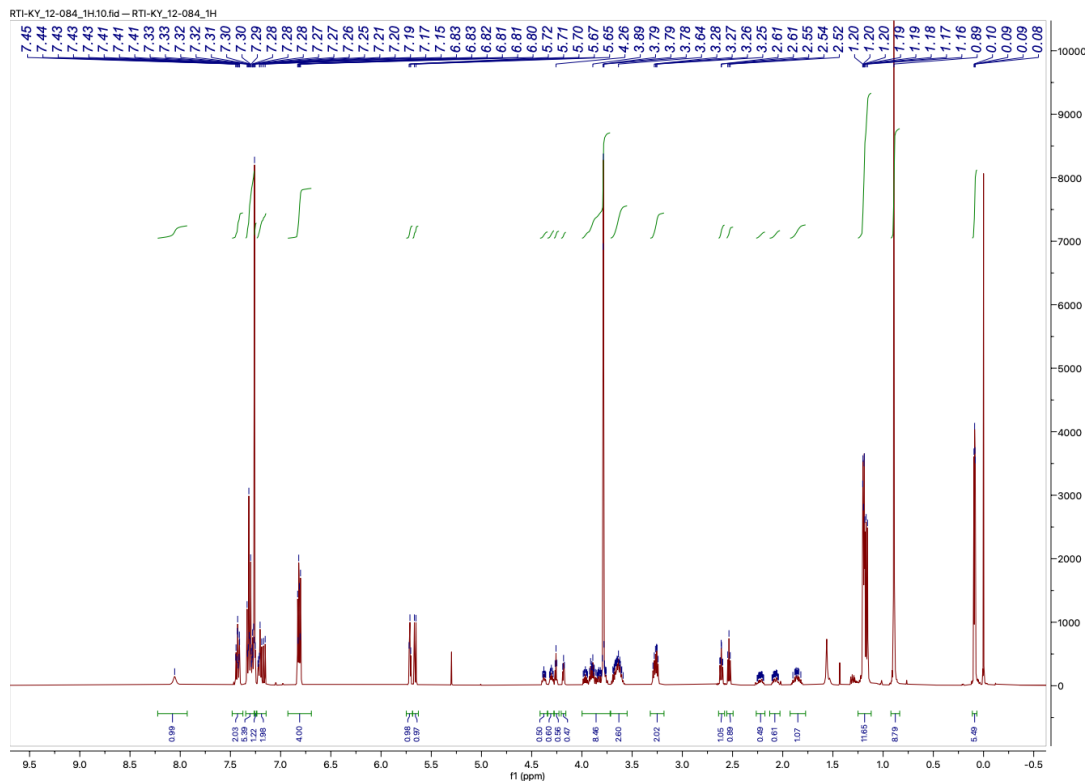
Compound **8**, ^1H -NMR (DMSO- d_6 , 500 MHz)



Compound **8**, ^{13}C -NMR (DMSO- d_6 , 126 MHz)



Compound **9**, ^1H -NMR (CDCl_3 , 500 MHz)



Compound **9**, ^{31}P -NMR (CDCl_3 , 202 MHz)

