

Supplementary Information

Exploring Neutral Backbones in Phosphorodiamidate Morpholino Oligomers: Synthesis and Biophysical Properties

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1. Materials and Methods

1.1. General chemical methods

All reagents were purchased from commercially available sources and used without further purification, unless specified. Reactions were carried out in oven dried glassware under argon atmosphere. Solvents were purified and dried according to recommended procedures. Thin layer chromatography (TLC) was carried out on sheets of silica gel 60 F254 on aluminium (layer thickness 0.25 mm, Merck). Visualization of the TLC was achieved under UV light and staining of TLC was carried out in standard staining solutions (CAM, Ninhydrin, KMnO_4). Reflux and other heating were performed in oil bath. Chromatographic purification of products was accomplished by column chromatography on silica gel (mesh 60-120 and 100-200). Chlorophosphoramidate and phosphoramidite dimers were freshly purified and dried over P_2O_5 in vacuum desiccators. ^1H , ^{13}C and ^{31}P -NMR spectra were recorded on Bruker NMR spectrometer (300 MHz). Chemical shifts (δ) are given in ppm relative to the solvent residual peak or TMS as internal standard. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet. High Resolution Mass Spectra (HRMS) were recorded in a QTOF I (Quadrupole hexapole TOF) mass spectrometer with an orthogonal Z spray electrospray interface on Micro (YA 263) mass spectrometer (Manchester, UK). Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectra were recorded on Bruker UltrafleXtreme MALDI-TOF/ TOF system. α -Cyano-4-hydroxycinnamic acid (CHCA) was used as a matrix in 1:1 water-acetonitrile containing 0.1% TFA. HPLC of the 9-mer morpholino oligonucleotide was performed on Shimadzu SPD-20A and Waters 1525 binary pump system equipped with Waters 2998 PDA detector. The chromatogram was recorded with C18 (Waters SPHERISORB C18 ODS2 and Waters XBridge BEH Shield RP18) column using 0.1M Ammonium acetate buffer (in H_2O)- CH_3CN gradient system (20-50% in 20min) with a flow rate of 1ml/min or 2ml/min. HPLC analyses of Trityl-PMO 1 and 2 were conducted using HPLC Conditions 1 and 2 for standardization. The 12-mer oligonucleotides PMO3-PMO7 were analyzed under HPLC Condition 3, while PMO8-PMO12 were analyzed under HPLC Condition 4.

HPLC Condition 1: 0.1M ammonium acetate buffer (in H_2O)- CH_3CN gradient system (20-50% in 15min with flow rate of 1ml/min) in Waters SPHERISORB C18 ODS2 on Shimadzu SPD-20A system in Trityl-ON mode.

HPLC Condition 2: 0.1M ammonium acetate buffer (in H_2O)- CH_3CN gradient system (20-50% in 20min with flow rate of 2ml/min) in Waters XBridge BEH Shield RP18 OBD Prep column (10mm x 250mm) on Waters 1525 binary pump system equipped with Waters 2998 PDA detector in Trityl-ON mode.

HPLC Condition 3: 0.1M ammonium acetate buffer (in H_2O)- CH_3CN gradient system (10-50% in 20 min with flow rate of 1ml/min) in Waters SPHERISORB C18 ODS2 on Waters 1525 binary pump system equipped with Waters 2998 PDA detector system.

HPLC Condition 4: 0.1M triethylammonium acetate buffer (in H_2O)- CH_3CN gradient system (10-80% in 20 min with flow rate of 1ml/min) in Shiseido Capcell pak C18 ODS2 Waters 1525 binary pump system equipped with Waters 2998 PDA detector.

1.2. Synthesis of Morpholino Oligomers

9mer PMOs were synthesized manually using chlorophosphoramidate monomers with a coupling time of 1.5 h (30 min x 3 times) and 9 mer was also synthesized using phosphoramidite chemistry in automated Oligosynthesizer, K&A H-8 DNA/RNA/LNA Synthesizer, K & A Laborgeräte GbR, Germany with coupling time 18 min (6 min x 3 times). 12 mer polyT PMO (PMO3-7) was synthesized using 5'-

phosphoramidites. PMO8-12 mixed sequences were synthesized using chlorophosphoramidate monomers and the modified dimers were incorporated by amidite coupling method¹.

1.3. Thermal melting study of modified Morpholino Oligomers

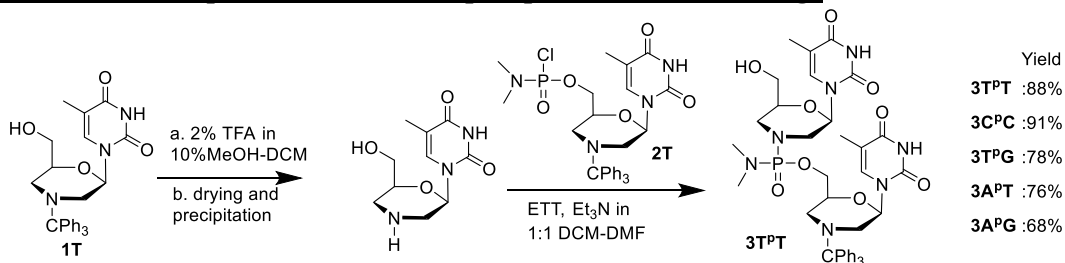
All PMO-DNA and PMO-RNA UV-melting experiments were performed using 1 μ M concentration of each strand and 40 mM phosphate buffer (pH 7) containing 100 mM NaCl. UV-Vis spectra were recorded on Cary 3500 UV-Visible spectrometer equipped with Peltier temperature controlling device and thermal software. All the samples were allowed to anneal at 80°C for 5 minutes and then cooled slowly to 5°C at a rate of 1°C/min at 260 nm absorption. Data collection for PMO3-7 with complementary DNA and RNA was performed at a rate of 1°C/min at 260 nm absorption. Data collection for PMO8-12 with complementary DNA and RNA was performed at a rate of 2°C/min at 260 nm absorption.

1.4. CD-spectral experiments

Circular Dichroism experiment of all PMO-DNA and PMO-RNA was performed using 1 μ M concentration of each strand and 0.04 M phosphate buffer containing 100 mM NaCl in a JASCO J-1500 Spectropolarimeter. All the samples were allowed to anneal at 80°C for 5 minutes and then cooled slowly to 10°C at a rate of 1°C/min. Then all the duplexes were stored at 4°C. All data collection were carried out at 10°C.

2. Synthetic procedure of dimer blocks and their amidate and amidites

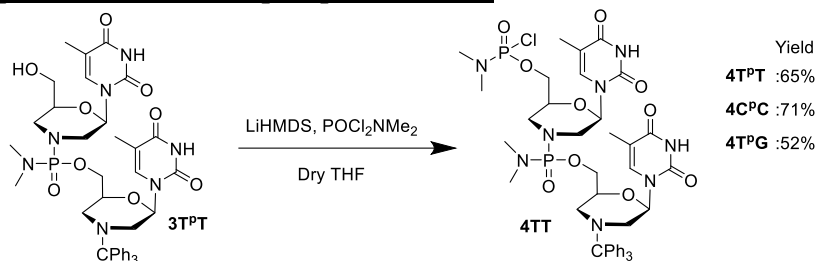
Synthesis of 5'-OH morpholino dimers with phosphorodiamidate linkage



Compound **1T** (500mg) was dissolved in DCM (9ml) containing 10% MeOH (1ml) and cooled in ice bath. Trifluoroacetic acid (300 μ L) was added drop wise and the reaction was left for 1hr at room temperature. After complete consumption of the starting material, the reaction mixture was evaporated to dryness and precipitated in diethyl ether. The precipitate was filtered and washed repeatedly with diethyl ether and hexane mixture (1:1). The collected precipitate was dried under vacuum and re-dissolved in dry DCM-DMF mixture (1:1, 20ml). The reaction mixture was cooled in ice bath followed by the sequential addition of triethylamine (4 equiv.), ETT (0.2 equiv.) and compound **2T** (1.2 equiv.) and left at room temperature for 1hr. After the completion of the reaction (TLC analysis), the mixture was diluted with ethyl acetate and washed repeatedly with water to remove the DMF and finally with saturated NH_4Cl . Collected organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was precipitated in diethyl ether to obtain pure product to afford pure white solids. Sometimes column chromatography was performed (60-120 mesh silica with MeOH-DCM as eluent) to remove traces of impurities. 740 mg of **3TPT** was obtained from 500 mg of **1T**. 733 mg of **3CPC** was obtained from 500 mg of **1C**. 733 mg of **3TPG** was obtained from

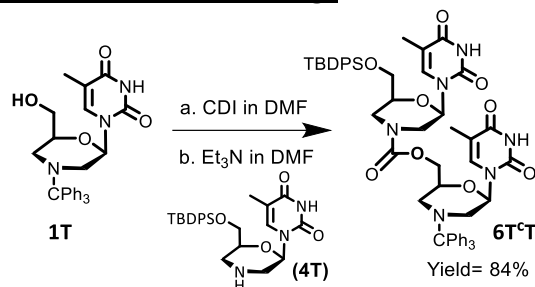
500 mg of **1T**. 637 mg of **3A^PT** was obtained from 500 mg of **1A**. 582 mg of **3A^PG** was obtained from 500 mg of **1A**.

Synthesis of morpholino dimer chlorophosphoramidates



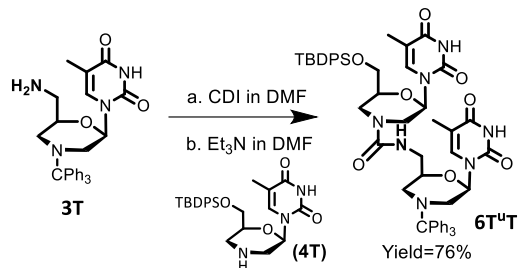
Compound **3T^PT** (200mg) was dissolved in dry THF (5ml) and cooled in an ice salt bath (-10°C approx). LiHMDS (1M in THF, 5 equiv.) was added in the reaction mixture dropwise under argon atmosphere followed by the slow addition of POCl₂NMe₂ (4 equiv.). The reaction mixture was monitored by TLC. After complete conversion of the starting material, the reaction mixture was quenched with saturated citric acid solution (1ml) and diluted with DCM. The organic layer was washed with half saturated NH₄Cl solution twice and dried over Na₂SO₄. The organic layer was concentrated and passed through celite bed. Then the crude product was purified by column chromatography (60-120 mesh silica with MeOH-acetone-DCM as eluent) to afford light brown solids. 150 mg of **4T^PT** was obtained from 200 mg of **3T^PT**. 160 mg of **4C^PC** was obtained from 200 mg of **3C^PC**. 120 mg of **4T^PG** was obtained from 200 mg of **3T^PG**.

Synthesis of morpholino dimer with carbamate linkage



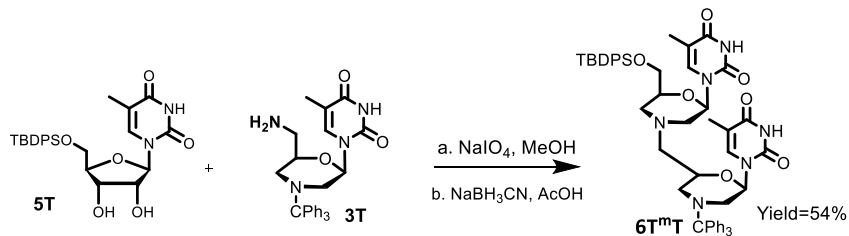
For the synthesis of compound **6T^cT**, compound **1T** (300mg) was dissolved in dry DMF (6ml) followed by the addition of *N,N'*-Carbonyldiimidazole (CDI) (1 equiv.) at ice cold condition. After that the reaction was stirred for 4 hr at room temperature. After complete consumption of **1T** (TLC analysis), the reaction mixture was cooled in an ice bath followed by the addition of Et₃N (2 equiv.) and compound **4T** (0.9 equiv.). The ice bath was removed, and the reaction mixture was stirred at 70°C for overnight. After that the reaction mixture was diluted with ethyl acetate and washed repeatedly with cold water and finally with brine. Collected organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude product was then purified by column chromatography using 60-120 mesh silica with Acetone-MeOH-DCM as eluent. 510 mg of **6T^cT** was obtained from 300 mg of **1T**.

Synthesis of morpholino dimer with urea linkage



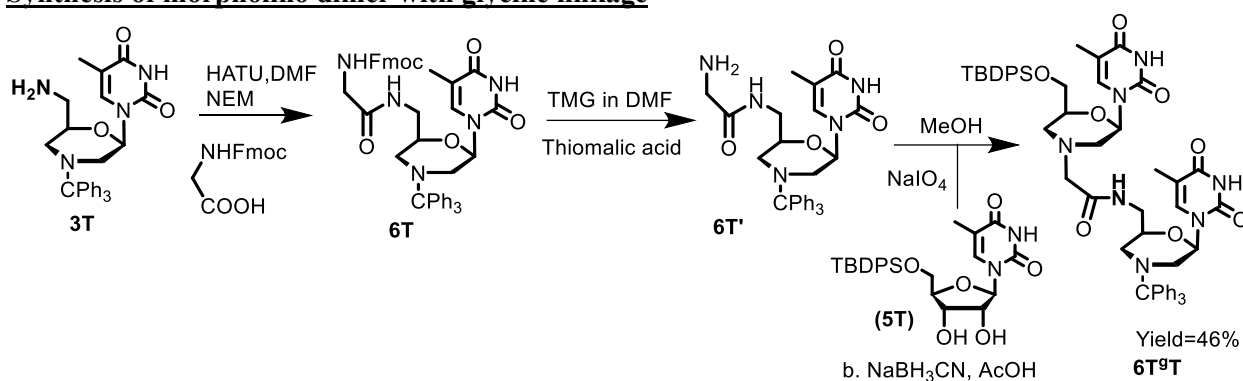
For the synthesis of compound **6T^uT**, *N,N'*-Carbonyldiimidazole (CDI) (1 equiv.) was dissolved in dry DMF (10ml) followed by the slow dropwise addition of **3T** (300mg in 10 ml DMF) in an ice bath and stirred for 1 hr at room temperature. After complete consumption of **3T** (TLC analysis), the reaction mixture was cooled in an ice bath followed by the addition of Et₃N (2 equiv.) and **4T** (0.9 equiv. dissolved in 6ml of DMF). The ice bath was removed, and the reaction mixture was stirred at room temperature for another 3 hr. After that the reaction mixture was diluted with ethyl acetate and washed repeatedly with cold water and finally with brine. Collected organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude product was then purified by column chromatography using 60-120 mesh silica with Acetone-MeOH-DCM as eluent. 467 mg of **6T^uT** was obtained from 300 mg of **3T**.

Synthesis of morpholino dimer with methylene linkage



Compounds **5T** (500mg) and **3T** (1.2 equiv) were dissolved in MeOH (20ml) followed by the addition of NaIO₄ and the reaction mixture was stirred for 8hr at room temperature. It was then filtered through a sintered funnel and washed residue was washed repeatedly with methanol. After that, 4Å molecular sieve powder was added followed by NaBH₃CN (2.2 equiv) to the reaction mixture. Then the reaction mixture was cooled in an ice bath and acetic acid (1 equiv) was added in a dropwise manner. After stirring overnight, the reaction mixture was filtered through a sintered funnel and concentrated in a rotary evaporator. The mixture was diluted with ethyl acetate and washed repeatedly with water and finally with brine. Collected organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude product was then purified by column chromatography using 60-120 mesh silica with Acetone-MeOH-DCM as eluent to obtain **6T^mT**. 515 mg of compound **6T^mT** was obtained from 500 mg of **5T**.

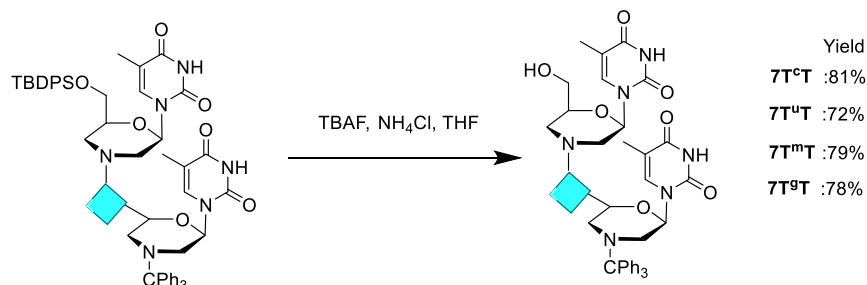
Synthesis of morpholino dimer with glycine linkage



Compound **3T** was subjected to standard peptide coupling condition with the Fmoc-glycine (1.3 equiv) in the presence of HATU (1.3 equiv) and 4-ethylmorpholine (2 equiv) in dry DMF solvent. After work up and purification, compound **6T** was obtained in 89% yield. The requisite amount for the reductive amination in the next stage was taken in a round bottom flask and Fmoc deprotection was performed using 5% TMG (1,1,3,3-tetramethylguanidine) in DMF (10ml) in the presence of thiomalic acid (1.5 equiv) at room temperature. After complete deprotection, the mixture was diluted with ethyl acetate washed repeatedly with water and saturated NaHCO₃. The organic layer was dried over Na₂SO₄ and evaporated to dryness. This (compound **6T'**) was dried and used for next reaction without further purification.

Compound **5T** (500mg) and **6T'** (1.2 equiv) was dissolved in MeOH (20ml) followed by the addition of NaIO₄ and the reaction mixture was stirred for 8 hr. It was then filtered through a sintered funnel and the residue was dissolved multiple times with methanol 4Å molecular sieve powder was added followed by NaBH₃CN (2.2 equiv) to it. After that, the reaction mixture was cooled in an ice bath. Then acetic acid (1 equiv) was added in dropwise manner. After stirring overnight, the reaction mixture was filtered through a sintered funnel and concentrated in a rotary evaporator. The mixture was diluted with ethyl acetate and washed repeatedly with water and finally with brine. Collected organic layer was dried over Na₂SO₄ and evaporated to dryness. The crude mixture was then purified by column chromatography using 60-120 mesh silica with Acetone-MeOH-DCM as eluent to obtain **6T^{9T}**. 465 mg of **6T^{9T}** was obtained from 500 mg of **5T**.

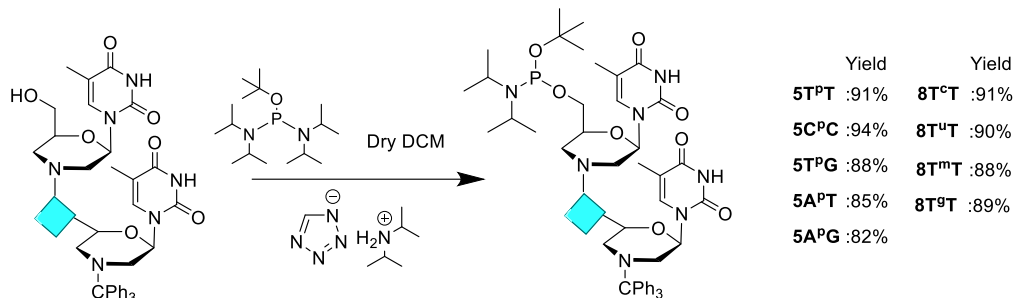
General method for TBDPS deprotection



The 5'-OTBDPS dimers were dissolved in dry THF followed by the addition of solid NH₄Cl (2 equiv.) and *tetra*-butylammonium fluoride (1.5 equiv., 1M in THF) and stirred for 3hr at room temperature. After completion of the reaction (TLC analysis), THF was removed in a rotary evaporator and re-dissolved in ethyl acetate. It was then washed repeatedly with water and finally with brine. Collected organic layer was

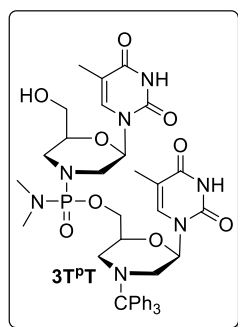
dried over Na_2SO_4 and evaporated to dryness. The crude product was then purified by column chromatography using 60-120 mesh silica with Acetone-MeOH-DCM as eluent to obtain compound .

General method for the synthesis of morpholino dimer phosphoramidites



Compound **3TT** (dried over P_2O_5) was dissolved in dry DCM (10ml) and 4Å molecular sieve powder was added in to it under argon atmosphere followed by the addition of 1-*tert*-butoxy-*N,N,N',N'*-tetraisopropylphosphanediamine (2 equiv.) and diisopropylammonium tetrazolide (0.5 equiv.). After overnight stirring the reaction mixture at room temperature, TLC showed complete conversion of the starting material. The reaction mixture was passed through celite bed and the clear solution was concentrated to dryness. The crude product was precipitated in hexane (twice) to obtain the product as a pure white solid. 455 mg of **5T^PT** was obtained from 400 mg of **3T^PT**. 453 mg of **5C^PC** was obtained from 400 mg of **3C^PC**. 430 mg of **5T^PG** was obtained from 400 mg of **3T^PG**. 414 mg of **5A^PT** was obtained from 400 mg of **3A^PT**. 393 mg of **5A^PG** was obtained from 400 mg of **3A^PG**.

3. Spectral Data

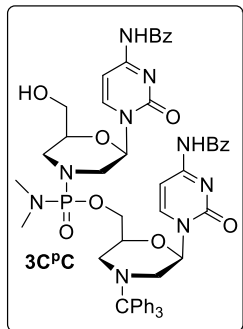


¹H NMR (300 MHz, Chloroform-*d*) δ 9.54 (s, 2H), 7.45 (d, $J = 6.9$ Hz, 5H), 7.37 – 7.22 (m, 8H), 7.16 (t, $J = 7.3$ Hz, 3H), 7.02 (dd, $J = 8.7, 1.4$ Hz, 1H), 6.17 (ddd, $J = 9.6, 7.0, 2.3$ Hz, 1H), 5.65 (ddd, $J = 12.9, 9.9, 2.7$ Hz, 1H), 4.38 (s, 1H), 4.05 – 3.92 (m, 1H), 3.85 (dq, $J = 7.6, 4.0$ Hz, 2H), 3.62 (dt, $J = 21.1, 4.5$ Hz, 2H), 3.47 – 3.22 (m, 3H), 3.18 – 3.03 (m, 1H), 2.93 – 2.80 (m, 1H), 2.61 (t, $J = 10.0$ Hz, 8H), 1.90 (dd, $J = 6.9, 1.2$ Hz, 3H), 1.79 (d, $J = 1.1$ Hz, 3H), 1.53 – 1.37 (m, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 164.2, 150.4, 150.1, 135.7, 129.3, 128.1, 126.6, 111.0, 110.9, 110.7, 80.3, 79.7, 78.3, 77.7, 77.4, 77.2, 76.9, 76.8, 75.6, 67.1 65.8, 62.8, 51.7, 49.0, 47.2, 46.1, 44.9, 36.7, 12.5.

³¹P NMR (121 MHz, CDCl₃) δ 16.65, 16.43.

HRMS Data [M+H]⁺: Mass calculated for C₄₁H₅₀N₇O₉P is 814.3329, found 814.3328.



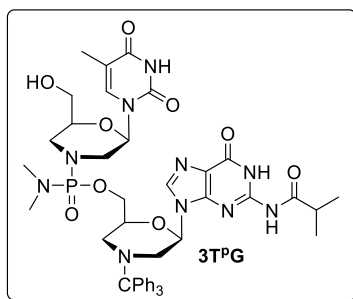
^1H NMR (300 MHz, Chloroform-*d*) δ 9.39 (s, 3H), 7.98 (d, J = 7.5 Hz, 1H), 7.91 – 7.78 (m, 4H), 7.68 (d, J = 7.6 Hz, 1H), 7.56 – 7.34 (m, 12H), 7.31 – 7.21 (m, 7H), 7.16 (d, J = 7.3 Hz, 3H), 6.25 (ddd, J = 12.4, 9.3, 2.2 Hz, 1H), 5.67 (td, J = 9.8, 2.6 Hz, 1H), 4.49 – 4.30 (m, 1H), 4.07 – 3.77 (m, 3H), 3.65 (ddt, J = 17.8, 12.4, 6.3 Hz, 3H), 3.52 – 3.41 (m, 1H), 3.41 – 3.27 (m, 1H), 3.15 – 3.01 (m, 1H), 2.59 (dd, J = 23.8, 9.9 Hz, 7H), 2.47 – 2.32 (m, 1H), 1.58 – 1.22 (m, 2H).

^{13}C NMR (75 MHz, CDCl_3) δ 167.4, 167.1, 163.0, 162.8, 154.4, 154.2, 145.1, 144.8, 133.3, 133.2, 133.1, 133.0, 129.3, 128.9, 128.9, 128.8, 128.7, 128.1, 128.0, 126.6, 97.5, 97.1, 82.3, 81.8, 81.6, 81.4, 78.3, 77.8, 77.5, 77.3, 77.0, 76.9, 75.7, 65.9,

62.8, 58.1, 52.4, 48.9, 47.8, 45.2, 45.0, 36.8, 36.7.

^{31}P NMR (121 MHz, CDCl_3) δ 16.49, 16.12.

HRMS Data $[\text{M}+\text{H}]^+$: Mass calculated for $\text{C}_{53}\text{H}_{55}\text{N}_9\text{O}_9\text{P}$ is 992.3860, found 992.3861.

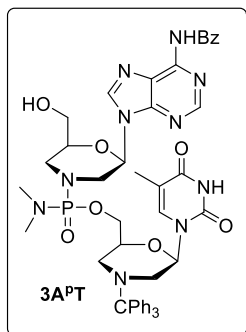


^1H NMR (300 MHz, Chloroform-*d*) δ 12.20 (d, J = 22.6 Hz, 1H), 10.34 – 9.49 (m, 2H), 7.57 (d, J = 9.2 Hz, 1H), 7.51 – 7.06 (m, 17H), 5.99 (t, J = 11.5 Hz, 1H), 5.80 – 5.46 (m, 1H), 4.39 (s, 1H), 4.02 (d, J = 9.8 Hz, 2H), 3.83 (d, J = 7.4 Hz, 1H), 3.62 (dd, J = 14.7, 6.4 Hz, 3H), 3.47 – 3.18 (m, 2H), 3.14 – 3.03 (m, 1H), 2.91 – 2.73 (m, 2H), 2.62 (dd, J = 18.9, 9.7 Hz, 7H), 2.39 (d, J = 10.4 Hz, 1H), 1.80 (d, J = 10.9 Hz, 3H), 1.72 – 1.37 (m, 2H), 1.35 – 1.14 (m, 6H).

^{13}C NMR (75 MHz, CDCl_3) δ 180.3, 180.2, 164.7, 164.6, 156.5, 156.3, 150.6, 149.1, 148.9, 148.4, 148.3, 136.4, 129.7, 129.3, 128.5, 128.4, 127.2, 121.2, 121.0, 111.4, 111.1, 81.3, 80.2, 78.1, 66.1, 63.5, 53.8, 53.7, 49.3, 48.2, 45.5, 37.3, 37.2, 36.7, 30.3, 27.4, 20.0, 19.2, 19.2, 13.0, 12.9.

^{31}P NMR (121 MHz, CDCl_3) δ 16.48, 15.54.

HRMS Data $[\text{M}+\text{H}]^+$: Mass calculated for $\text{C}_{45}\text{H}_{54}\text{N}_{10}\text{O}_9\text{P}$ is 909.3813, found 909.3812.

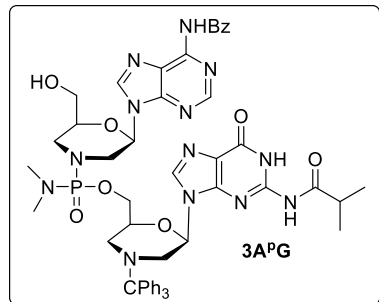


^1H NMR (300 MHz, Chloroform-*d*) δ 10.52 (d, J = 36.8 Hz, 1H), 10.03 (d, J = 36.7 Hz, 1H), 8.74 (d, J = 2.1 Hz, 1H), 8.32 (s, 1H), 8.15 – 8.01 (m, 2H), 7.58 – 7.09 (m, 21H), 6.94 (dd, J = 20.8, 1.4 Hz, 1H), 6.12 (dd, J = 9.7, 2.3 Hz, 1H), 5.85 (ddd, J = 25.6, 10.0, 2.8 Hz, 1H), 4.35 (s, 1H), 4.15 – 3.98 (m, 1H), 3.98 – 3.77 (m, 2H), 3.75 – 3.42 (m, 4H), 3.26 (t, J = 8.7 Hz, 2H), 3.17 – 2.98 (m, 3H), 2.63 (dd, J = 18.4, 10.0 Hz, 7H), 1.66 (d, J = 7.9 Hz, 3H), 1.54 – 1.33 (m, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 166.3, 164.7, 163.2, 153.2, 151.8, 151.7, 151.0, 150.9, 150.6, 144.5, 141.5, 135.8, 134.3, 134.2, 133.1, 129.7, 129.3, 129.1, 129.0, 128.5, 128.3, 127.5, 127.1, 123.4, 123.2, 111.5, 111.3, 80.7, 78.4, 78.1, 77.9, 77.5, 77.4, 76.1, 66.4, 63.1, 58.5, 52.7, 52.3, 49.4, 48.6, 46.4, 45.5, 37.3, 37.2, 37.1, 32.0, 19.0, 12.9, 9.2.

^{31}P NMR (121 MHz, CDCl_3) δ 16.55, 16.48, 16.47.

HRMS Data $[\text{M}+\text{H}]^+$: Mass calculated for $\text{C}_{48}\text{H}_{52}\text{N}_{10}\text{O}_8\text{P}$ is 927.3707, found 927.3705.

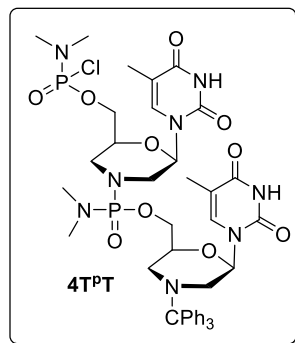


¹H NMR (300 MHz, Chloroform-*d*) δ 12.17 (d, $J = 17.6$ Hz, 1H), 10.16 (d, $J = 14.0$ Hz, 1H), 9.86 (d, $J = 11.6$ Hz, 1H), 8.68 (s, 1H), 8.22 (d, $J = 29.1$ Hz, 1H), 8.11 – 8.01 (m, 2H), 7.58 – 7.06 (m, 20H), 5.98 (t, $J = 10.4$ Hz, 1H), 5.90 – 5.68 (m, 1H), 4.96 (s, 1H), 4.43 (s, 1H), 4.11 – 3.44 (m, 7H), 3.31 (d, $J = 11.8$ Hz, 2H), 3.18 (d, $J = 8.2$ Hz, 2H), 3.00 – 2.76 (m, 3H), 2.61 (dd, $J = 28.6, 10.0$ Hz, 6H), 1.73 – 1.42 (m, 2H), 1.26 – 1.08 (m, 6H).

¹³C NMR (75 MHz, CDCl₃) δ 179.8, 165.6, 162.8, 156.0, 155.9, 152.7, 151.1, 151.0, 149.9, 148.4, 148.1, 147.9, 141.1, 136.3, 133.6, 132.7, 129.1, 128.7, 128.3, 128.0, 126.7, 123.0, 120.5, 120.5, 80.1, 77.8, 77.6, 77.4, 77.0, 76.8, 75.3, 65.6, 62.6, 58.0, 53.3, 48.1, 48.1, 46.2, 45.1, 19.4, 18.7, 18.5.

³¹P NMR (121 MHz, CDCl₃) δ 16.55, 16.48, 16.47.

HRMS Data [M+H]⁺: Mass calculated for C₄₅H₅₄N₁₀O₉P is 909.3813, found 909.3812.

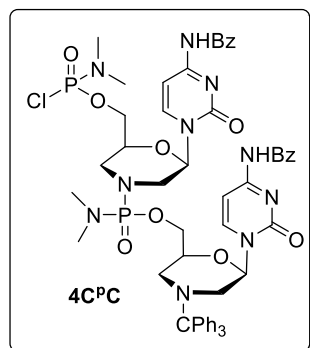


¹H NMR (300 MHz, Chloroform-*d*) δ 9.53 – 9.17 (m, 2H), 7.52 – 7.11 (m, 16H), 7.08 – 7.00 (m, 1H), 6.25 – 6.06 (m, 1H), 5.65 (ddd, $J = 13.3, 9.9, 2.9$ Hz, 1H), 4.48 – 3.72 (m, 6H), 3.70 – 3.52 (m, 1H), 3.37 (dd, $J = 21.3, 9.4$ Hz, 3H), 3.13 (d, $J = 11.3$ Hz, 1H), 2.89 – 2.51 (m, 13H), 1.98 – 1.87 (m, 3H), 1.80 (s, 3H), 1.43 (td, $J = 10.2, 3.8$ Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 163.7, 150.1, 149.9, 129.3, 128.9, 128.0, 126.6, 110.83, 80.5, 79.8, 77.6, 77.3, 77.1, 76.9, 76.7, 75.5, 65.7, 53.9, 51.8, 49.0, 47.3, 36.8, 36.7, 36.7, 29.8, 29.4, 14.2, 13.8, 12.6, 12.5, 12.5.

³¹P NMR (121 MHz, CDCl₃) δ 18.75, 18.71, 18.16, 18.05, 16.60, 16.44, 16.39, 16.13.

HRMS Data [M+Na]⁺: Mass calculated for C₄₃H₅₃ClN₈O₁₀P₂Na is 961.2946, found 961.2945.



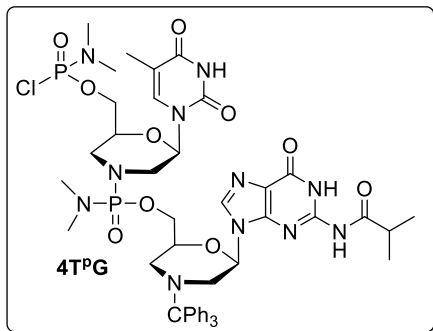
¹H NMR (300 MHz, Chloroform-*d*) δ 9.66 (s, 2H), 8.12 – 7.83 (m, 5H), 7.73 (d, $J = 7.5$ Hz, 1H), 7.62 – 7.15 (m, 23H), 6.33 – 6.21 (m, 1H), 5.69 (dd, $J = 9.4, 2.8$ Hz, 1H), 4.48 – 4.33 (m, 1H), 4.33 – 4.10 (m, 2H), 4.10 – 3.95 (m, 2H), 3.89 (s, 1H), 3.76 – 3.56 (m, 2H), 3.50 (t, $J = 9.7$ Hz, 1H), 3.37 (t, $J = 10.0$ Hz, 1H), 3.12 (t, $J = 11.8$ Hz, 1H), 2.82 – 2.51 (m, 11H), 2.40 (td, $J = 13.0, 6.4$ Hz, 1H), 1.48 (t, $J = 10.9$ Hz, 1H), 1.36 – 1.29 (m, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 175.7, 167.2, 163.1, 163.0, 162.8, 154.2, 154.1, 144.8, 144.5, 133.1, 129.2, 128.8, 128.1, 128.0, 126.6, 97.3, 82.2, 81.9, 81.6, 77.8, 77.6, 77.4, 77.0, 75.4, 67.3, 65.8, 52.5, 49.0, 47.7, 44.4, 36.8, 36.7, 31.5,

29.7, 29.4, 21.2.

³¹P NMR (121 MHz, CDCl₃) δ 18.70, 18.65, 18.01, 17.98, 16.45, 16.25, 16.06, 15.85.

HRMS Data [M+H]⁺: Mass calculated for C₅₅H₆₀ClN₁₀O₁₀P₂ is 1117.3658, found 1118.3655.



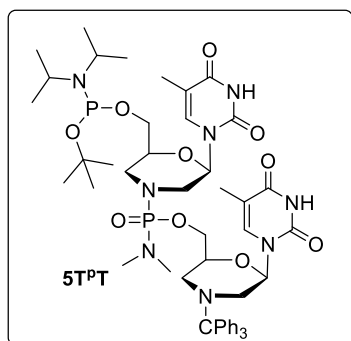
^1H NMR (300 MHz, Chloroform-*d*) δ 12.15 (s, 1H), 9.90 – 9.17 (m, 1H), 7.75 – 7.02 (m, 16H), 6.04 (dd, $J = 14.7, 7.8$ Hz, 1H), 5.69 – 5.48 (m, 1H), 4.46 (s, 1H), 4.26 – 3.58 (m, 6H), 3.39 (d, $J = 9.9$ Hz, 2H), 3.17 (q, $J = 12.3, 10.9$ Hz, 1H), 2.94 – 2.37 (m, 16H), 2.08 – 1.77 (m, 3H), 1.67 (d, $J = 9.9$ Hz, 1H), 1.25 (s, 6H).

^{13}C NMR (75 MHz, CDCl_3) δ 180.4, 164.6, 156.3, 149.1, 148.4, 147.8, 137.0, 129.7, 129.2, 129.1, 128.5, 128.3, 128.3, 127.5, 127.4, 127.1, 120.8, 111.5, 80.8, 78.4, 78.1, 77.9, 77.5, 77.4, 75.7, 70.2,

67.5, 66.2, 54.7, 53.7, 53.6, 49.8, 49.4, 47.8, 47.7, 44.9, 37.3, 37.2, 29.9, 19.9, 19.3, 13.0.

^{31}P NMR (121 MHz, CDCl_3) δ 18.97, 18.91, 18.69, 18.42, 18.24, 17.99, 17.81, 16.72, 16.62, 16.15.

HRMS Data [M+H] $^+$: Mass calculated for $\text{C}_{47}\text{H}_{59}\text{ClN}_{11}\text{O}_{10}\text{P}_2$ is 1034.3610, found 1034.3610.

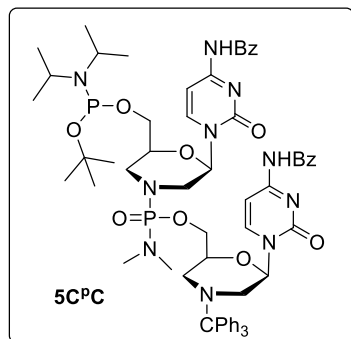


^1H NMR (300 MHz, Chloroform-*d*) δ 7.59 – 7.12 (m, 16H), 7.12 – 7.02 (m, 1H), 6.17 (ddd, $J = 14.4, 9.7, 2.4$ Hz, 1H), 5.74 – 5.56 (m, 1H), 4.50 – 4.31 (m, 1H), 3.92 (tdt, $J = 11.3, 7.4, 3.6$ Hz, 3H), 3.72 – 3.26 (m, 7H), 3.16 (d, $J = 11.6$ Hz, 1H), 2.84 – 2.71 (m, 1H), 2.62 (t, $J = 10.2$ Hz, 7H), 2.00 – 1.91 (m, 3H), 1.56 – 1.33 (m, 14H), 1.19 (dq, $J = 4.5, 2.8$ Hz, 10H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 164.2, 164.1, 150.0, 136.0, 135.2, 127.9, 126.5, 80.5, 79.8, 65.6, 51.8, 49.2, 47.3, 46.6, 45.5, 43.1, 42.9, 36.7, 36.6, 31.0, , 30.9, 30.8, 30.4, 24.8, 24.7, 24.6, 24.2, 19.9, 12.5, 12.4.

^{31}P NMR (121 MHz, CDCl_3) δ 139.03, 138.88, 138.80, 138.77, 16.45, 16.42, 16.28, 16.22, 16.15.

HRMS Data [M-H] $^-$: Mass calculated for $\text{C}_{51}\text{H}_{69}\text{N}_8\text{O}_{10}\text{P}_2$ is 1015.4617, found 1015.4611.

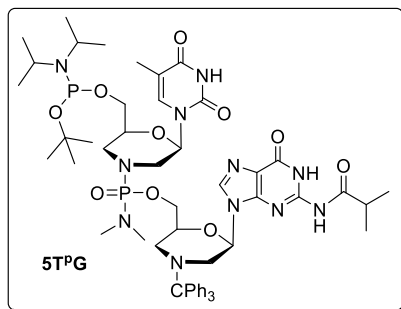


^1H NMR (300 MHz, Chloroform-*d*) δ 7.99 – 7.72 (m, 6H), 7.64 – 7.36 (m, 13H), 7.33 – 7.10 (m, 10H), 6.24 (dd, $J = 9.3, 2.3$ Hz, 1H), 5.78 – 5.58 (m, 1H), 4.40 (s, 1H), 4.16 – 3.79 (m, 3H), 3.79 – 3.29 (m, 7H), 3.12 (t, $J = 10.3$ Hz, 1H), 2.92 – 2.73 (m, 1H), 2.62 (dd, $J = 23.0, 9.8$ Hz, 7H), 2.46 – 2.21 (m, 2H), 1.53 (dd, $J = 8.4, 6.2$ Hz, 2H), 1.38 – 1.06 (m, 20H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 167.6, 167.4, 162.9, 154.3, 143.9, 133.1, 132.8, 128.6, 128.5, 128.1, 128.0, 127.8, 126.4, 97.5, 97.0, 82.0, 81.5, 76.8, 75.3, 65.5, 62.6, 52.5, 49.0, 47.9, 46.5, 45.6, 43.1, 42.9, 36.6, 30.9, 30.9, 30.8, 30.8, 30.3, 24.8, 24.7, 24.6, 24.2, 24.1, 19.5.

^{31}P NMR (121 MHz, CDCl_3) δ 139.09, 138.99, 138.87, 138.81, 16.34, 16.15, 15.84, 15.78.

HRMS Data [M-H] $^-$: Mass calculated for $\text{C}_{63}\text{H}_{75}\text{N}_{10}\text{O}_{10}\text{P}_2$ is 1193.5148, found 1193.5144.

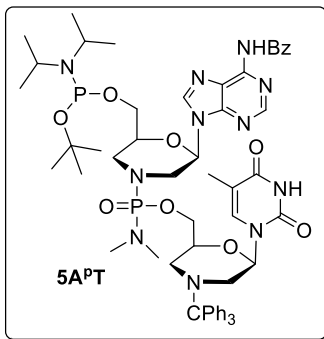


^1H NMR (300 MHz, Chloroform-*d*) δ 10.47 (s, 2H), 7.68 – 7.32 (m, 7H), 7.31 – 7.08 (m, 10H), 6.07 (ddd, J = 35.0, 7.4, 2.5 Hz, 1H), 5.81 – 5.48 (m, 1H), 4.52 (d, J = 42.7 Hz, 1H), 4.15 – 3.88 (m, 2H), 3.88 – 3.71 (m, 2H), 3.57 (ddd, J = 14.8, 7.3, 4.6 Hz, 3H), 3.42 (p, J = 6.3 Hz, 3H), 3.20 (dd, J = 21.2, 11.1 Hz, 2H), 3.03 – 2.91 (m, 1H), 2.82 (td, J = 6.8, 3.0 Hz, 1H), 2.75 – 2.68 (m, 1H), 2.67 – 2.50 (m, 6H), 1.92 (d, J = 7.7 Hz, 3H), 1.75 – 1.59 (m, 1H), 1.57 – 1.46 (m, 2H), 1.40 – 0.88 (m, 26H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 156.3, 150.1, 149.9, 149.4, 148.0, 136.3, 127.9, 126.5, 75.1, 46.9, 45.6, 43.1, 42.9, 36.6, 36.5, 36.5, 35.9, 30.9, 30.9, 30.8, 30.4, 24.8, 24.7, 24.1, 19.6, 19.4, 18.8, 12.5.

^{31}P NMR (121 MHz, CDCl_3) δ 139.04, 138.80, 138.77, 138.69, 16.83, 16.56, 16.46.

HRMS Data [M-H] $^-$: Mass calculated for $\text{C}_{55}\text{H}_{74}\text{N}_{11}\text{O}_{10}\text{P}_2$ is 1110.5010, found 1110.5094.

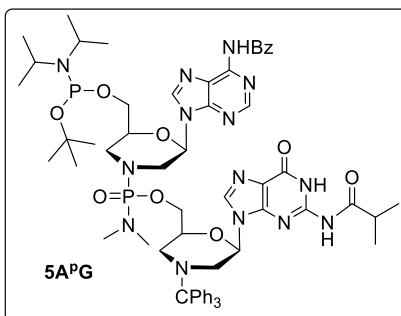


^1H NMR (300 MHz, Chloroform-*d*) δ 8.76 (d, J = 15.7 Hz, 1H), 8.26 – 8.14 (m, 1H), 8.06 (td, J = 5.9, 4.8, 3.1 Hz, 2H), 7.59 (d, J = 7.1 Hz, 1H), 7.52 (t, J = 7.8 Hz, 2H), 7.42 (s, 3H), 7.26 (s, 2H), 7.26 – 7.06 (m, 7H), 7.05 – 6.96 (m, 1H), 6.16 – 6.01 (m, 1H), 5.87 (t, J = 9.3 Hz, 1H), 4.36 (s, 1H), 3.94 (d, J = 6.8 Hz, 3H), 3.77 – 3.48 (m, 5H), 3.48 – 3.25 (m, 3H), 3.18 – 3.07 (m, 1H), 2.90 – 2.74 (m, 2H), 2.64 (t, J = 10.0 Hz, 6H), 1.74 (d, J = 6.6 Hz, 2H), 1.44 (d, J = 5.5 Hz, 2H), 1.37 – 1.28 (m, 13H), 1.26 – 1.19 (m, 5H), 1.16 – 1.09 (m, 8H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 165.5, 164.0, 152.5, 151.4, 150.2, 140.6, 135.1, 133.6, 132.6, 129.1, 128.5, 127.8, 126.5, 123.1, 110.6, 75.1, 62.7, 51.8, 49.1, 48.2, 46.6, 45.9, 45.1, 43.1, 42.9, 36.6, 30.9, 30.9, 30.8, 30.8, 30.4, 30.3, 24.7, 24.7, 24.6, 24.2, 24.1, 23.0, 19.3, 12.3.

^{31}P NMR (121 MHz, CDCl_3) δ 139.20, 139.08, 138.97, 138.94, 16.43, 16.39.

HRMS Data [M-H] $^-$: Mass calculated for $\text{C}_{58}\text{H}_{72}\text{N}_{11}\text{O}_9\text{P}_2$ is 1128.4995, found 1128.4991.

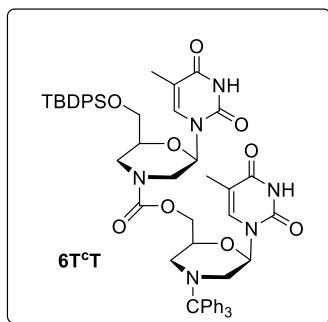


^1H NMR (300 MHz, Chloroform-*d*) δ 8.92 – 8.70 (m, 1H), 8.11 – 7.99 (m, 2H), 7.57 (d, J = 11.9 Hz, 3H), 7.53 – 7.44 (m, 3H), 7.43 (s, 3H), 7.25 (q, J = 7.3 Hz, 8H), 7.13 (d, J = 8.1 Hz, 1H), 5.95 (dd, J = 10.3, 2.8 Hz, 1H), 5.84 (dd, J = 10.1, 2.7 Hz, 1H), 4.46 (s, 1H), 4.19 – 3.77 (m, 5H), 3.72 – 3.49 (m, 4H), 3.38 (q, J = 5.7 Hz, 2H), 3.25 – 2.94 (m, 3H), 2.79 (dt, J = 14.8, 7.5 Hz, 2H), 2.62 (dd, J = 16.9, 10.0 Hz, 6H), 1.88 – 1.62 (m, 2H), 1.56 – 1.42 (m, 3H), 1.40 – 1.05 (m, 23H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 179.9, 165.7, 156.2, 152.5, 151.4, 150.0, 148.6, 148.0, 133.4, 132.6, 128.5, 128.2, 126.5, 123.5, 120.4, 76.7, 36.6, 35.7, 30.8, 30.3, 30.3, 24.6, 24.2, 24.1, 19.4, 18.8.

^{31}P NMR (121 MHz, CDCl_3) δ 139.16, 139.09, 138.88, 138.83, 16.77, 16.74, 16.60, 16.56, 16.48.

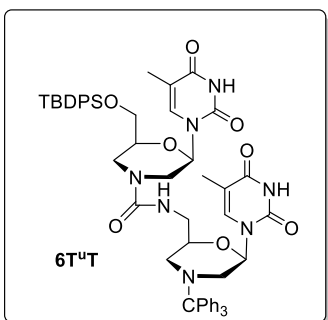
HRMS Data [M-H] $^-$: Mass calculated for $\text{C}_{62}\text{H}_{77}\text{N}_{14}\text{O}_9\text{P}_2$ is 1223.5479, found 1223.5475.



^1H NMR (300 MHz, Chloroform-*d*) δ 10.25 – 9.59 (m, 2H), 7.71 – 7.62 (m, 4H), 7.52 – 7.12 (m, 27H), 6.27 – 6.10 (m, 1H), 5.67 (d, J = 9.8 Hz, 1H), 4.41 (d, J = 13.1 Hz, 1H), 4.24 – 3.99 (m, 3H), 3.78 (s, 3H), 3.38 (d, J = 10.8 Hz, 1H), 3.12 (d, J = 11.8 Hz, 1H), 2.98 (s, 1H), 2.69 (dd, J = 13.0, 10.2 Hz, 1H), 1.87 (d, J = 23.1 Hz, 6H), 1.53 – 1.34 (m, 3H), 1.08 (s, 9H).

^{13}C NMR (75 MHz, CDCl_3) δ 164.0, 154.5, 149.8, 135.5, 135.5, 134.9, 132.9, 129.9, 129.2, 127.9, 127.8, 126.7, 126.5, 111.1, 110.8, 80.4, 78.8, 77.5, 77.3, 77.1, 76.8, 76.8, 76.7, 75.1, 74.6, 66.1, 64.1, 51.9, 49.1, 48.9, 44.3, 26.7, 19.3, 12.6, 12.4.

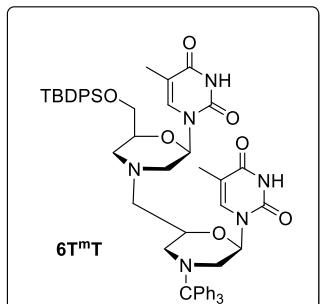
HRMS Data [M-H]⁻: Mass calculated for $\text{C}_{56}\text{H}_{59}\text{N}_6\text{O}_9\text{Si}$ is 987.4118, found 987.4112.



^1H NMR (300 MHz, Chloroform-*d*) δ 10.07 (s, 1H), 7.80 – 7.04 (m, 27H), 6.18 (d, J = 9.1 Hz, 1H), 5.97 (s, 1H), 5.71 (d, J = 9.5 Hz, 1H), 4.28 (d, J = 12.1 Hz, 3H), 3.81 (d, J = 10.0 Hz, 3H), 3.68 (d, J = 15.7 Hz, 1H), 3.30 (d, J = 10.7 Hz, 1H), 3.20 (s, 1H), 2.99 (s, 1H), 2.84 – 2.70 (m, 1H), 1.70 (s, 6H), 1.43 (d, J = 11.0 Hz, 2H), 1.05 (s, 9H).

^{13}C NMR (75 MHz, CDCl_3) δ 163.9, 163.8, 157.4, 150.4, 150.2, 135.5, 135.4, 133.0, 129.9, 127.9, 127.8, 126.5, 80.4, 79.1, 77.5, 77.3, 77.1, 76.8, 76.7, 64.4, 52.0, 50.0, 47.4, 44.3, 43.3, 36.5, 26.8, 19.3, 12.4, 12.3.

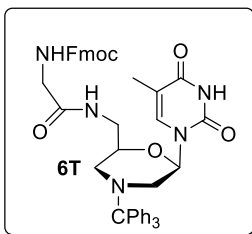
HRMS Data [M+Na]⁺: Mass calculated for $\text{C}_{56}\text{H}_{61}\text{N}_7\text{O}_8\text{SiNa}$ is 1010.4249, found 1010.4248.



^1H NMR (300 MHz, Chloroform-*d*) δ 9.72 (d, J = 99.9 Hz, 2H), 7.75 – 7.61 (m, 4H), 7.61 – 7.15 (m, 23H), 6.94 (d, J = 1.4 Hz, 1H), 6.15 (dd, J = 9.7, 2.3 Hz, 1H), 5.79 (dd, J = 9.8, 2.5 Hz, 1H), 4.36 (s, 1H), 3.92 (d, J = 9.1 Hz, 1H), 3.75 (qd, J = 11.0, 4.6 Hz, 2H), 3.34 (d, J = 11.2 Hz, 1H), 3.24 – 3.06 (m, 2H), 2.94 (d, J = 11.1 Hz, 1H), 2.60 – 2.36 (m, 2H), 2.17 (t, J = 10.9 Hz, 1H), 1.99 (t, J = 10.4 Hz, 1H), 1.88 (d, J = 1.2 Hz, 3H), 1.78 (d, J = 1.1 Hz, 3H), 1.40 (td, J = 11.0, 3.4 Hz, 2H), 1.08 (s, 9H).

^{13}C NMR (75 MHz, CDCl_3) δ 164.1, 163.9, 150.2, 150.1, 135.7, 135.3, 133.3, 133.3, 130.0, 129.3, 128.1, 127.9, 126.7, 110.8, 110.8, 80.5, 79.4, 77.6, 77.4, 77.2, 76.9, 76.8, 76.4, 74.6, 64.7, 60.7, 56.5, 54.6, 52.2, 51.1, 26.9, 19.5, 12.7, 12.6.

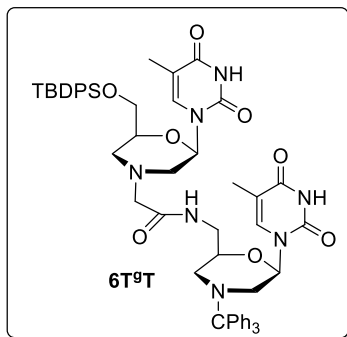
HRMS Data [M+Na]⁺: Mass calculated for $\text{C}_{55}\text{H}_{60}\text{N}_6\text{O}_7\text{SiNa}$ is 967.4190, found 967.4193.



^1H NMR (300 MHz, Chloroform-*d*) δ 9.58 (s, 1H), 7.82 – 7.14 (m, 24H), 6.98 (s, 2H), 6.13 (d, J = 9.3 Hz, 1H), 5.75 (s, 1H), 4.36 (d, J = 7.1 Hz, 2H), 4.21 (dt, J = 14.0, 8.2 Hz, 2H), 3.82 (d, J = 5.6 Hz, 2H), 3.62 (dd, J = 13.5, 7.1 Hz, 1H), 3.31 (d, J = 11.1 Hz, 1H), 3.10 (d, J = 11.6 Hz, 2H), 2.81 (s, 1H), 1.74 (s, 3H), 1.40 (d, J = 10.9 Hz, 1H).

^{13}C NMR (75 MHz, CDCl_3) δ 169.4, 164.0, 156.7, 150.0, 143.9, 141.4, 135.6, 129.2, 128.0, 127.9, 127.2, 126.7, 125.2, 120.1, 110.6, 80.5, 77.6, 77.4, 77.2, 76.9, 76.8, 76.0, 67.3, 52.0, 50.0, 47.2, 44.5, 41.8, 38.7, 12.5.

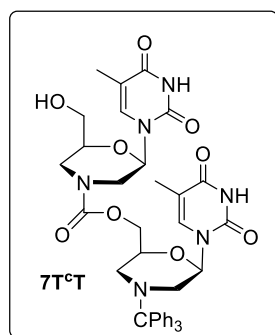
HRMS Data [M+Na]⁺: Mass calculated for $\text{C}_{46}\text{H}_{43}\text{N}_5\text{O}_6\text{Na}$ is 784.3111, found 784.3112.



¹H NMR (300 MHz, Chloroform-*d*) δ 10.01 (s, 1H), 9.31 (s, 1H), 7.71 – 7.08 (m, 29H), 6.15 (dd, $J = 9.6, 2.1$ Hz, 1H), 5.70 (dd, $J = 9.7, 2.4$ Hz, 1H), 4.37 – 4.23 (m, 1H), 3.98 (dt, $J = 11.0, 3.3$ Hz, 1H), 3.76 (d, $J = 4.4$ Hz, 2H), 3.56 – 3.42 (m, 1H), 3.41 – 3.25 (m, 2H), 3.17 – 3.04 (m, 2H), 3.00 (s, 1H), 2.98 – 2.87 (m, 1H), 2.68 (d, $J = 10.4$ Hz, 1H), 2.28 (t, $J = 11.0$ Hz, 1H), 2.06 (s, 1H), 2.00 – 1.87 (m, 4H), 1.80 (s, 3H), 1.52 (t, $J = 10.4$ Hz, 1H), 1.39 (t, $J = 11.1$ Hz, 1H), 1.08 (s, 9H).

¹³C NMR (75 MHz, CDCl₃) δ 169.5, 164.0, 150.0, 135.5, 135.1, 133.0, 129.9, 129.2, 127.8, 127.7, 126.4, 111.0, 110.5, 80.6, 79.5, 77.5, 77.3, 77.0, 76.8, 76.6, 75.7, 64.4, 61.3, 56.5, 54.3, 51.3, 49.9, 41.5, 26.8, 19.3, 12.5, 12.2.

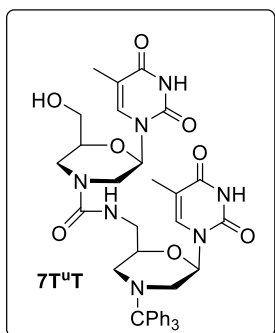
HRMS Data [M-H]⁻: Mass calculated for C₅₇H₆₂N₇O₈Si is 1000.4435, found 1000.4428.



¹H NMR (300 MHz, Chloroform-*d*) δ 10.01 (d, $J = 65.6$ Hz, 2H), 7.60 – 6.91 (m, 18H), 6.17 (d, $J = 9.3$ Hz, 1H), 5.70 (dd, $J = 9.9, 3.1$ Hz, 1H), 4.42 (t, $J = 6.2$ Hz, 1H), 4.10 (d, $J = 20.7$ Hz, 4H), 3.95 – 3.65 (m, 3H), 3.35 (d, $J = 10.5$ Hz, 1H), 3.11 (d, $J = 11.0$ Hz, 1H), 2.80 (d, $J = 11.1$ Hz, 2H), 1.85 (d, $J = 24.9$ Hz, 6H), 1.45 (q, $J = 9.4$ Hz, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 164.1, 154.6, 150.3, 149.8, 135.6, 135.1, 129.2, 127.9, 126.5, 111.2, 110.8, 80.3, 78.8, 77.5, 77.3, 77.1, 76.8, 76.7, 74.6, 66.1, 62.6, 52.5, 51.7, 48.9, 12.5, 12.4.

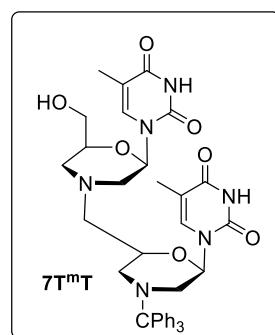
HRMS Data [M+Na]⁺: Mass calculated for C₄₀H₄₂N₆O₉Na is 773.2911, found 773.2910.



¹H NMR (300 MHz, DMSO-*d*₆) δ 11.46 (s, 1H), 11.31 (s, 1H), 7.60 (s, 1H), 7.53 – 7.08 (m, 15H), 6.85 (t, $J = 5.7$ Hz, 1H), 5.96 (dd, $J = 9.2, 2.4$ Hz, 1H), 5.52 (dd, $J = 10.1, 2.8$ Hz, 1H), 4.87 (t, $J = 5.2$ Hz, 1H), 4.16 (q, $J = 8.6, 6.8$ Hz, 1H), 3.96 – 3.78 (m, 2H), 3.43 (d, $J = 6.3$ Hz, 3H), 3.31 – 3.09 (m, 3H), 3.09 – 2.93 (m, 2H), 2.80 (dd, $J = 12.7, 10.2$ Hz, 1H), 2.70 – 2.55 (m, 1H), 1.81 (s, 3H), 1.67 (s, 3H), 1.27 (dd, $J = 20.9, 8.6$ Hz, 3H).

¹³C NMR (75 MHz, DMSO) δ 164.1, 164.0, 157.7, 150.5, 150.2, 136.6, 136.2, 129.3, 128.2, 126.7, 110.0, 109.6, 80.4, 78.5, 77.1, 76.6, 76.0, 62.0, 51.9, 50.4, 46.3, 45.2, 42.6, 19.6, 13.9, 12.5.

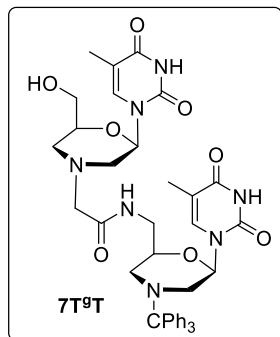
HRMS Data [M+Na]⁺: Mass calculated for C₄₀H₄₃N₇O₈Na is 772.3071, found 772.3070.



¹H NMR (300 MHz, Chloroform-*d*) δ 10.17 (s, 1H), 9.84 – 9.62 (m, 1H), 7.65 – 7.11 (m, 16H), 6.98 – 6.78 (m, 1H), 6.23 – 6.03 (m, 1H), 5.88 – 5.67 (m, 1H), 4.42 (s, 1H), 4.04 – 3.83 (m, 1H), 3.82 – 3.63 (m, 2H), 3.28 (d, $J = 10.3$ Hz, 1H), 3.06 (q, $J = 14.5, 13.5$ Hz, 4H), 2.61 (dd, $J = 13.1, 8.3$ Hz, 1H), 2.31 (d, $J = 12.4$ Hz, 1H), 2.16 – 1.96 (m, 2H), 1.85 (s, 3H), 1.75 – 1.65 (m, 3H), 1.48 – 1.31 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 164.0, 150.1, 150.0, 135.8, 135.5, 129.3, 128.0, 126.7, 110.9, 110.7, 80.3, 79.3, 77.6, 77.4, 77.2, 76.9, 76.8, 73.5, 63.4, 60.5, 56.5, 53.1, 52.4, 51.9, 50.7, 25.3, 20.2, 13.7, 12.6, 12.4.

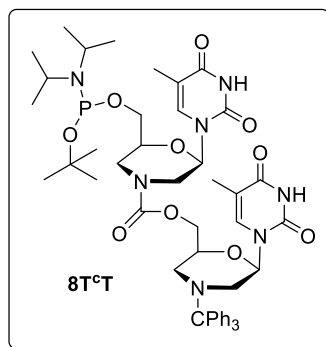
HRMS Data [M+Na]⁺: Mass calculated for C₃₉H₄₂N₆O₇Na is 729.3013, found 729.3012.



¹H NMR (300 MHz, Chloroform-*d*) δ 10.19 (d, J = 66.5 Hz, 1H), 7.56 – 7.05 (m, 17H), 6.96 (s, 2H), 6.26 – 6.01 (m, 1H), 5.69 (dd, J = 9.1, 2.6 Hz, 1H), 4.17 (t, J = 9.7 Hz, 1H), 3.88 (t, J = 6.0 Hz, 1H), 3.81 – 3.61 (m, 3H), 3.27 (d, J = 10.8 Hz, 1H), 3.11 (d, J = 14.1 Hz, 2H), 3.04 – 2.91 (m, 2H), 2.91 – 2.74 (m, 2H), 2.24 – 2.10 (m, 1H), 2.03 (t, J = 11.1 Hz, 1H), 1.83 (s, 3H), 1.78 (s, 3H), 1.58 (t, J = 10.4 Hz, 1H), 1.37 (t, J = 11.1 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 169.2, 164.2, 164.1, 150.7, 150.0, 135.6, 135.5, 129.2, 127.9, 126.5, 111.2, 110.8, 80.1, 79.5, 77.5, 77.3, 77.1, 76.9, 76.7, 76.1, 63.1, 61.0, 56.2, 53.6, 51.1, 49.9, 40.8, 12.4.

HRMS Data [M-H]⁻: Mass calculated for C₄₁H₄₄N₇O₈ is 762.3257, found 762.3250.



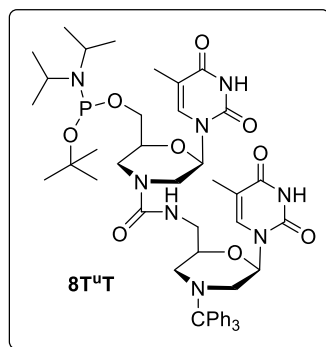
¹H NMR (500 MHz, Chloroform-*d*) δ 9.03 (s, 1H), 7.63 – 7.09 (m, 16H), 7.06 (s, 1H), 6.24 – 6.07 (m, 1H), 5.67 (d, J = 18.6 Hz, 1H), 4.42 (s, 1H), 4.35 – 3.94 (m, 4H), 3.86 (s, 1H), 3.76 – 3.67 (m, 1H), 3.63 (pd, J = 6.9, 3.4 Hz, 2H), 3.38 (d, J = 11.6 Hz, 1H), 3.11 (d, J = 11.8 Hz, 1H), 2.86 (t, J = 12.3 Hz, 1H), 2.75 – 2.62 (m, 1H), 1.97 (s, 3H), 1.86 (s, 3H), 1.53 – 1.42 (m, 4H), 1.37 (d, J = 10.2 Hz, 7H), 1.30 – 1.14 (m, 13H).

¹³C NMR (75 MHz, Chloroform-*d*) δ 164.1, 154.5, 150.3, 149.9, 135.5, 135.1, 129.2, 127.9, 126.5, 111.1, 110.7, 80.4, 79.0, 76.8, 74.7, 66.1, 51.9, 49.1, 43.2, 43.0, 31.0, 30.9, 30.8, 30.4, 24.8, 24.7, 24.6, 24.3, 24.2, 24.2, 24.1,

19.8, 12.5, 12.4.

³¹P NMR (202 MHz, CDCl₃) δ 140.65, 139.05, 138.94, 138.33.

HRMS Data [M-H]⁻: Mass calculated for C₅₀H₆₃N₇O₁₀P is 952.4380, found 952.4375.



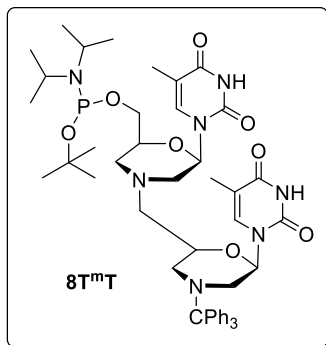
¹H NMR (500 MHz, Chloroform-*d*) δ 9.36 (s, 1H), 7.59 – 7.09 (m, 17H), 6.99 (d, J = 1.8 Hz, 1H), 6.19 – 6.06 (m, 1H), 5.66 (ddd, J = 10.0, 6.8, 2.8 Hz, 1H), 5.61 – 5.36 (m, 1H), 4.25 (d, J = 9.4 Hz, 1H), 4.19 – 4.01 (m, 2H), 3.85 (dt, J = 12.2, 3.7 Hz, 1H), 3.80 – 3.68 (m, 1H), 3.61 (tt, J = 11.5, 7.8, 7.2, 4.0 Hz, 3H), 3.35 – 3.27 (m, 1H), 3.14 (d, J = 12.3 Hz, 1H), 3.04 – 2.91 (m, 1H), 2.91 – 2.79 (m, 1H), 2.79 – 2.66 (m, 1H), 2.01 – 1.70 (m, 6H), 1.48 – 1.40 (m, 3H), 1.35 (d, J = 18.4 Hz, 8H), 1.26 – 1.13 (m, 13H).

¹³C NMR (75 MHz, Chloroform-*d*) δ 157.3, 150.3, 135.4, 128.0, 126.5, 80.4, 79.3, 75.1, 75.0, 62.5, 58.9, 52.0, 50.0, 43.1, 43.0, 31.0, 30.8, 30.7, 24.7,

24.2, 24.1, 24.1, 19.7, 13.7, 12.4.

³¹P NMR (202 MHz, CDCl₃) δ 138.85, 138.81, 138.77, 138.71.

HRMS Data [M-H]⁻: Mass calculated for C₅₀H₆₄N₈O₉P is 951.4539, found 951.4535.

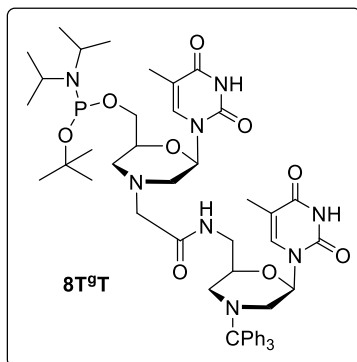


¹H NMR (500 MHz, Chloroform-*d*) δ 8.94 (s, 1H), 7.60 – 7.09 (m, 16H), 6.94 (dd, $J = 4.4, 1.4$ Hz, 1H), 6.13 (dt, $J = 9.7, 2.6$ Hz, 1H), 5.77 (dd, $J = 9.7, 2.6$ Hz, 1H), 4.40 – 4.28 (m, 1H), 3.96 – 3.84 (m, 1H), 3.61 (dtt, $J = 9.9, 7.4, 3.6$ Hz, 3H), 3.41 – 3.29 (m, 5H), 3.18 (d, $J = 11.9$ Hz, 1H), 3.12 (dd, $J = 9.0, 4.8$ Hz, 1H), 2.92 (t, $J = 11.2$ Hz, 1H), 2.51 (dt, $J = 12.4, 6.0$ Hz, 1H), 2.40 (ddd, $J = 13.0, 8.3, 4.5$ Hz, 1H), 2.17 (dt, $J = 18.1, 10.8$ Hz, 1H), 1.99 (dt, $J = 10.2, 5.1$ Hz, 1H), 1.93 (s, 3H), 1.83 – 1.79 (m, 2H), 1.69 (dd, $J = 10.5, 6.2$ Hz, 4H), 1.44 (dd, $J = 7.0, 2.7$ Hz, 5H), 1.37 (d, $J = 10.0$ Hz, 8H), 1.19 (ddd, $J = 10.7, 6.8, 3.2$ Hz, 8H).

¹³C NMR (75 MHz, Chloroform-*d*) δ 163.8, 150.0, 135.8, 135.2, 127.9, 126.5, 110.6, 80.3, 60.4, 58.9, 52.0, 50.9, 46.6, 43.1, 42.9, 30.9, 30.9, 30.8, 24.8, 24.7, 24.2, 24.1, 19.7, 13.7, 12.5, 12.4.

³¹P NMR (202 MHz, CDCl₃) δ 138.73, 138.71.

HRMS Data [M-H]⁻: Mass calculated for C₄₉H₆₃N₇O₈P is 908.4481, found 908.4475.

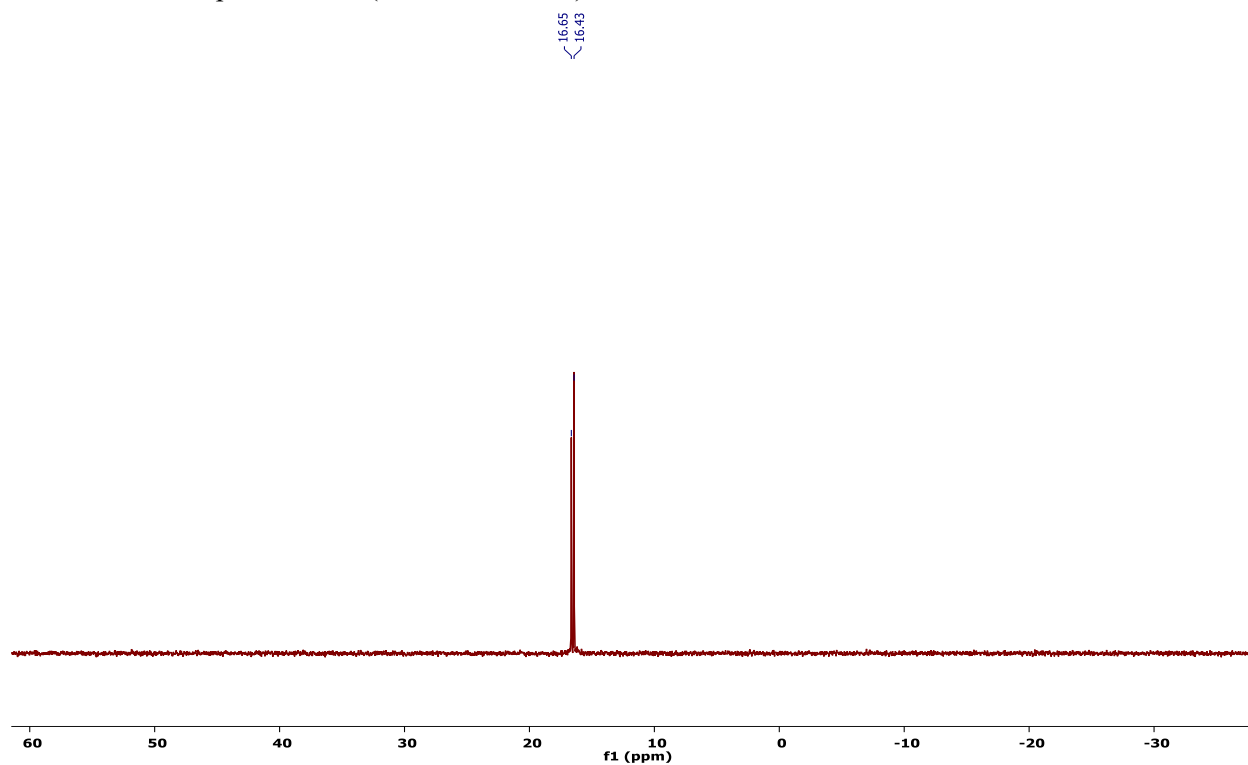
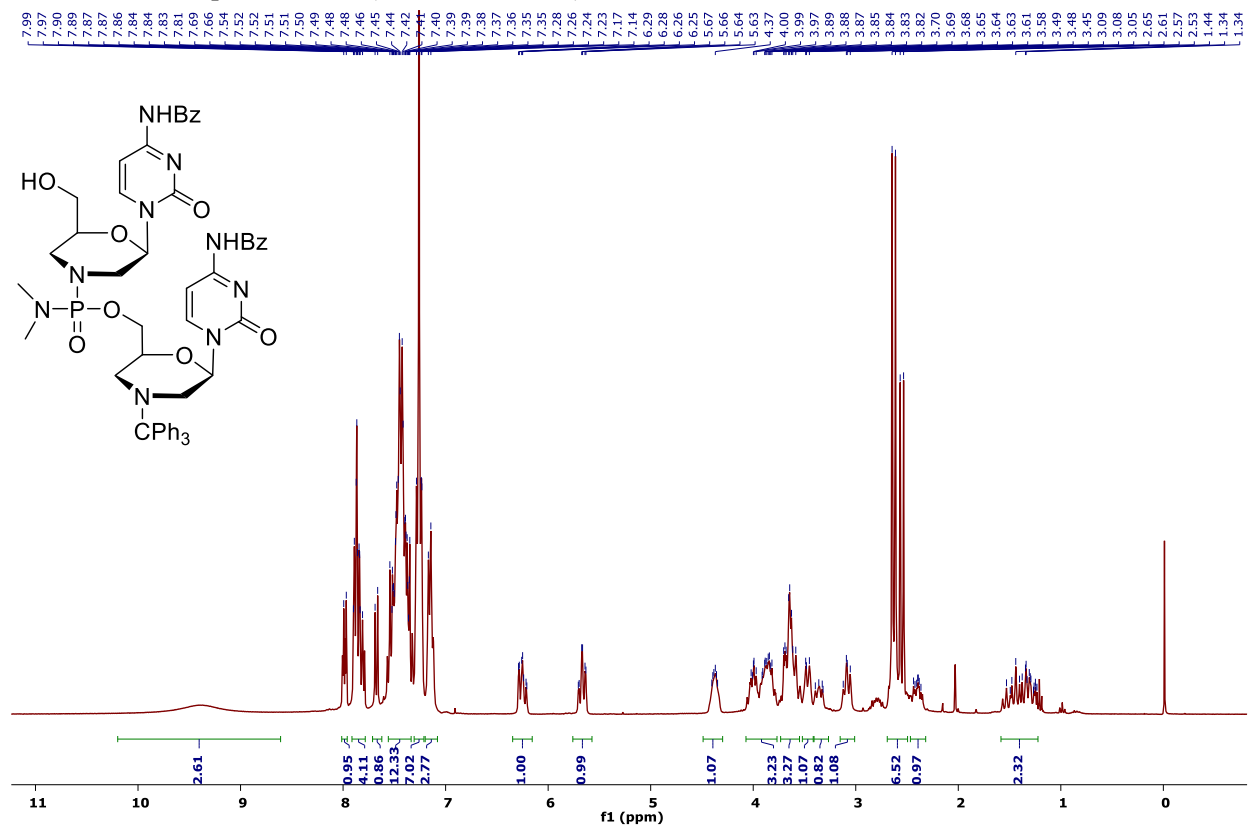


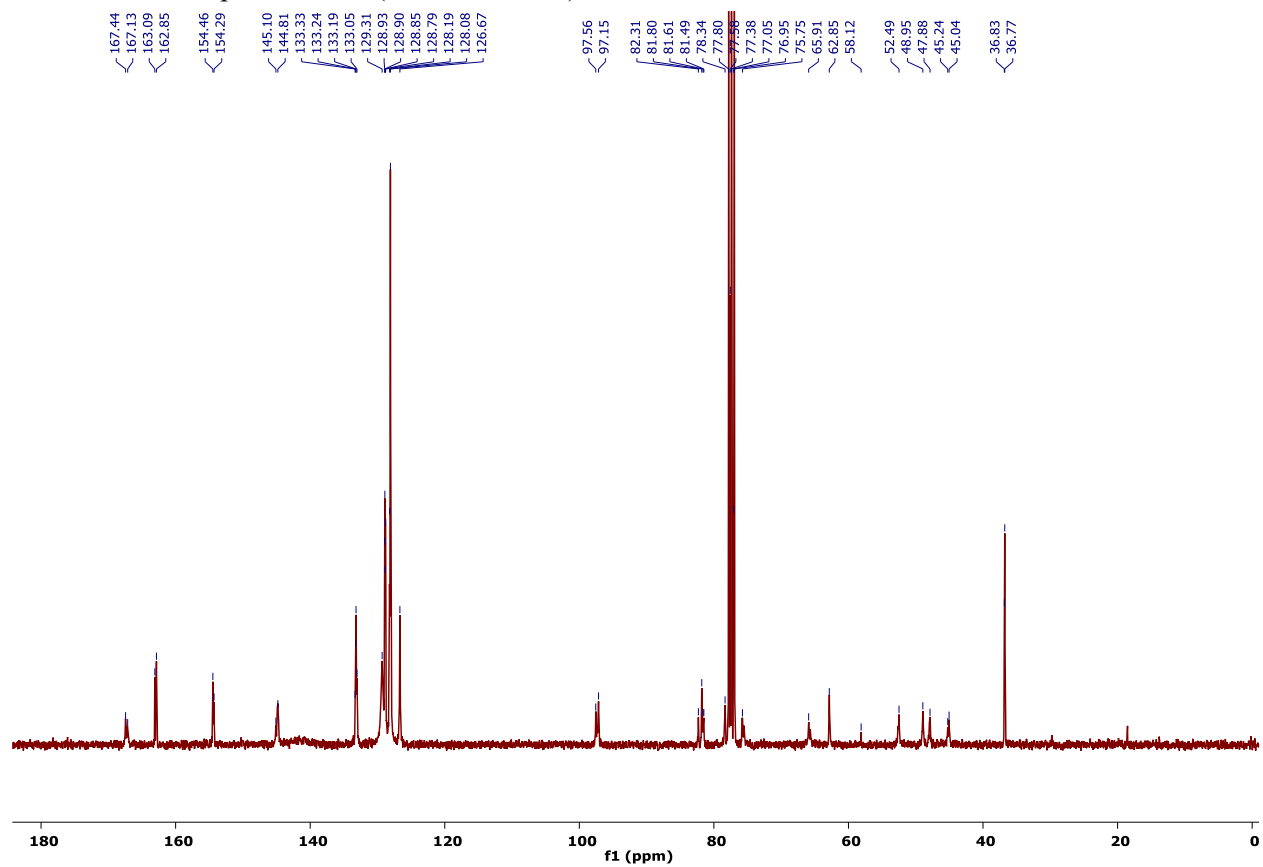
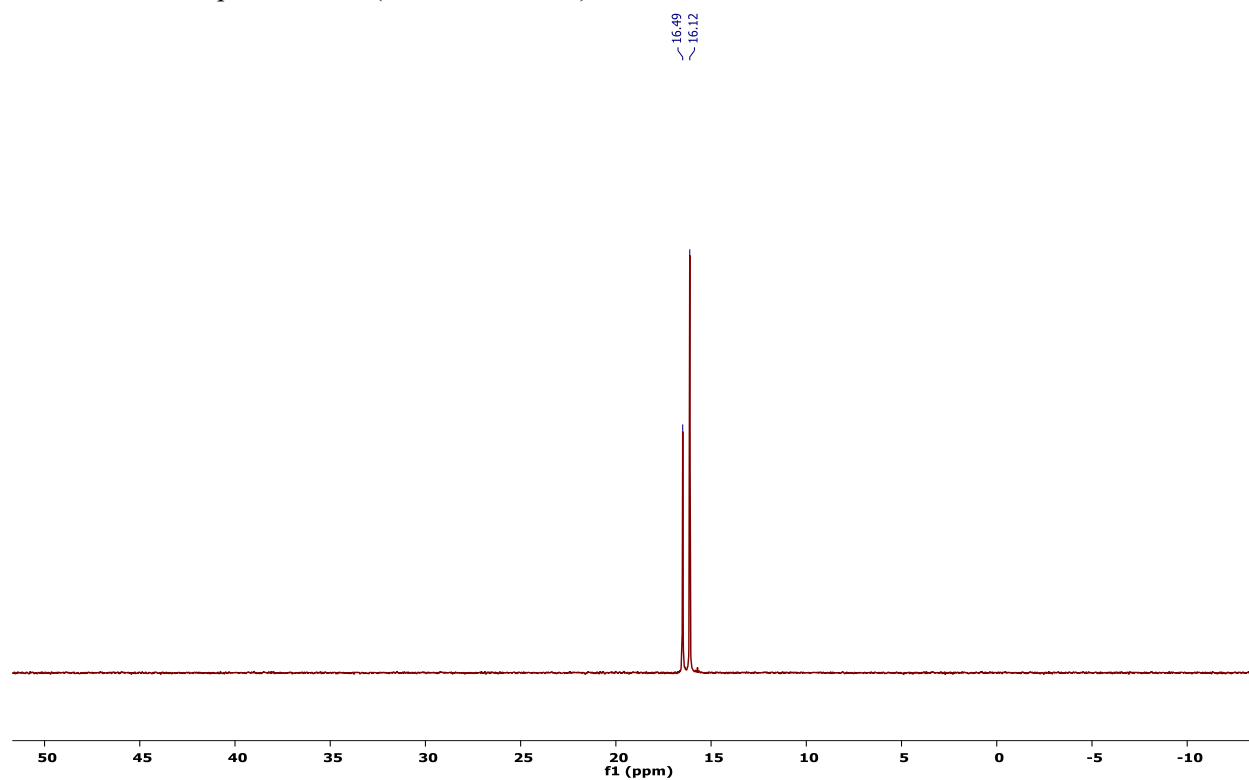
¹H NMR (300 MHz, Chloroform-*d*) δ 7.42 (s, 7H), 7.25 (q, $J = 7.2$ Hz, 8H), 7.17 – 7.04 (m, 4H), 6.13 (d, $J = 9.8$ Hz, 1H), 5.69 (t, $J = 6.9$ Hz, 1H), 4.24 (q, $J = 8.7$ Hz, 1H), 4.12 – 3.77 (m, 2H), 3.77 – 2.57 (m, 14H), 2.24 (t, $J = 10.8$ Hz, 1H), 2.09 (d, $J = 12.9$ Hz, 1H), 1.91 (s, 3H), 1.77 (s, 4H), 1.51 (s, 2H), 1.35 (t, $J = 6.3$ Hz, 10H), 1.16 (d, $J = 7.6$ Hz, 8H).

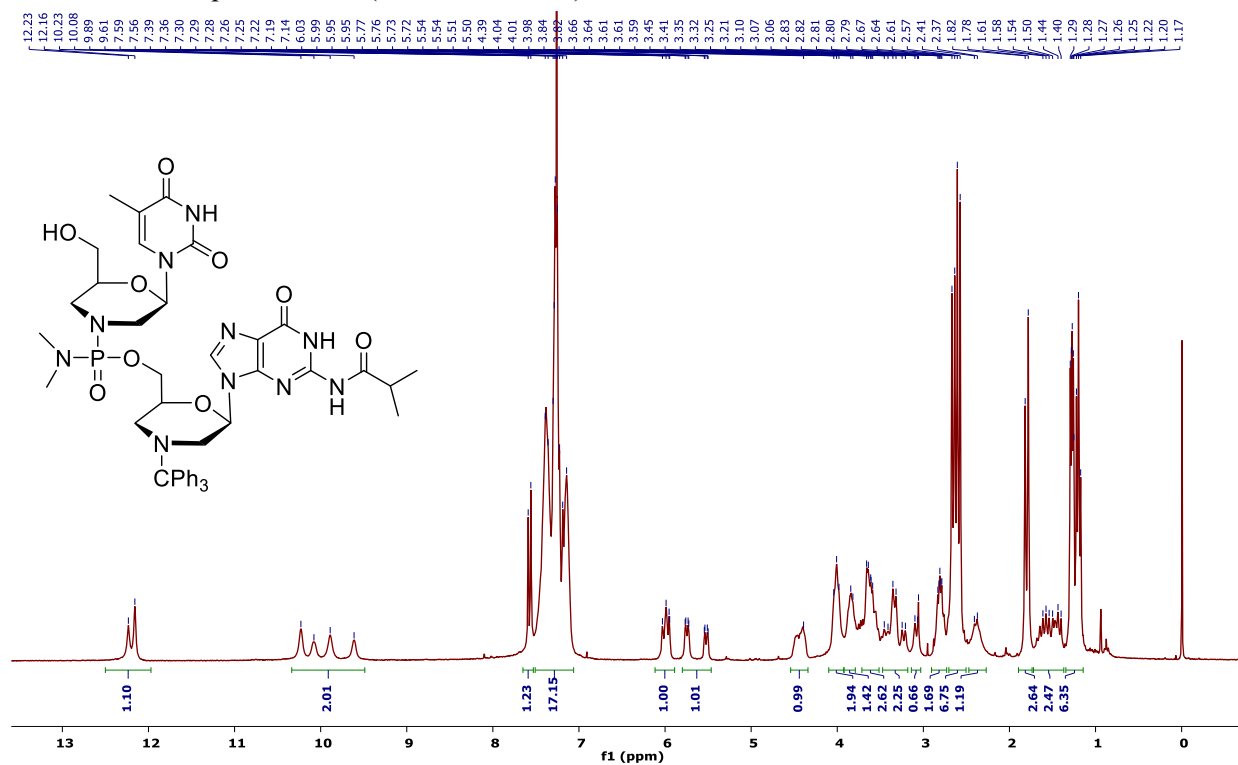
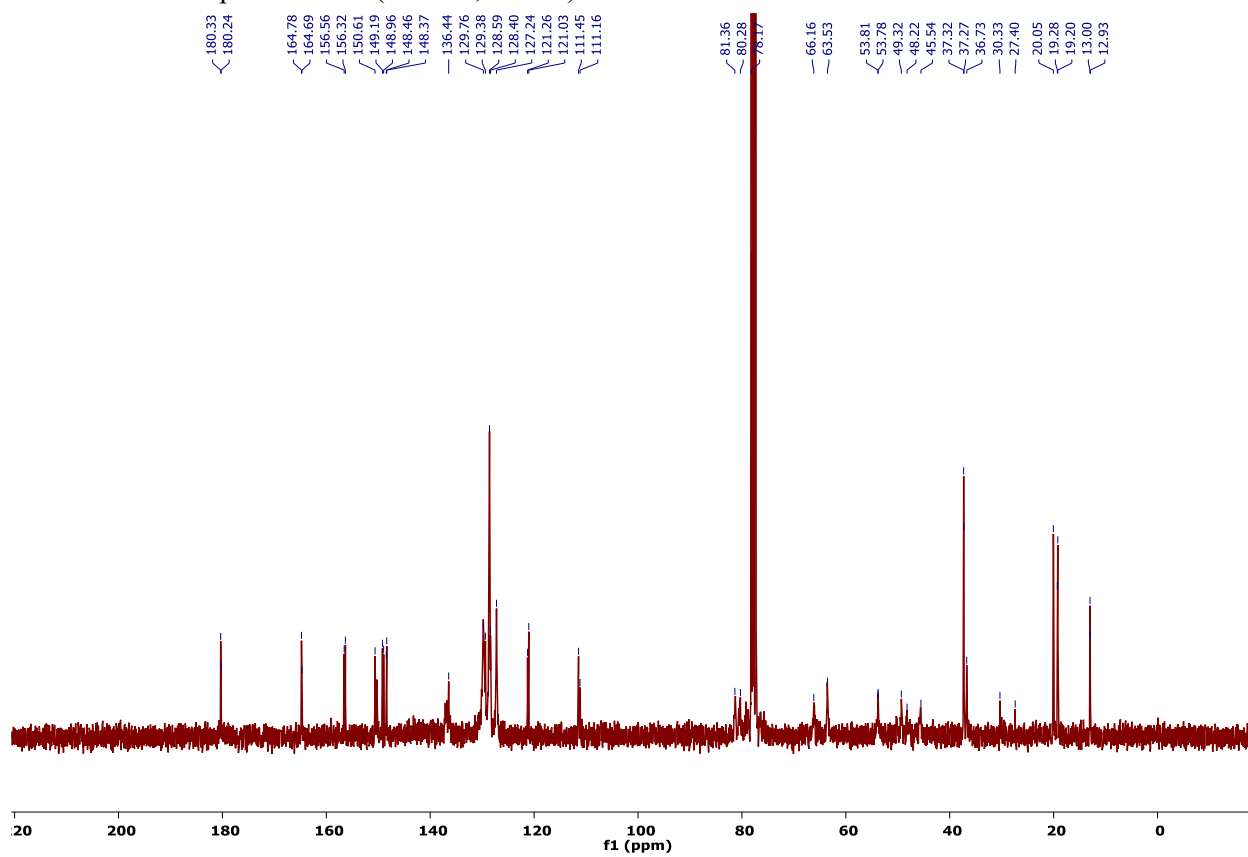
¹³C NMR (75 MHz, Chloroform-*d*) δ 164.1, 150.2, 149.9, 136.1, 135.3, 127.8, 126.4, 110.5, 80.7, 79.6, 76.8, 61.2, 58.8, 56.5, 53.5, 51.4, 49.8, 43.2, 43.0, 41.4, 31.0, 30.9, 30.8, 30.4, 24.8, 24.7, 24.6, 24.2, 24.1, 23.9, 19.7, 13.6, 12.5, 12.2.

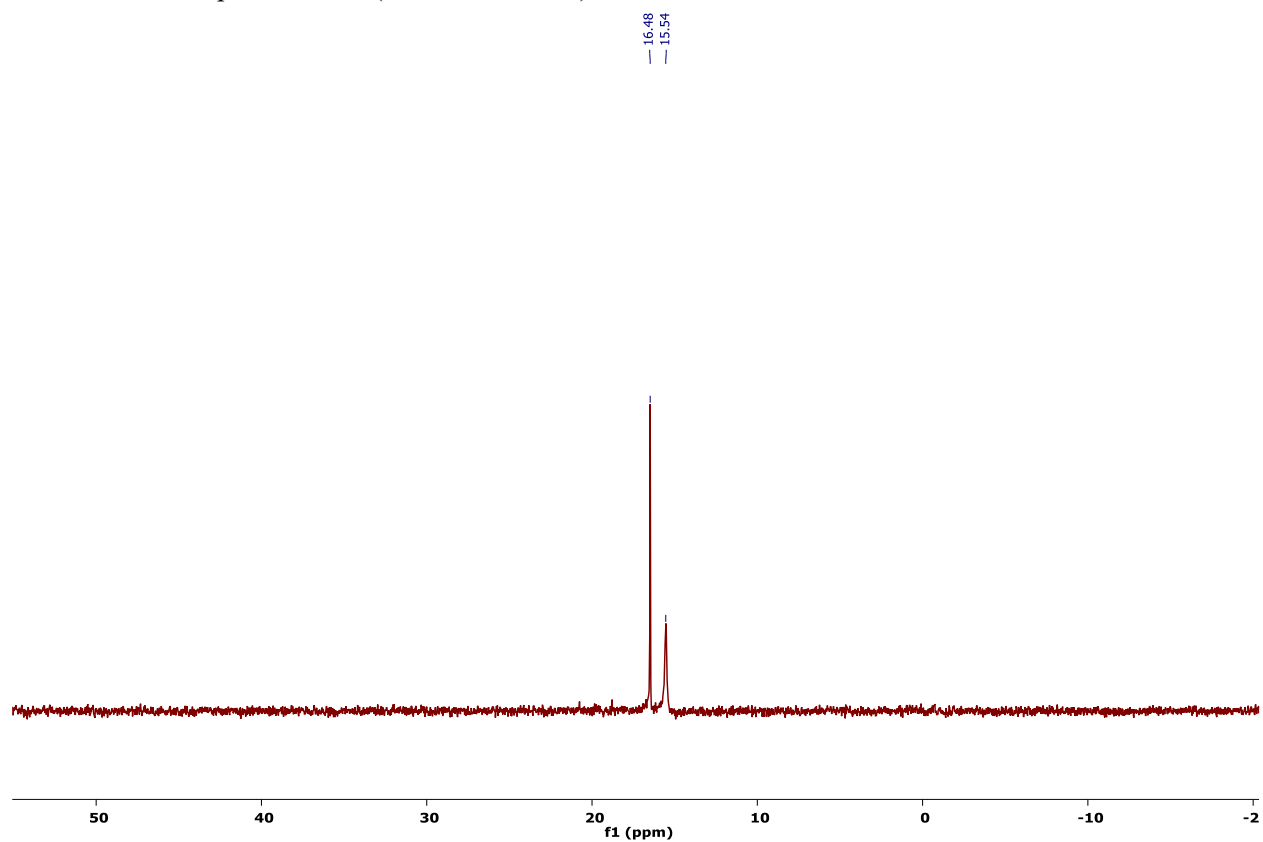
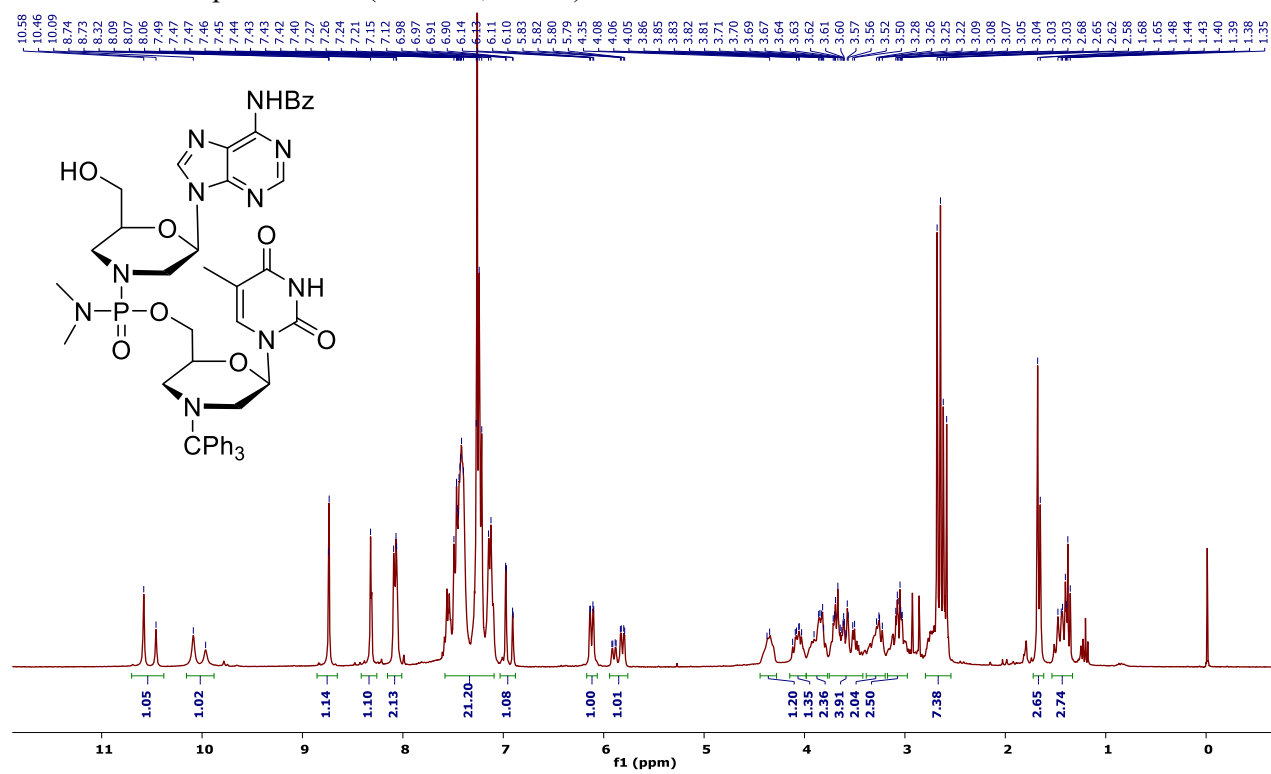
³¹P NMR (202 MHz, CDCl₃) δ 138.67, 138.66.

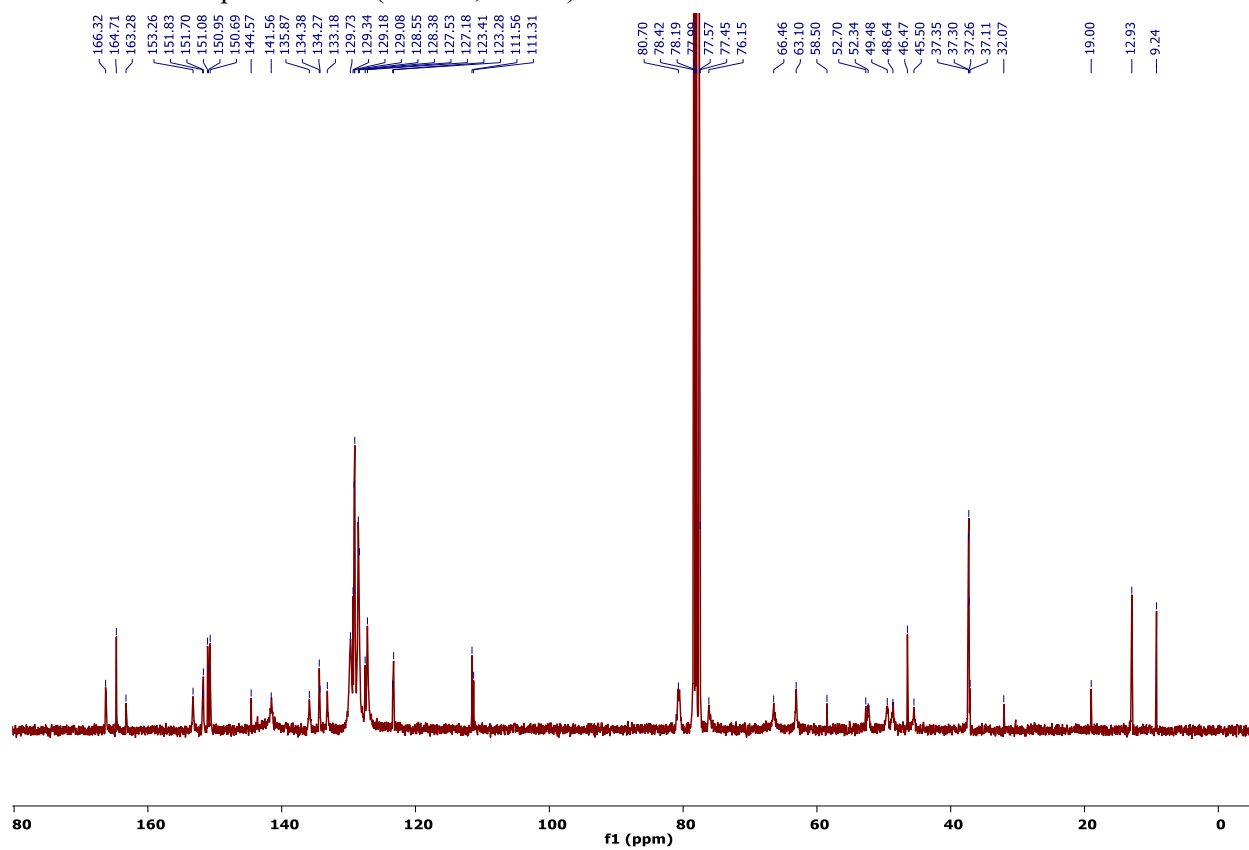
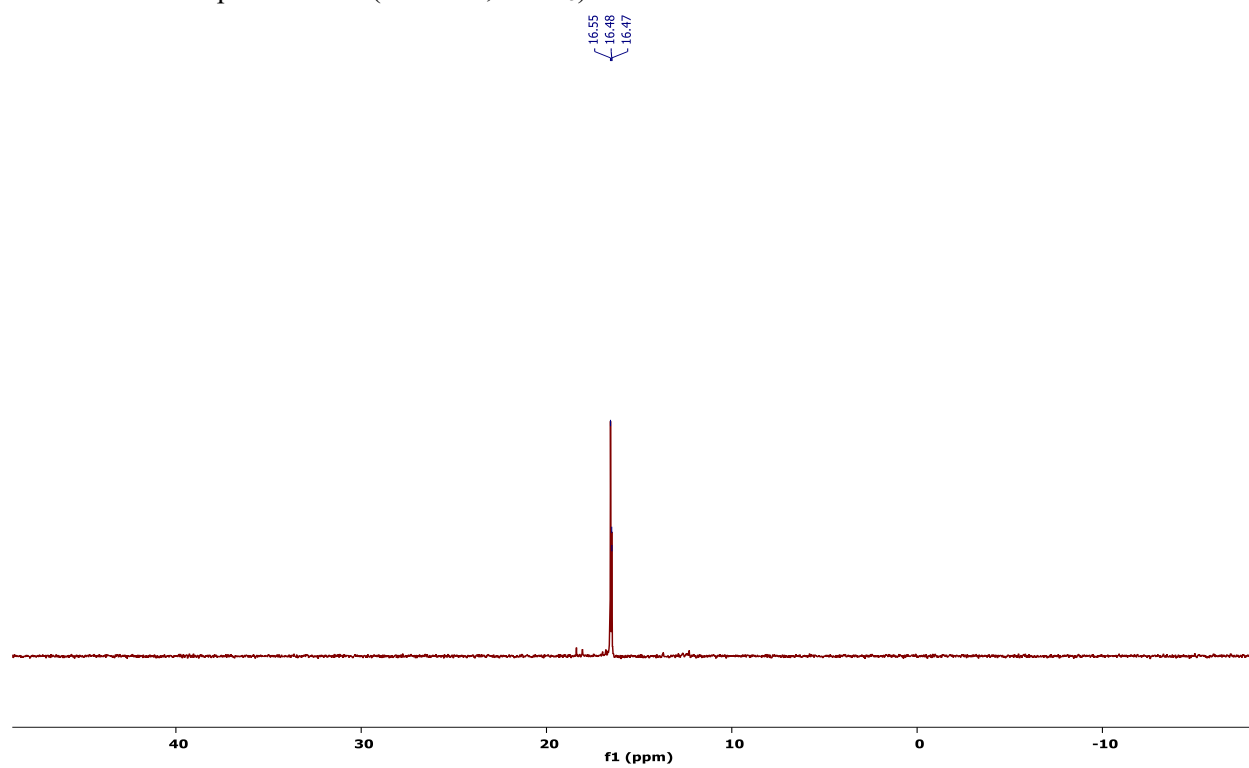
HRMS Data [M-H]⁻: Mass calculated for C₅₁H₆₆N₈O₉P is 965.4696, found 965.4690.

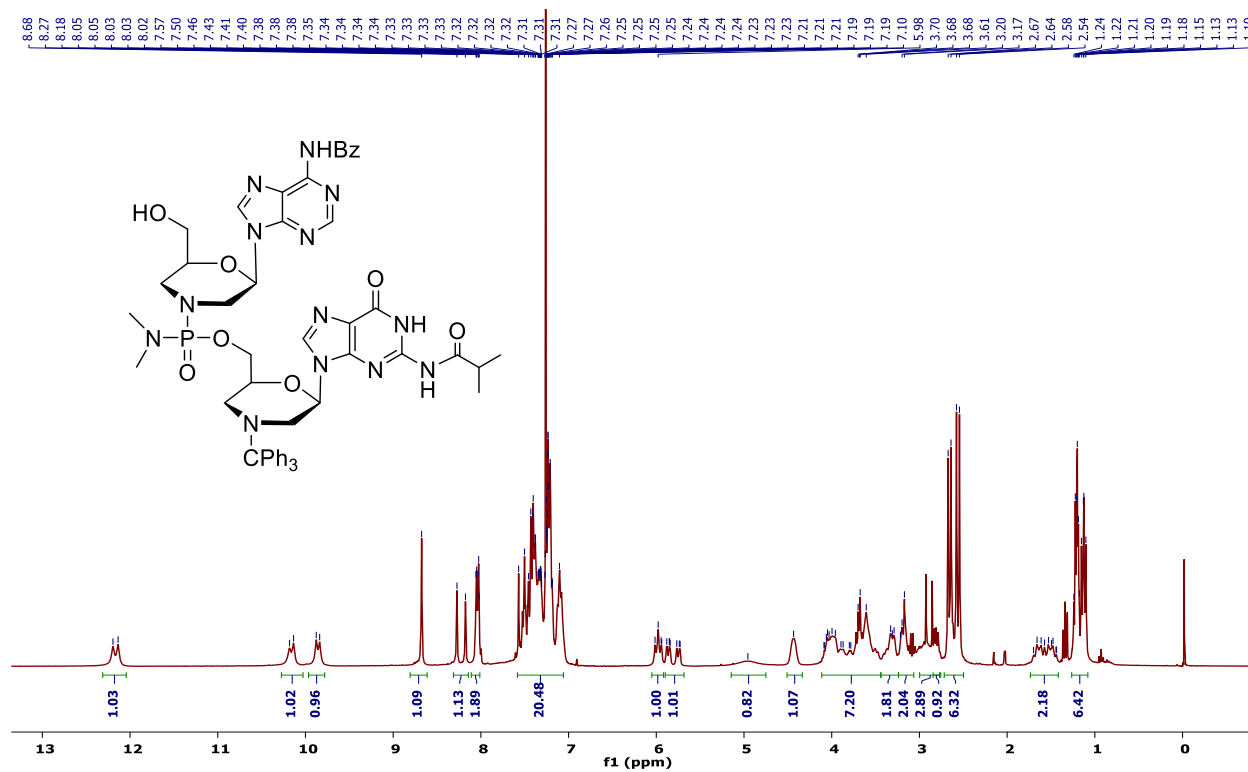
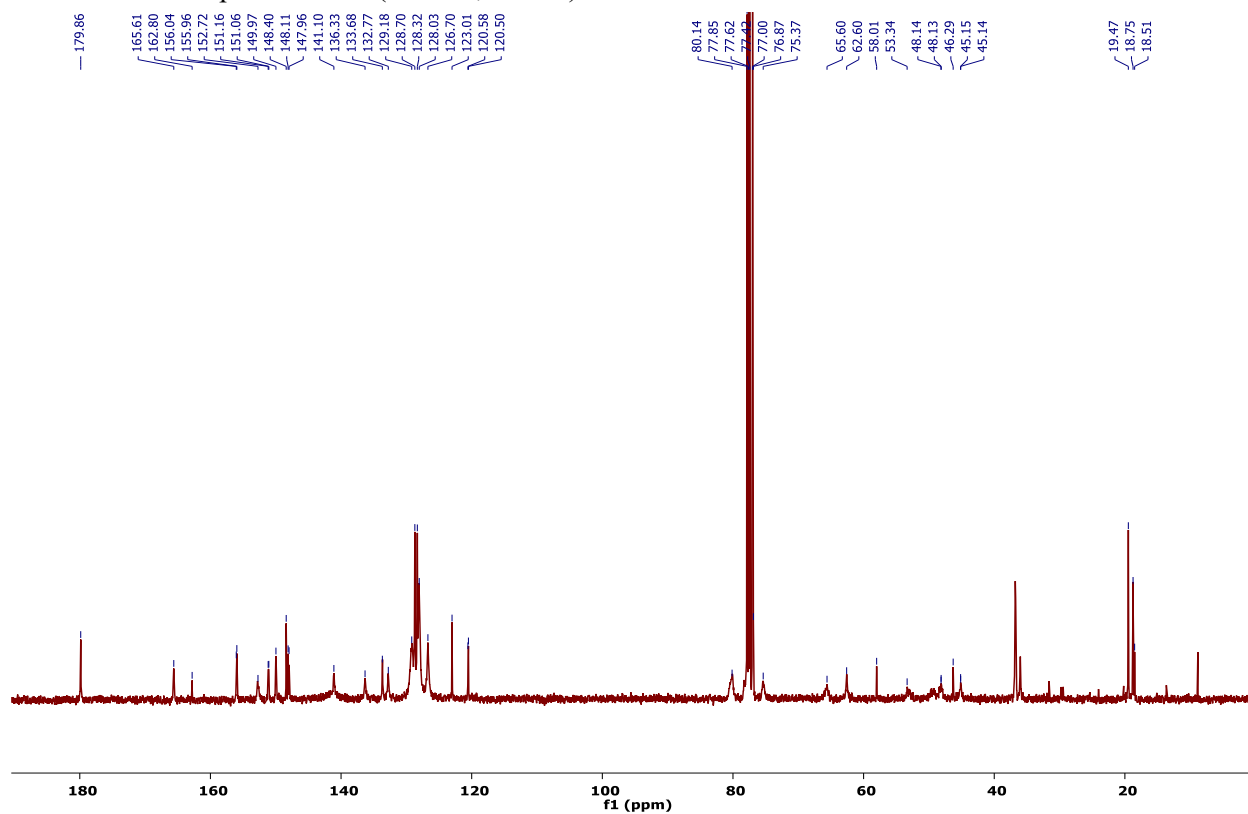
^{31}P NMR of Compound **3T^PT** (121MHz, CDCl_3) ^1H NMR of Compound **3C^PC** (300MHz, CDCl_3)

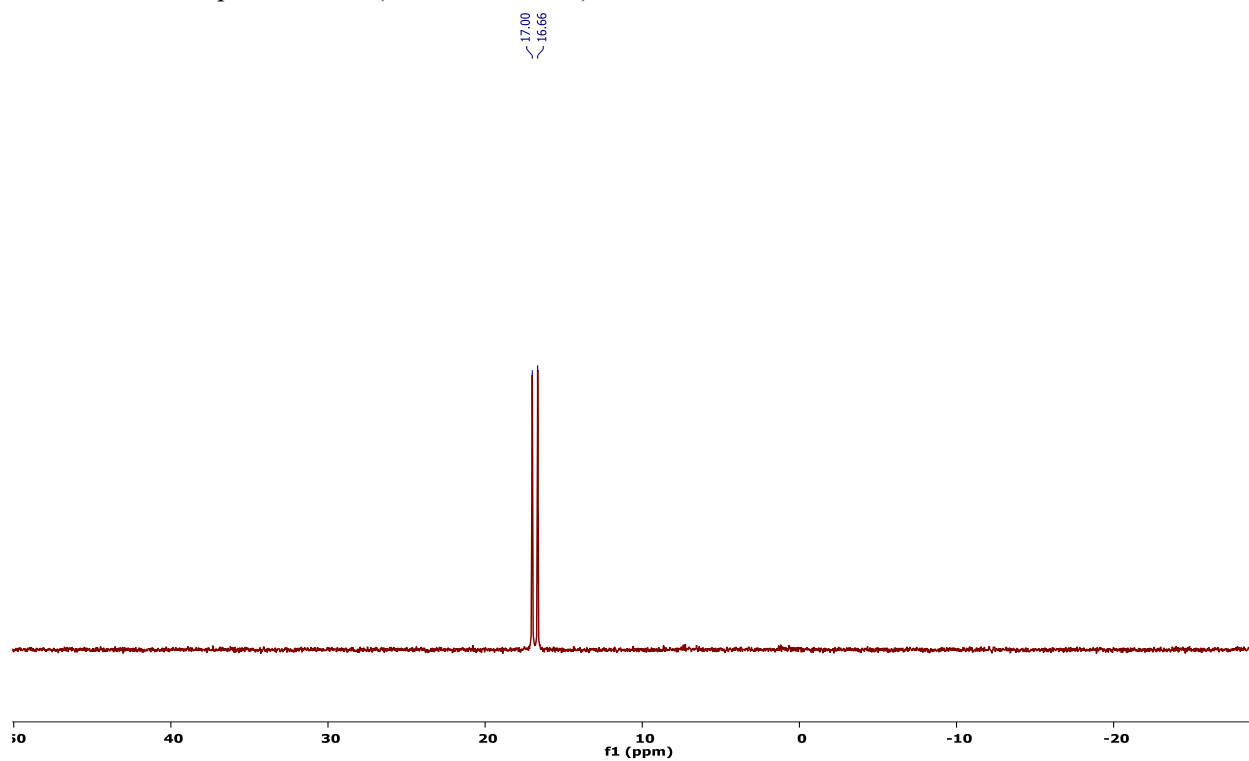
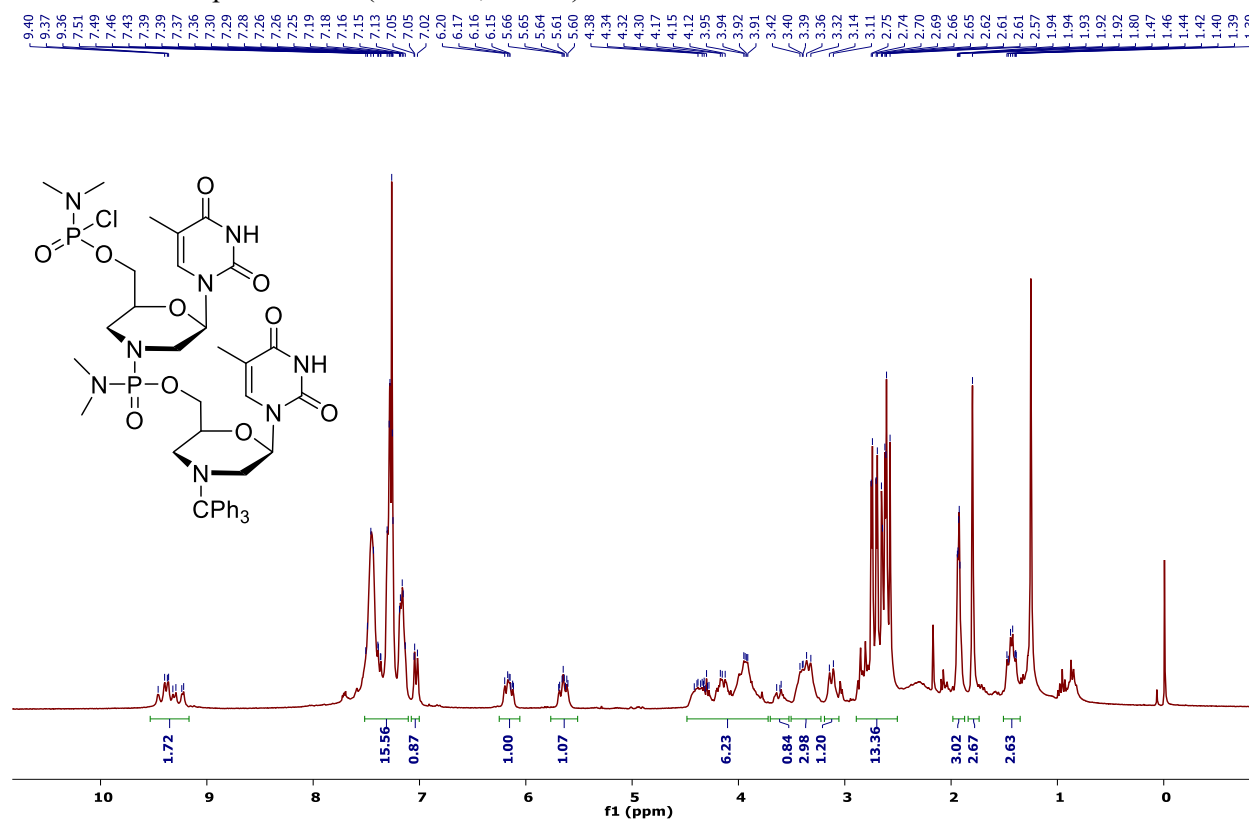
^{13}C NMR of Compound **3C^pC** (75MHz, CDCl_3) ^{31}P NMR of Compound **3C^pC** (121MHz, CDCl_3)

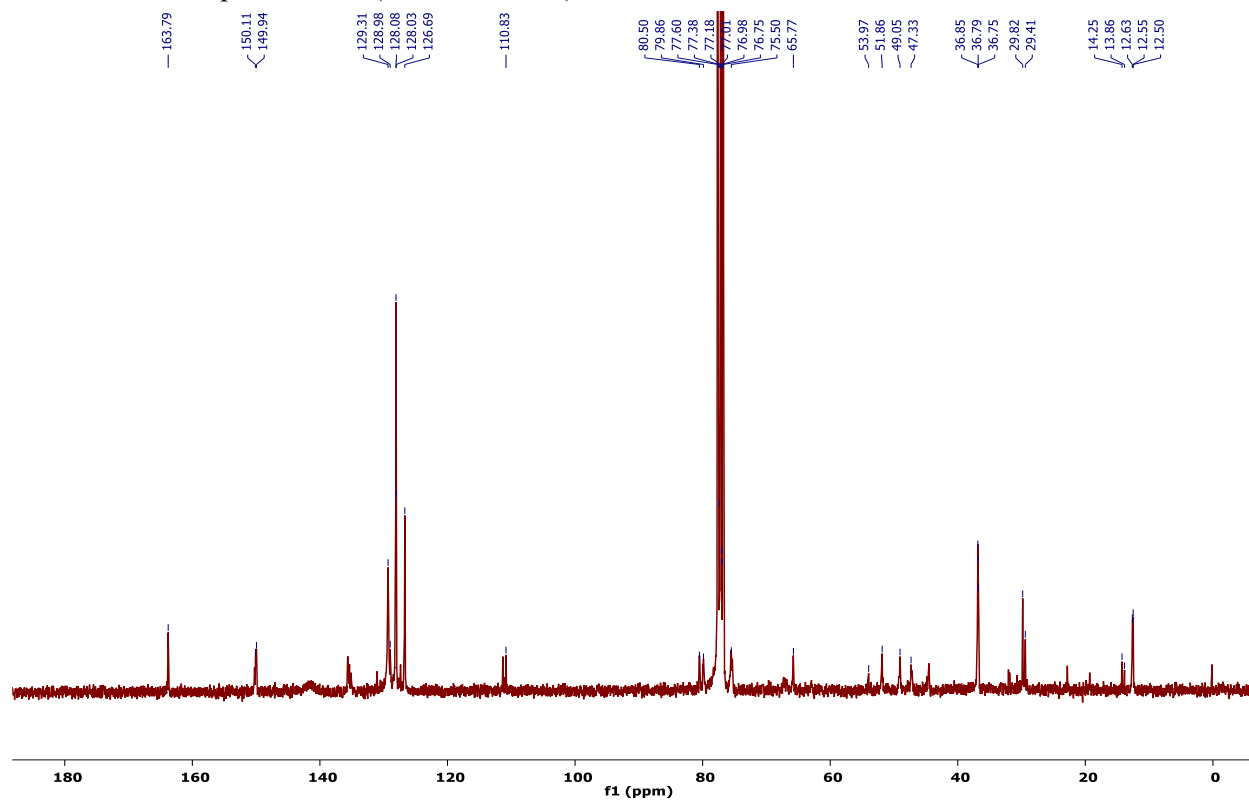
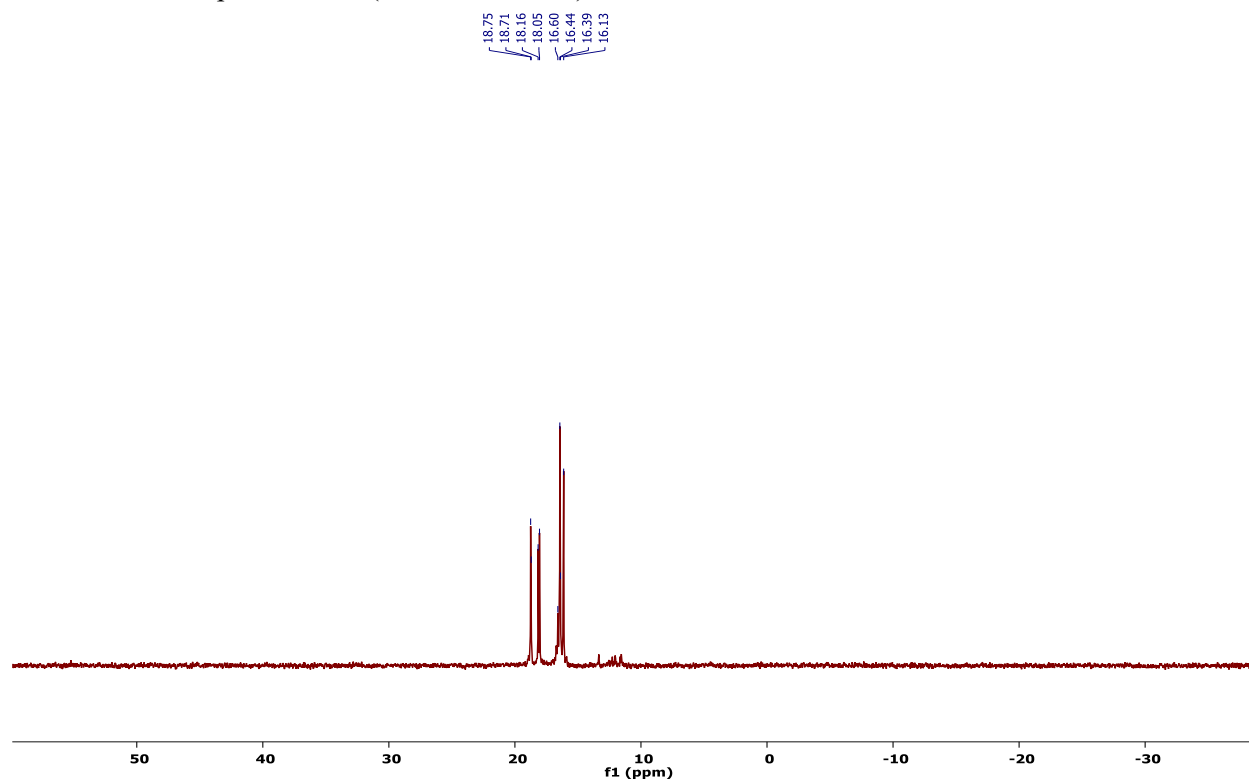
¹H NMR of Compound 3T^pG (300MHz, CDCl₃)¹³C NMR of Compound 3T^pG (75MHz, CDCl₃)

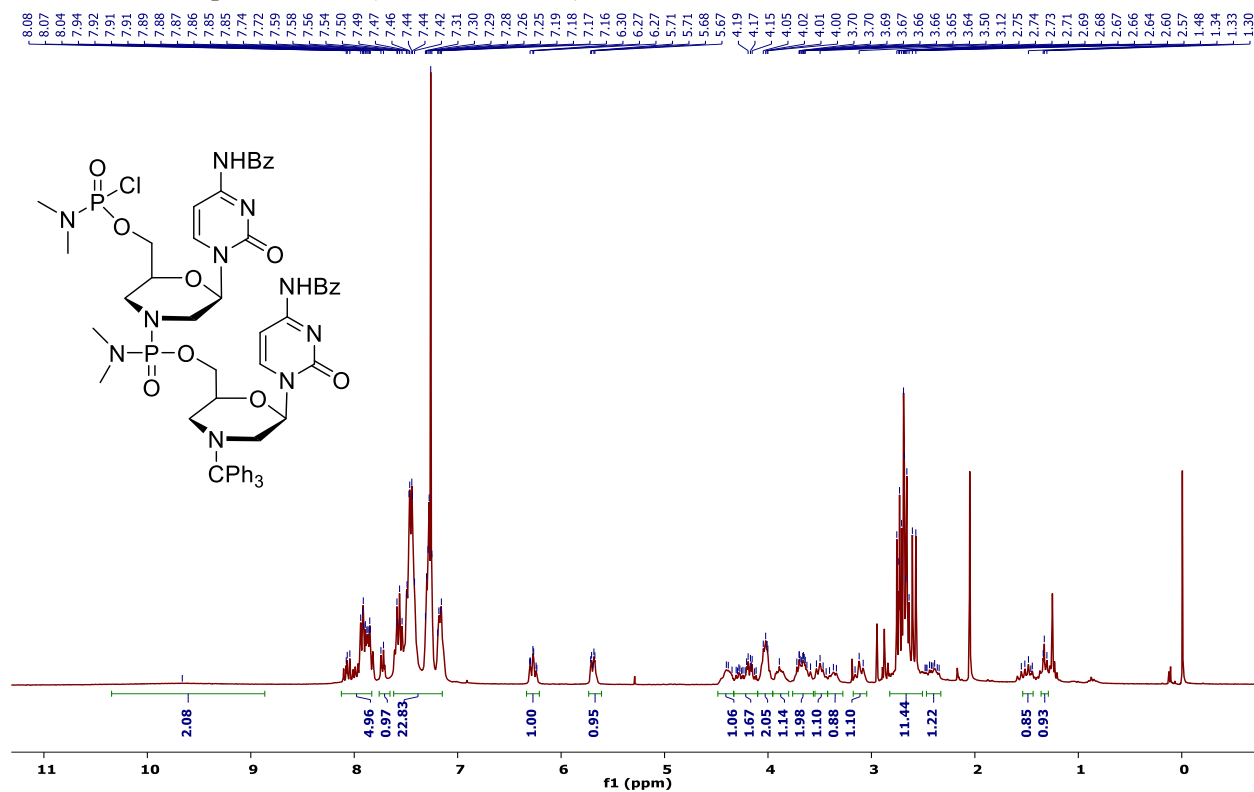
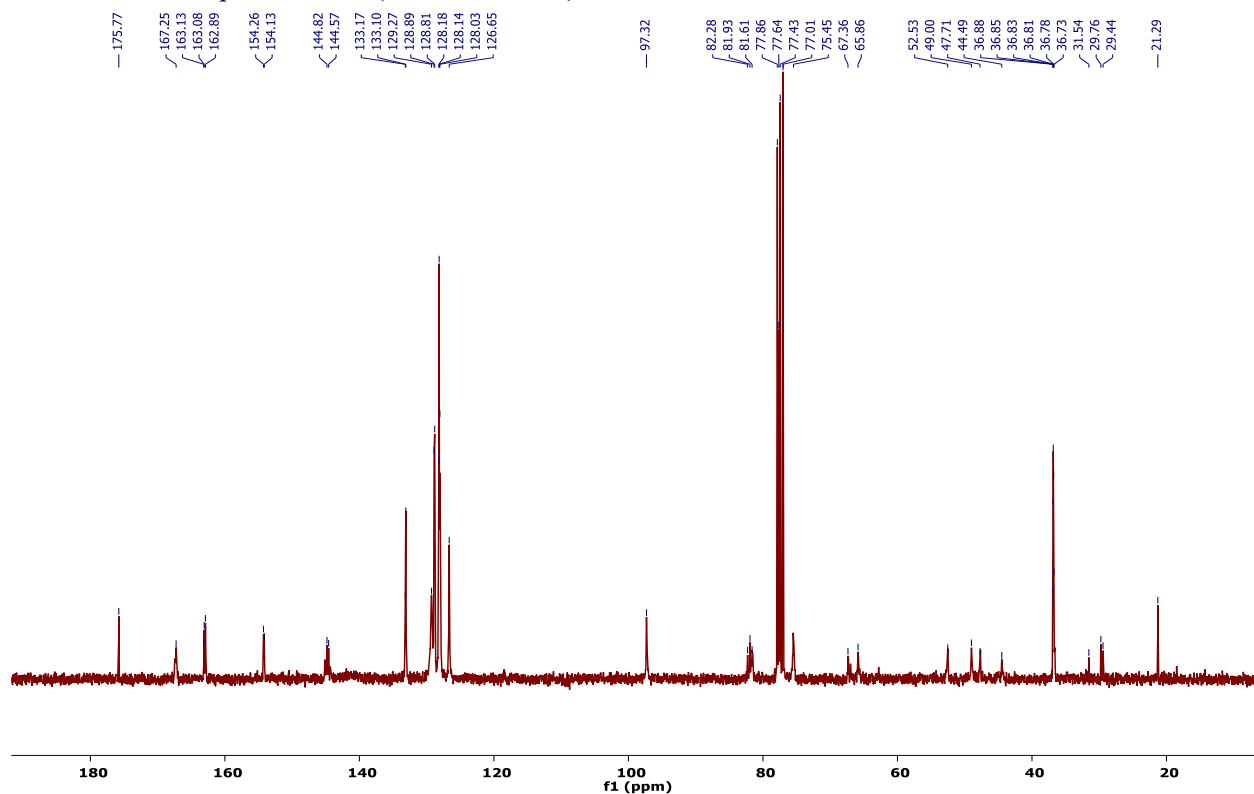
³¹P NMR of Compound **3T^pG** (121MHz, CDCl₃)¹H NMR of Compound **3A^pT** (300MHz, CDCl₃)

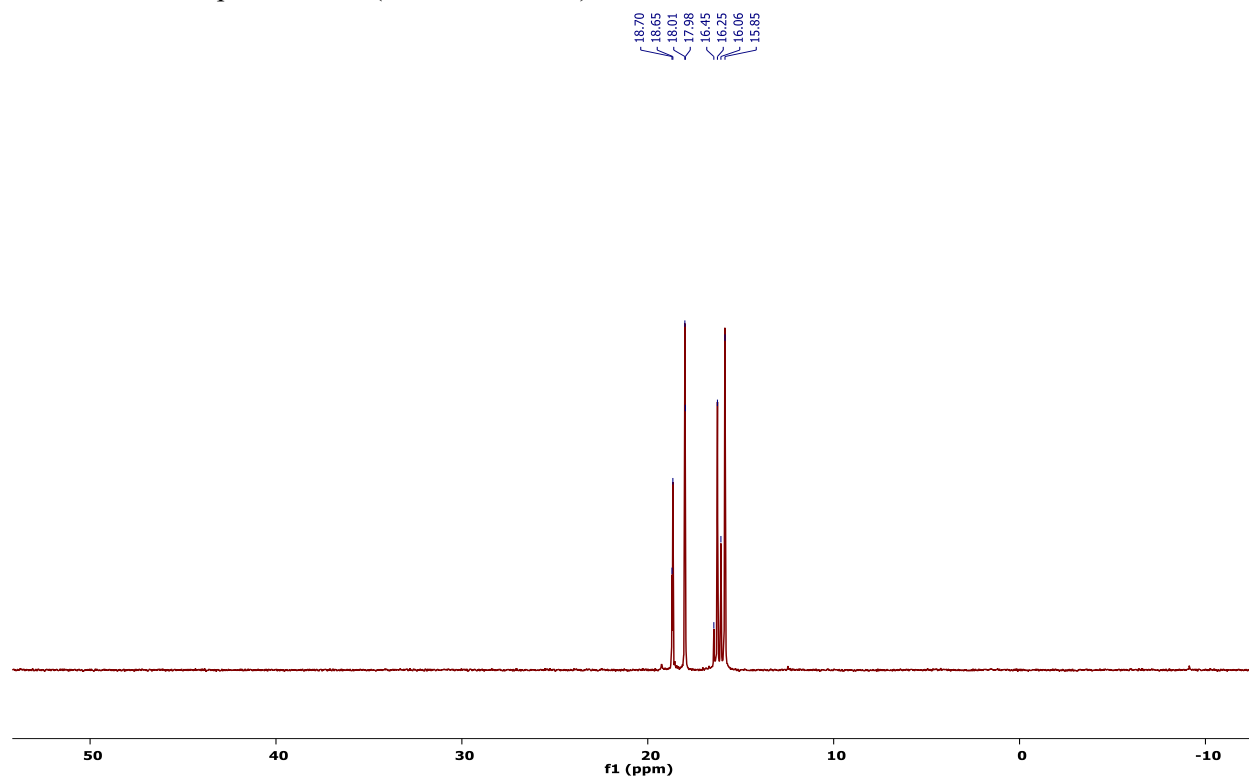
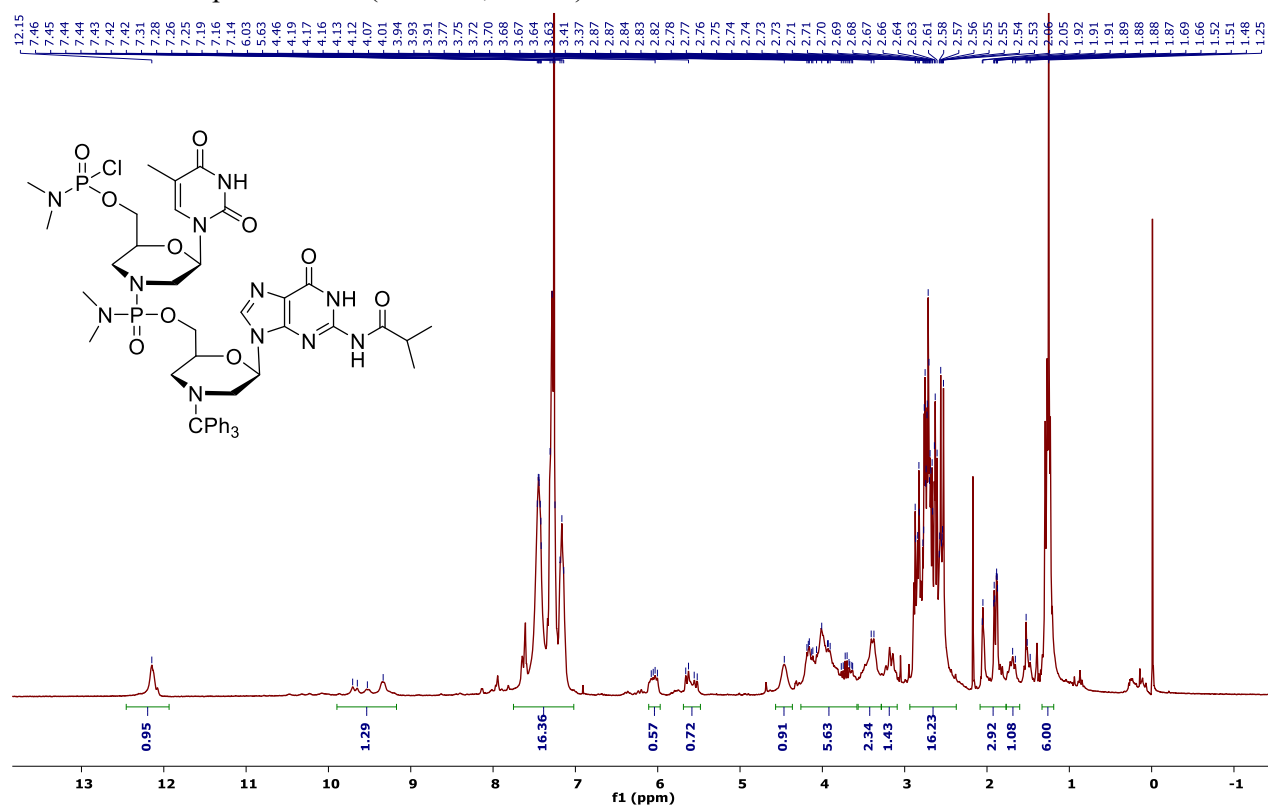
^{13}C NMR of Compound **3A^PT** (75MHz, CDCl_3) ^{31}P NMR of Compound **3A^PT** (121MHz, CDCl_3)

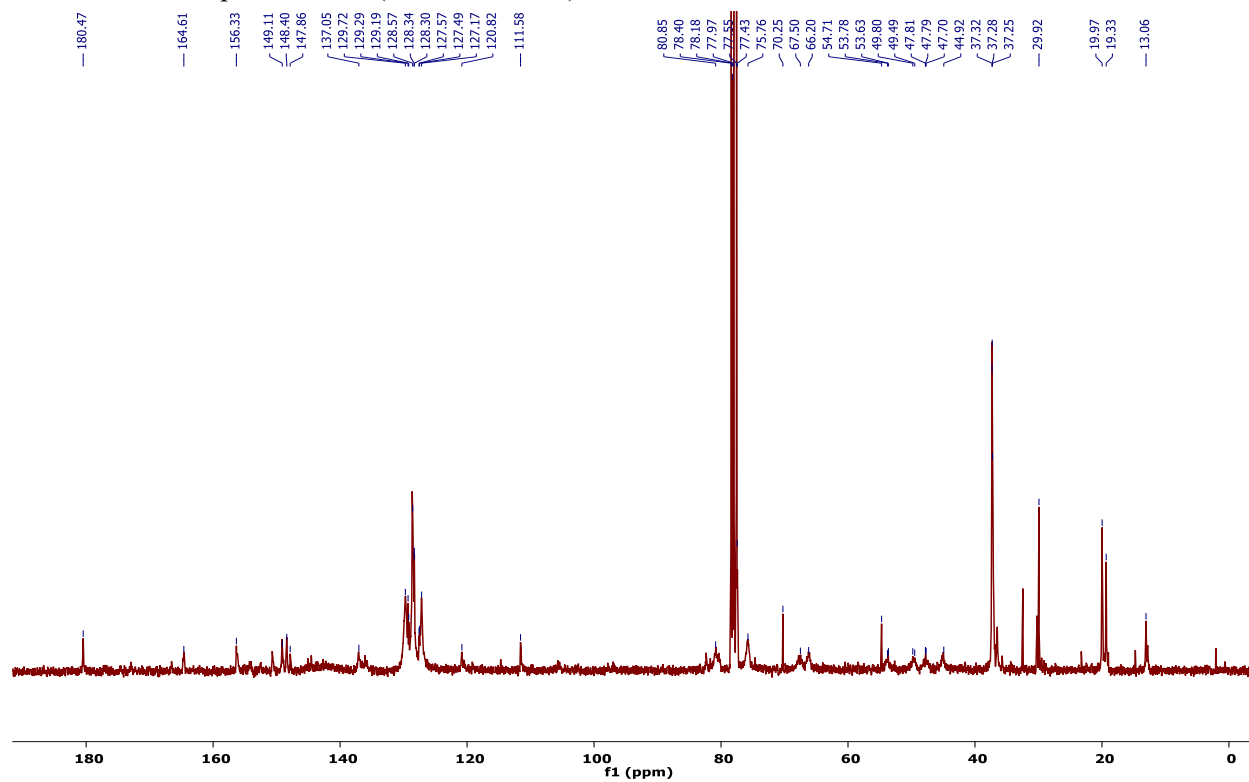
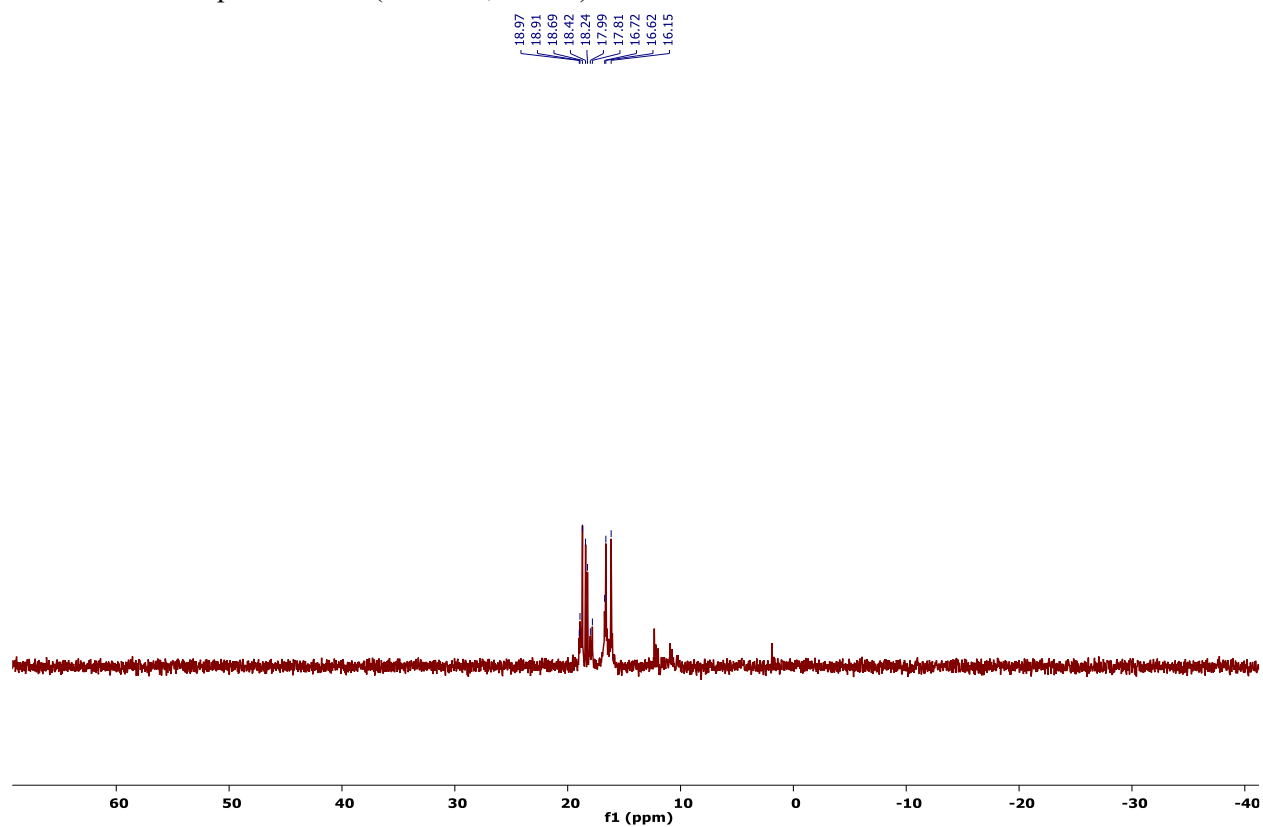
^1H NMR of Compound **3A^PG** (300MHz, CDCl_3) ^{13}C NMR of Compound **3A^PG** (75MHz, CDCl_3)

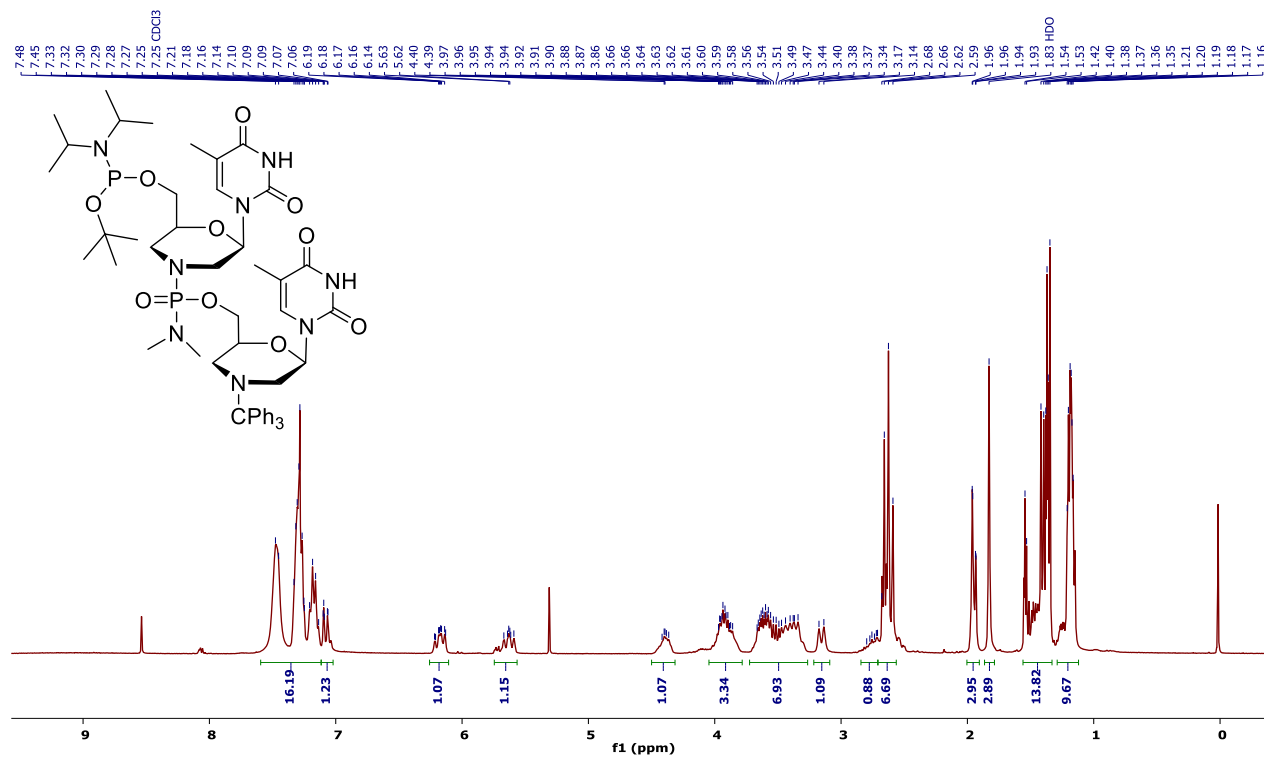
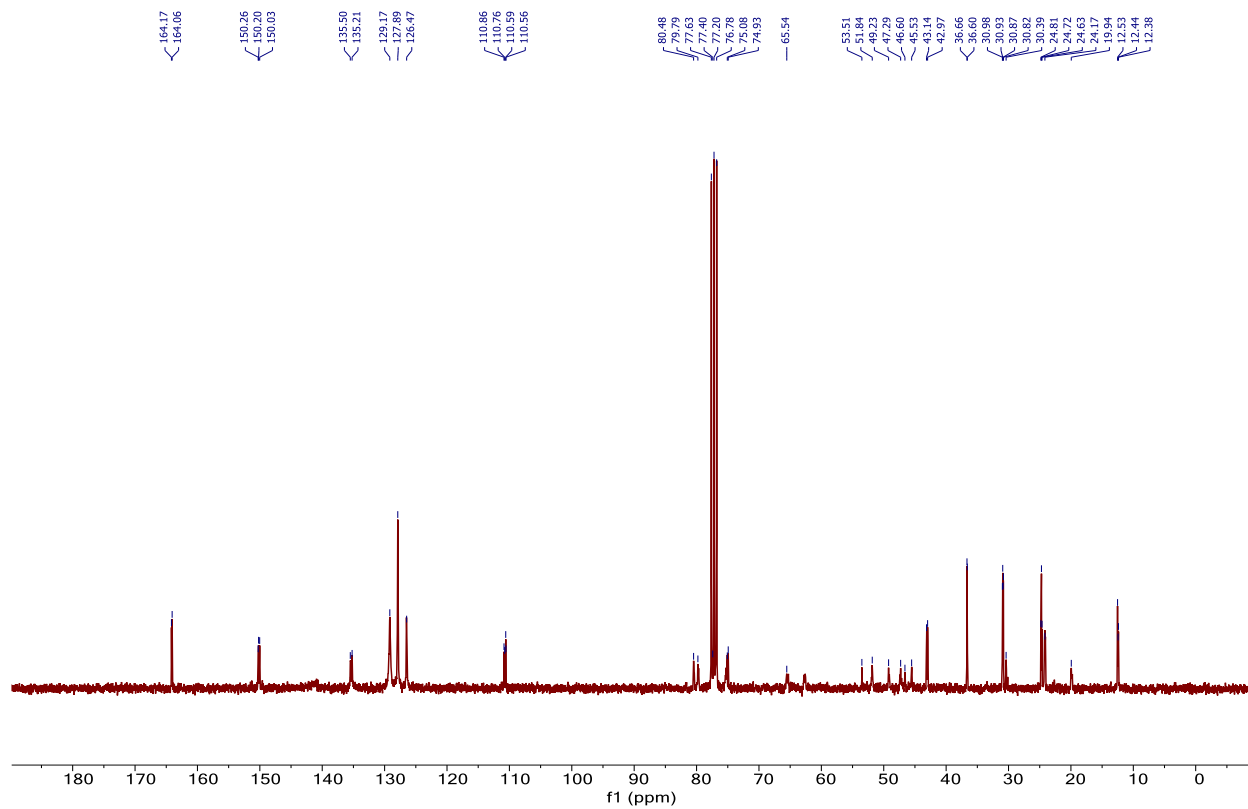
^{31}P NMR of Compound **3A^PG** (121MHz, CDCl_3) ^1H NMR of Compound **4T^PT** (300MHz, CDCl_3)

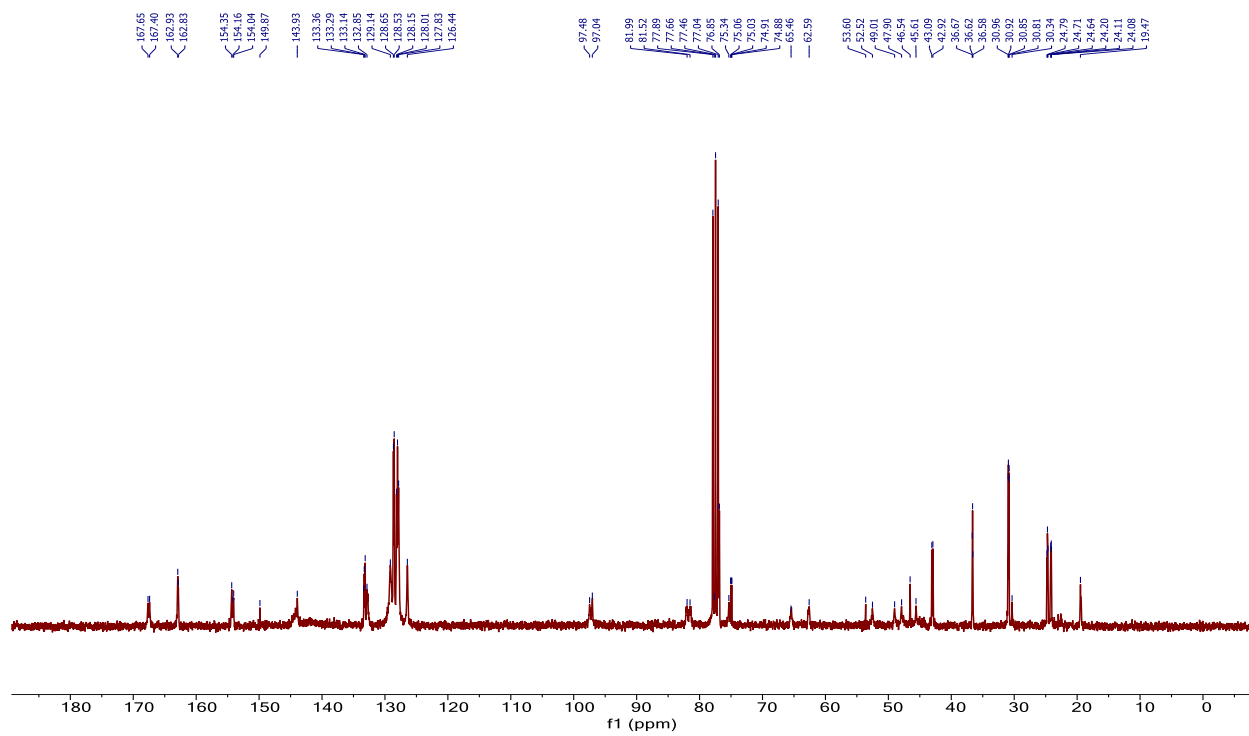
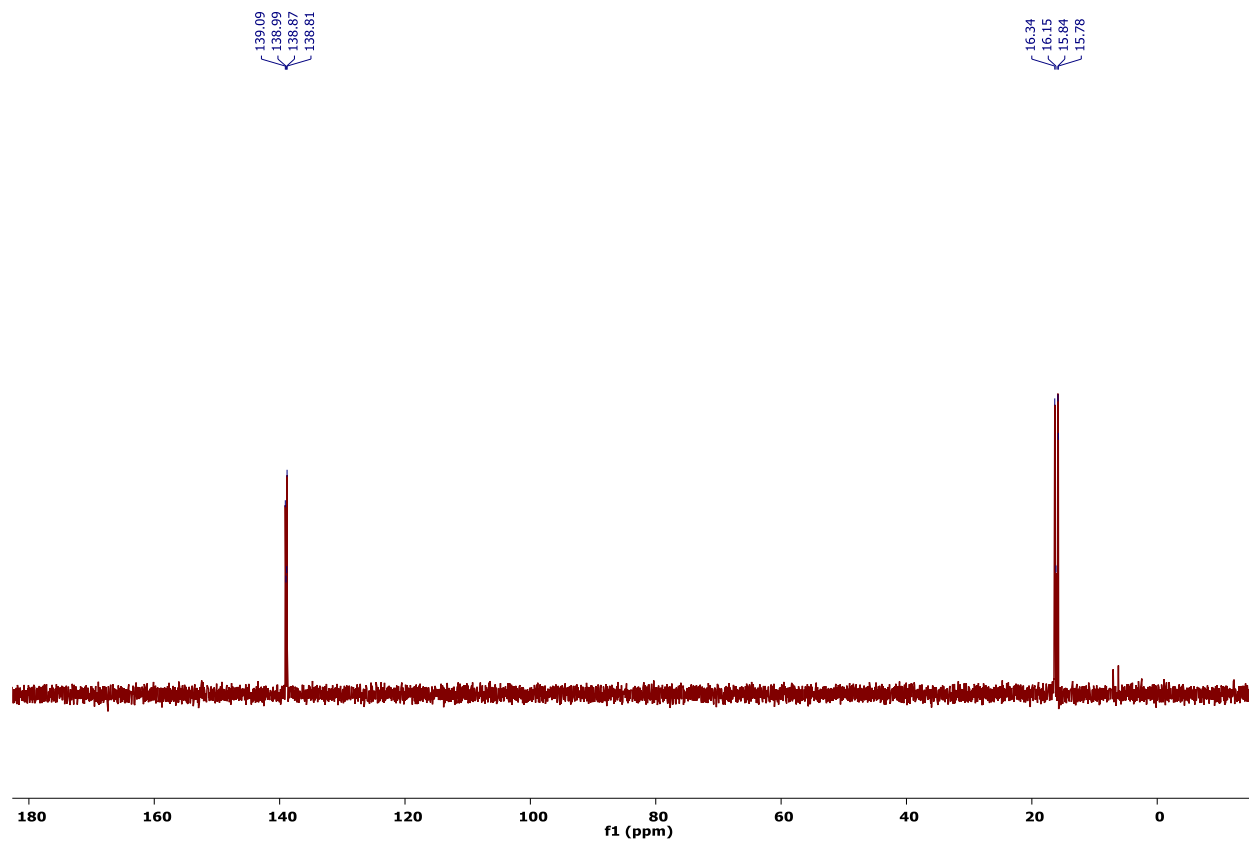
^{13}C NMR of Compound **4T^PT** (75MHz, CDCl_3) ^{31}P NMR of Compound **4T^PT** (121MHz, CDCl_3)

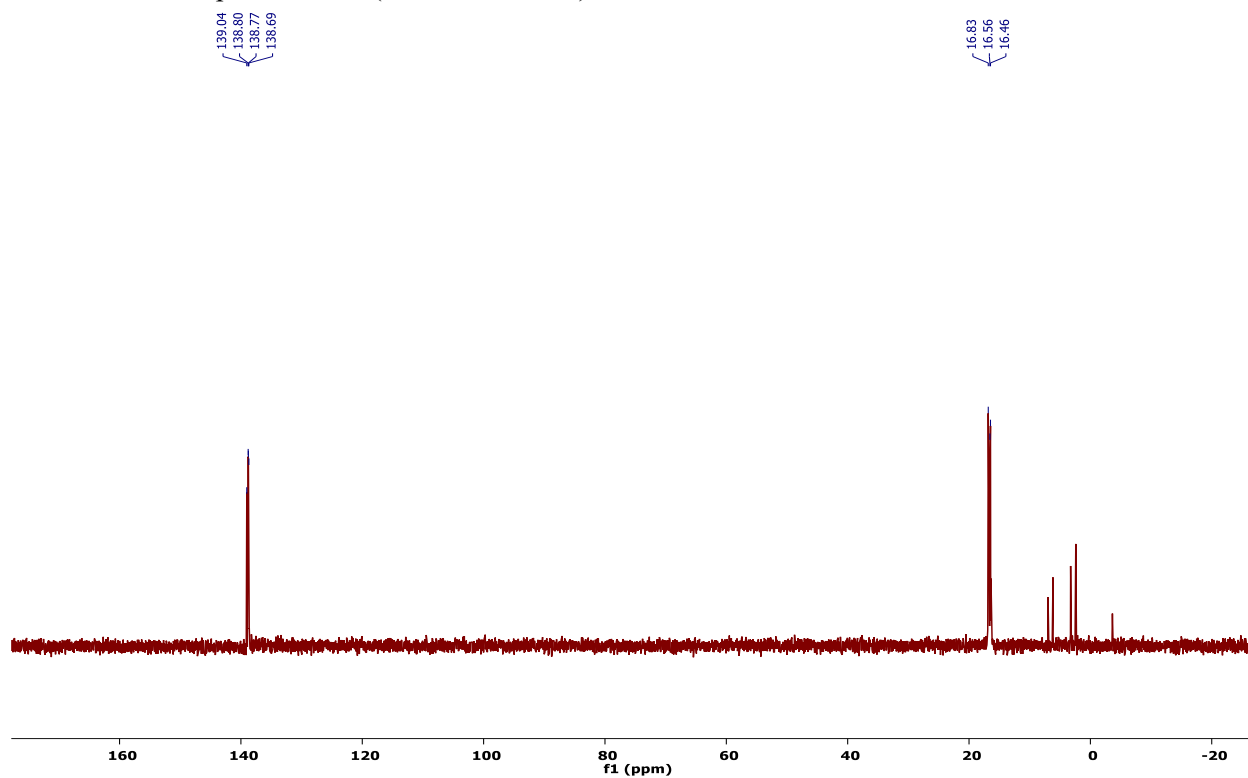
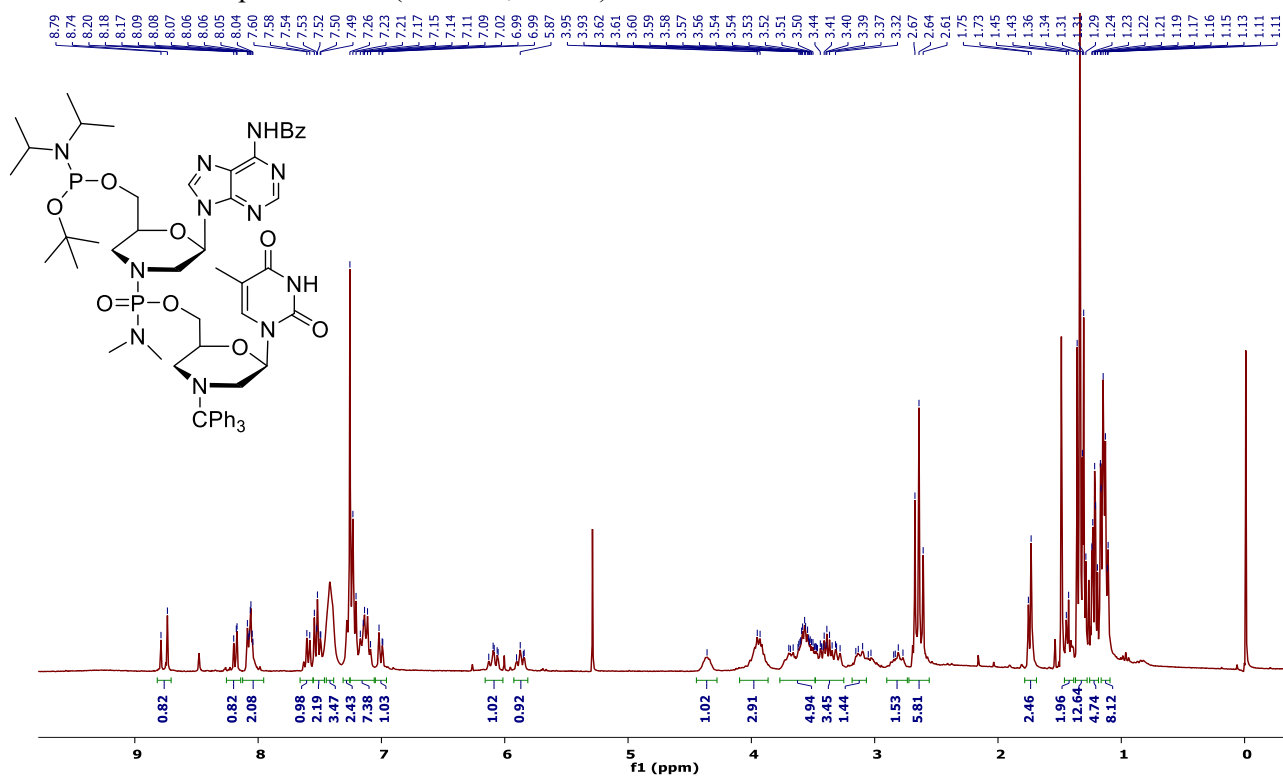
^1H NMR of Compound **4C**^PC (300MHz, CDCl_3) ^{13}C NMR of Compound **4C**^PC (75MHz, CDCl_3)

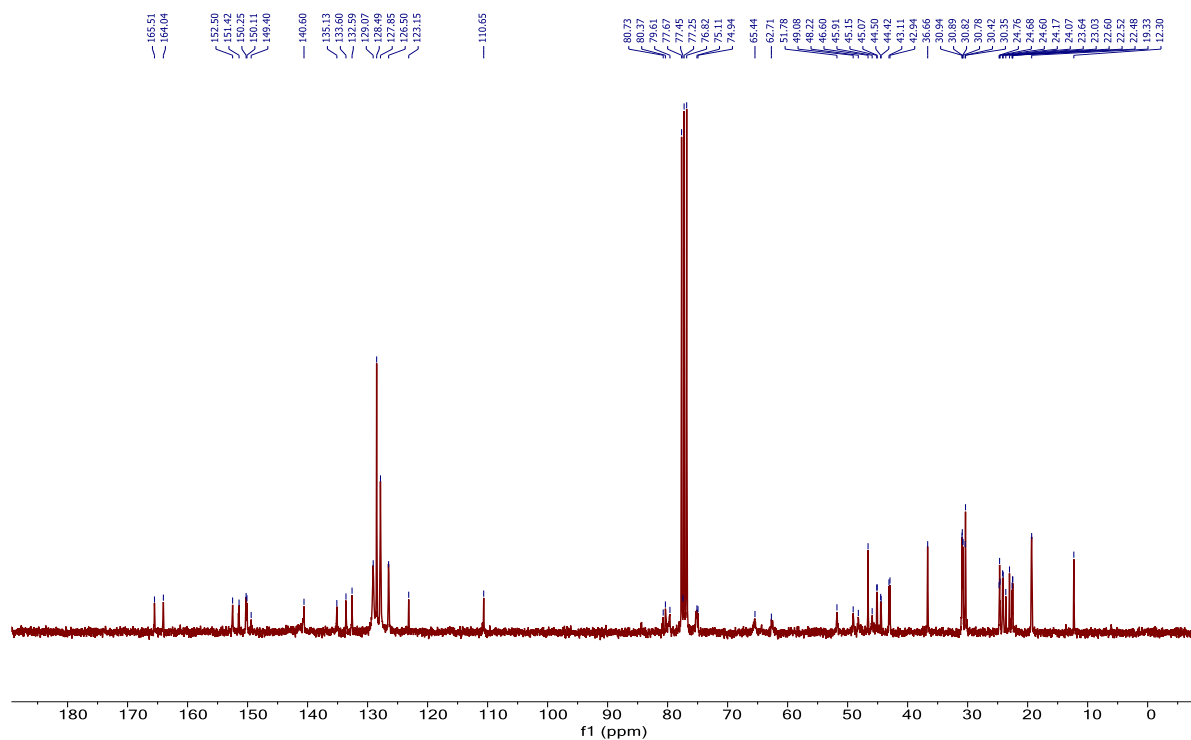
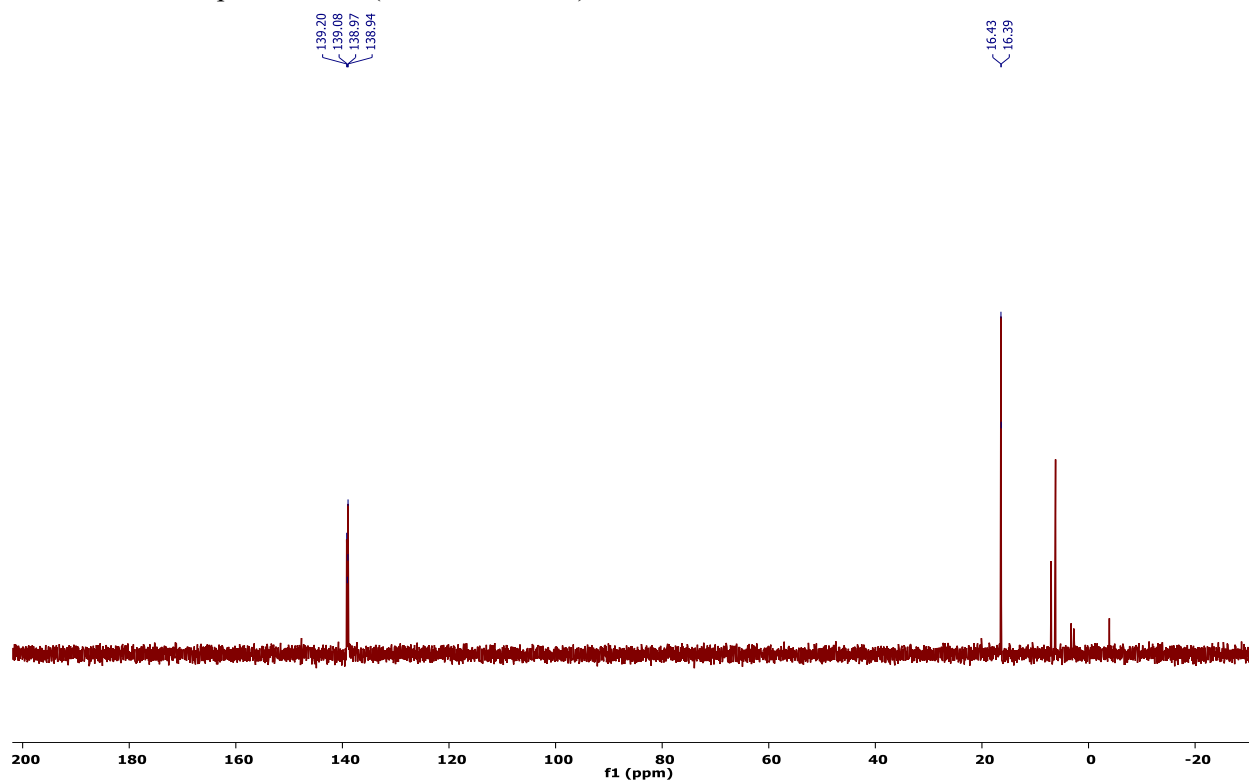
^{31}P NMR of Compound **4C^pC** (121MHz, CDCl_3) ^1H NMR of Compound **4T^pG** (300MHz, CDCl_3)

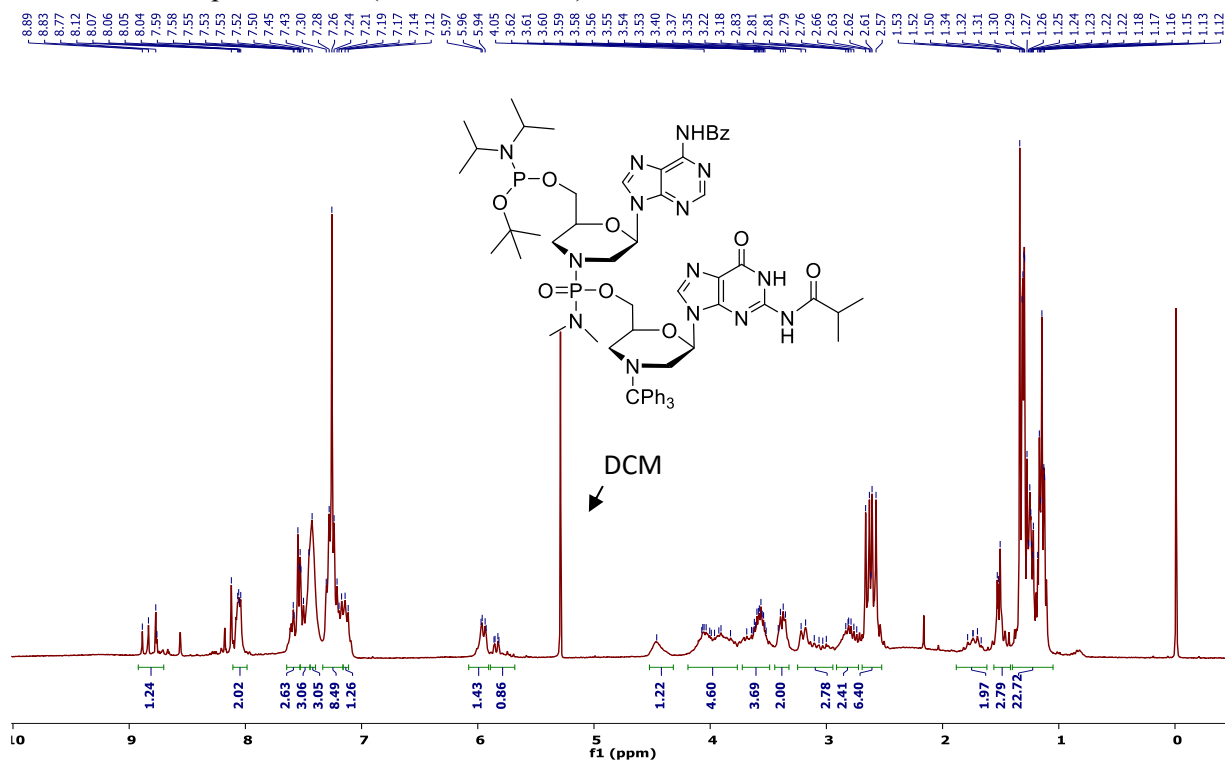
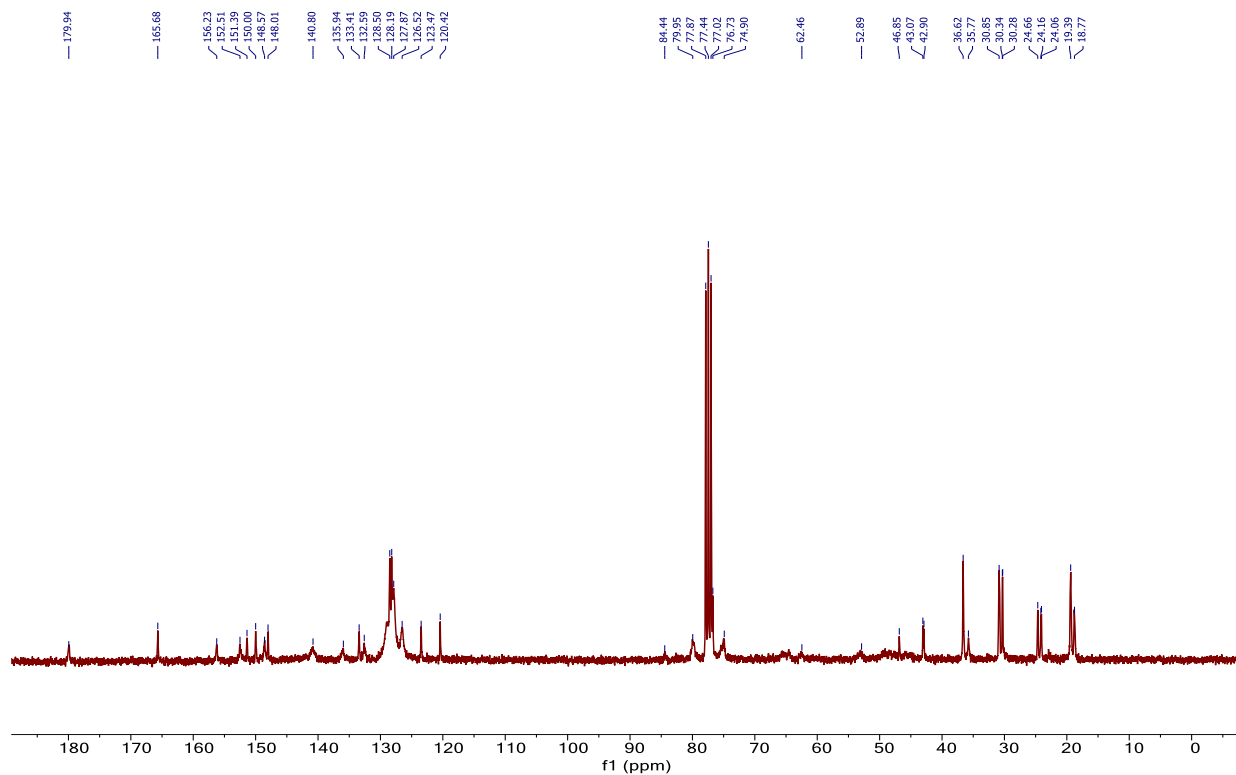
^{13}C NMR of Compound **4T^pG** (75MHz, CDCl_3) ^{31}P NMR of Compound **4T^pG** (121MHz, CDCl_3)

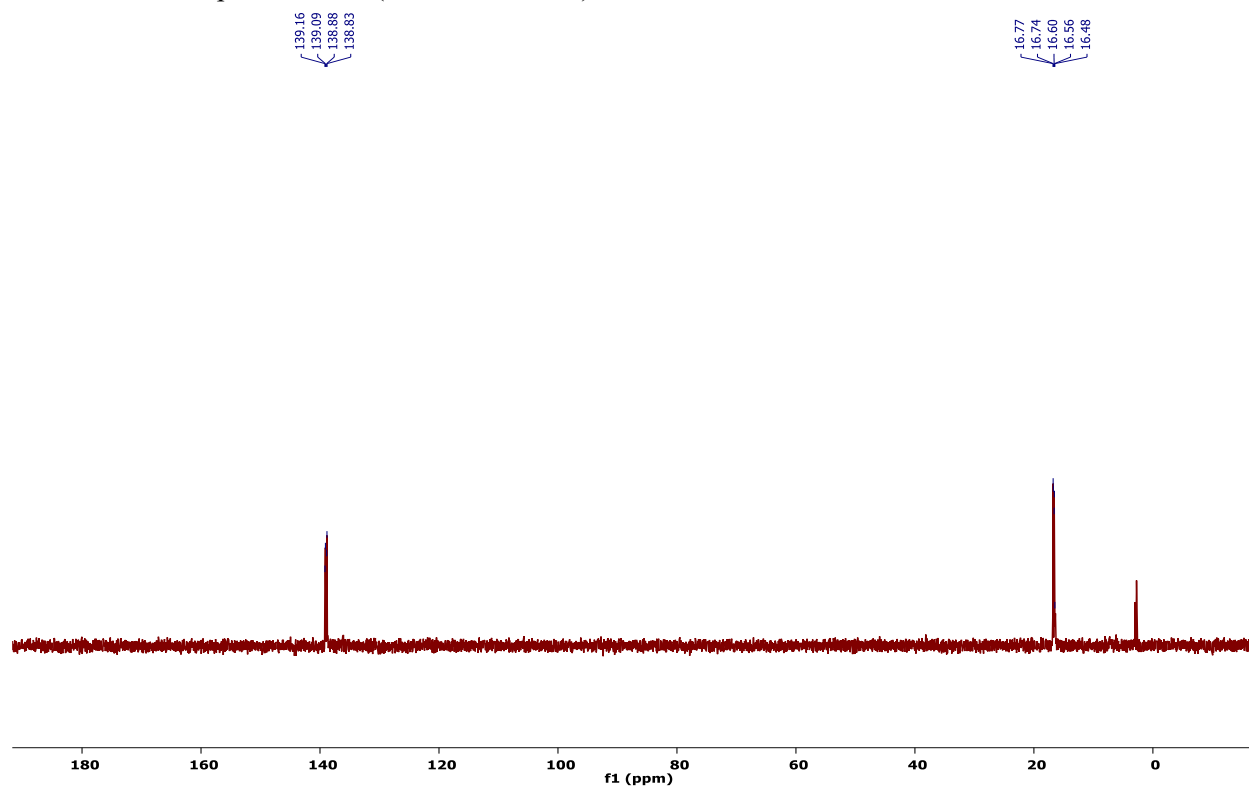
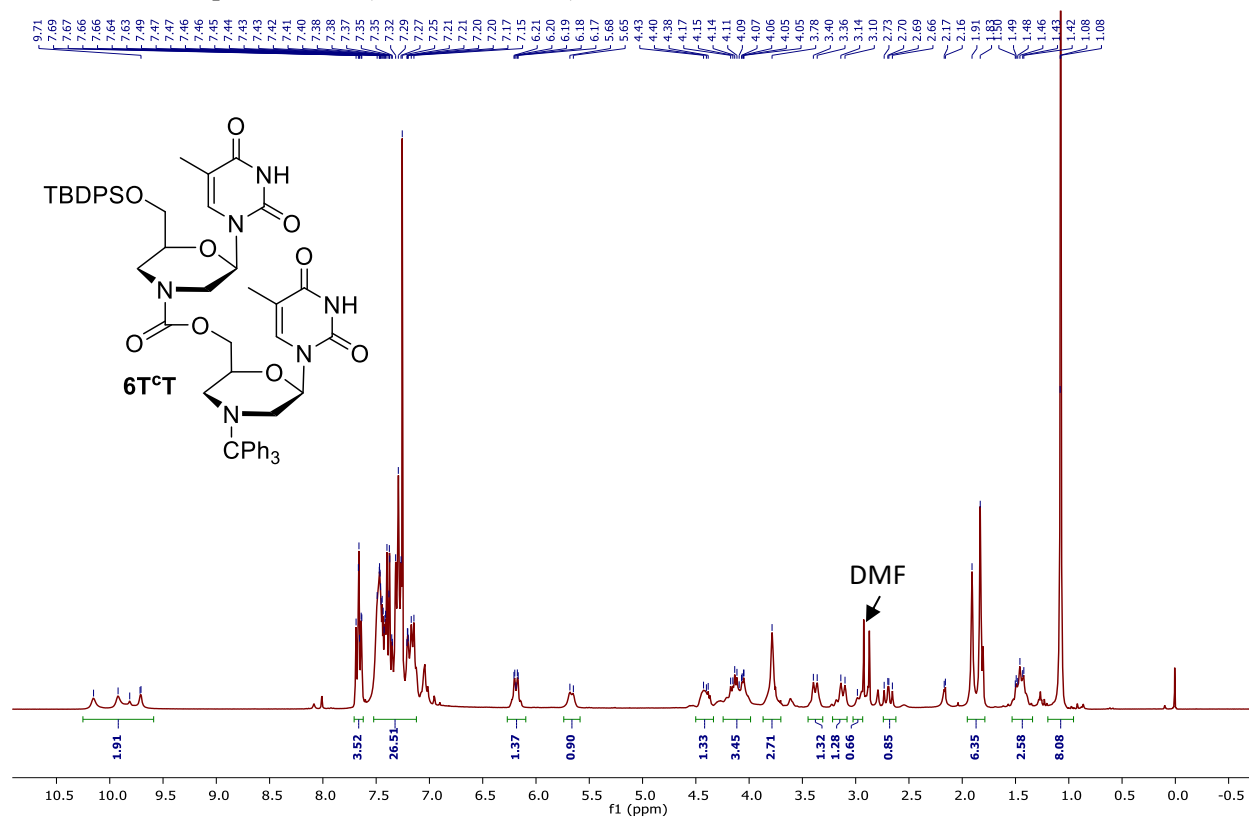
^1H NMR of Compound **5T^PT** (300MHz, CDCl_3) ^{13}C NMR of Compound **5T^PT** (300MHz, CDCl_3)

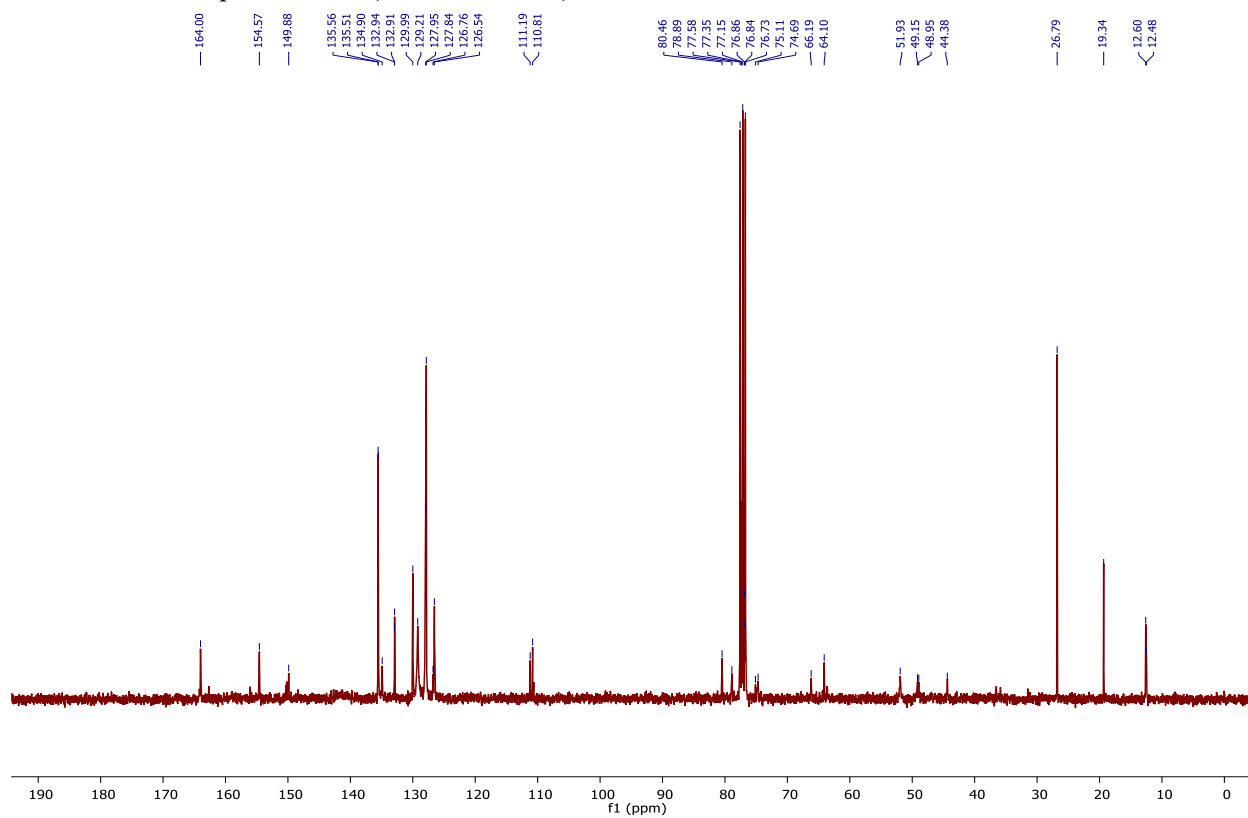
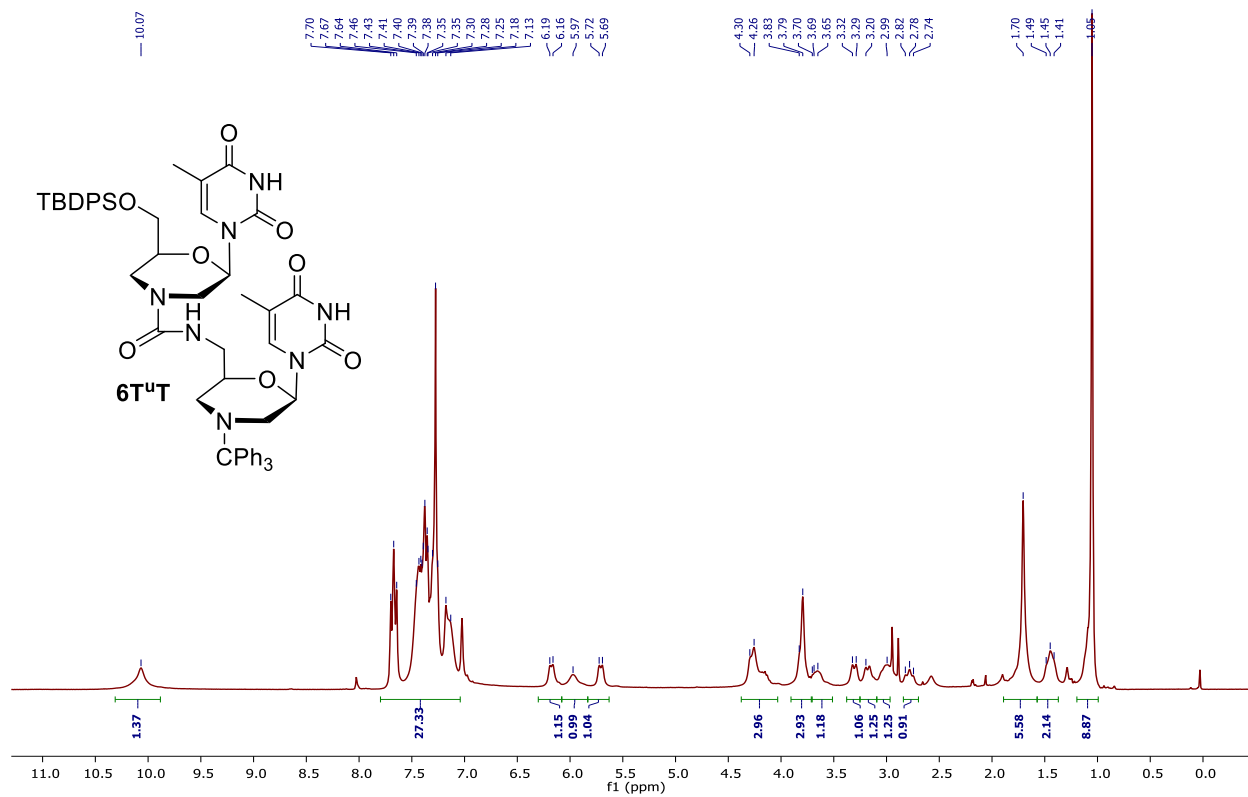
^{13}C NMR of Compound **5C^pC** (300MHz, CDCl_3) ^{31}P NMR of Compound **5C^pC** (121MHz, CDCl_3)

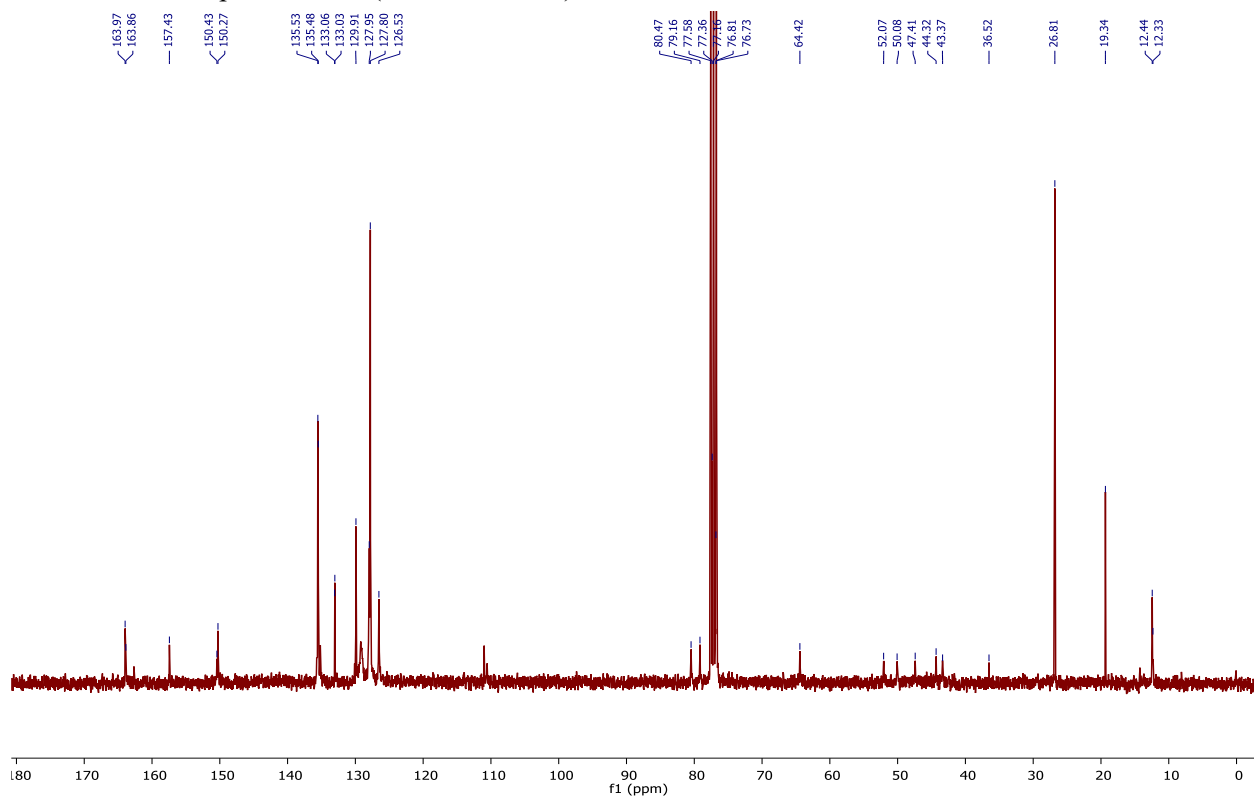
^{31}P NMR of Compound **5T^PG** (121MHz, CDCl_3) ^1H NMR of Compound **5A^PT** (300MHz, CDCl_3)

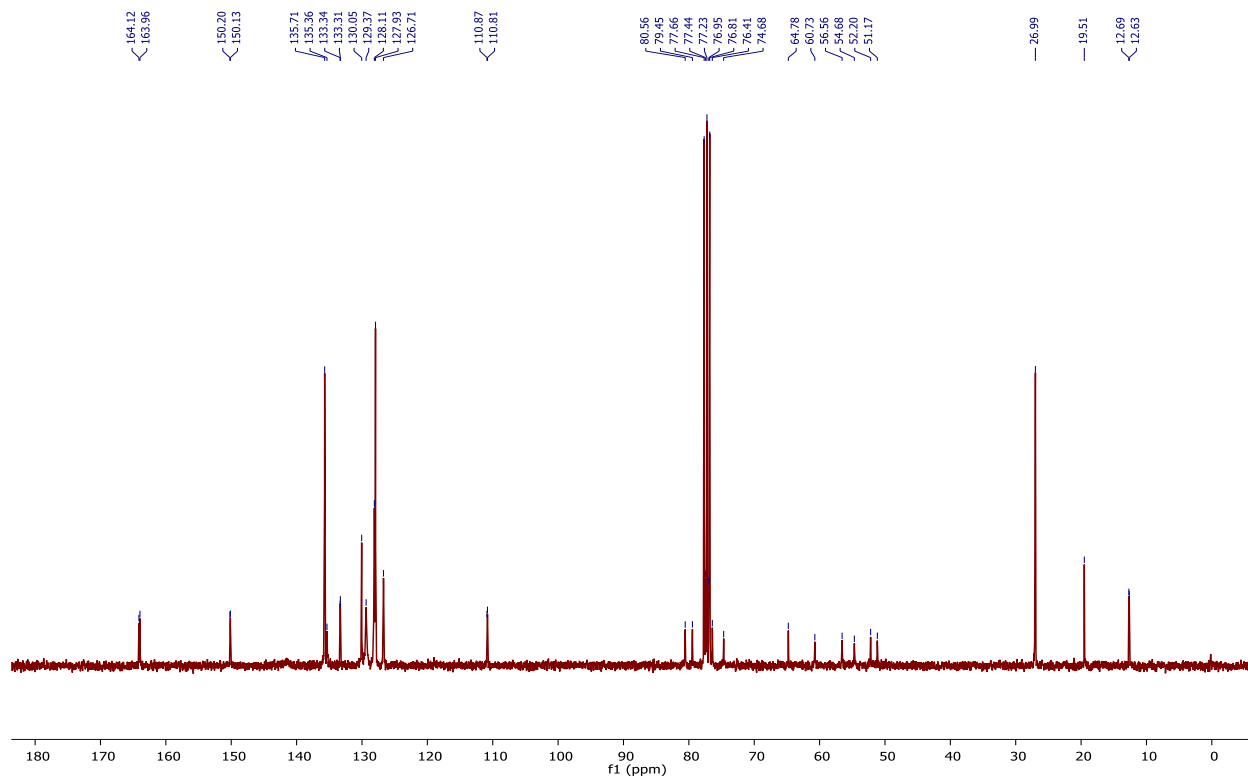
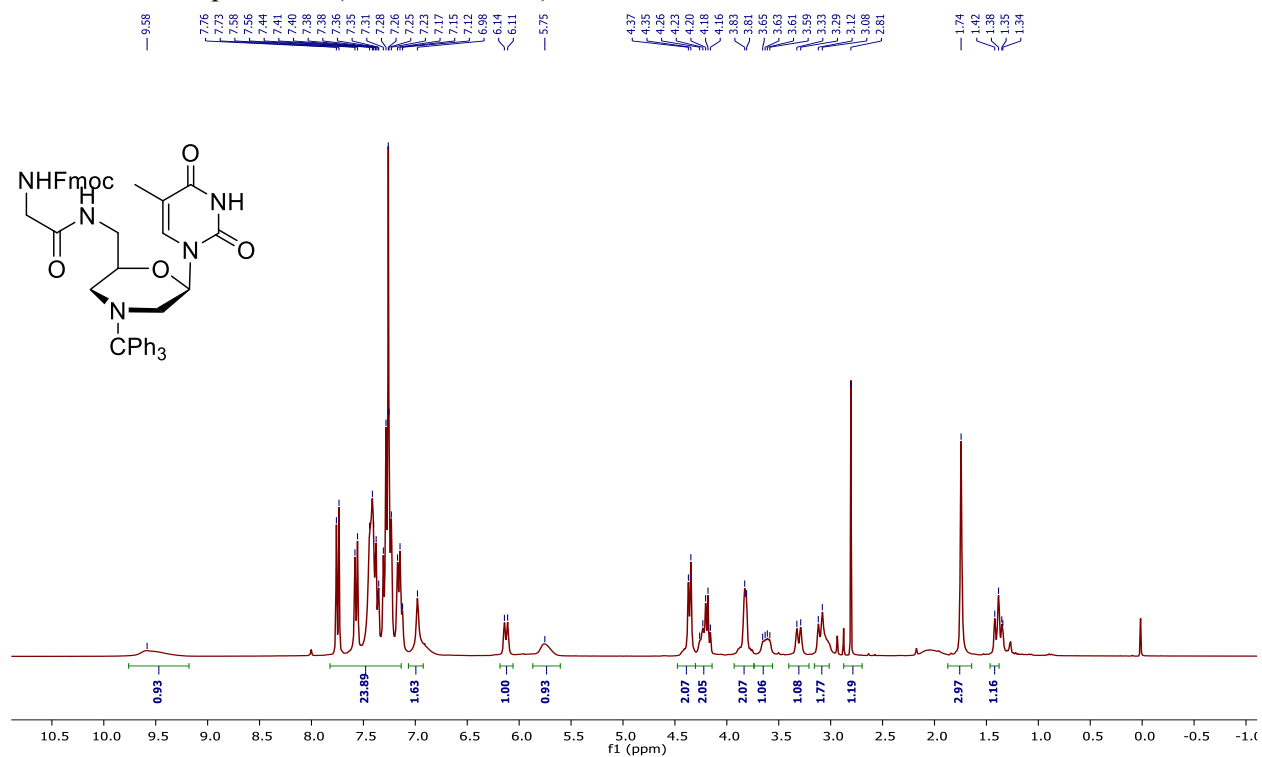
^{13}C NMR of Compound **5A^PT** (300MHz, CDCl_3) ^{31}P NMR of Compound **5A^PT** (121MHz, CDCl_3)

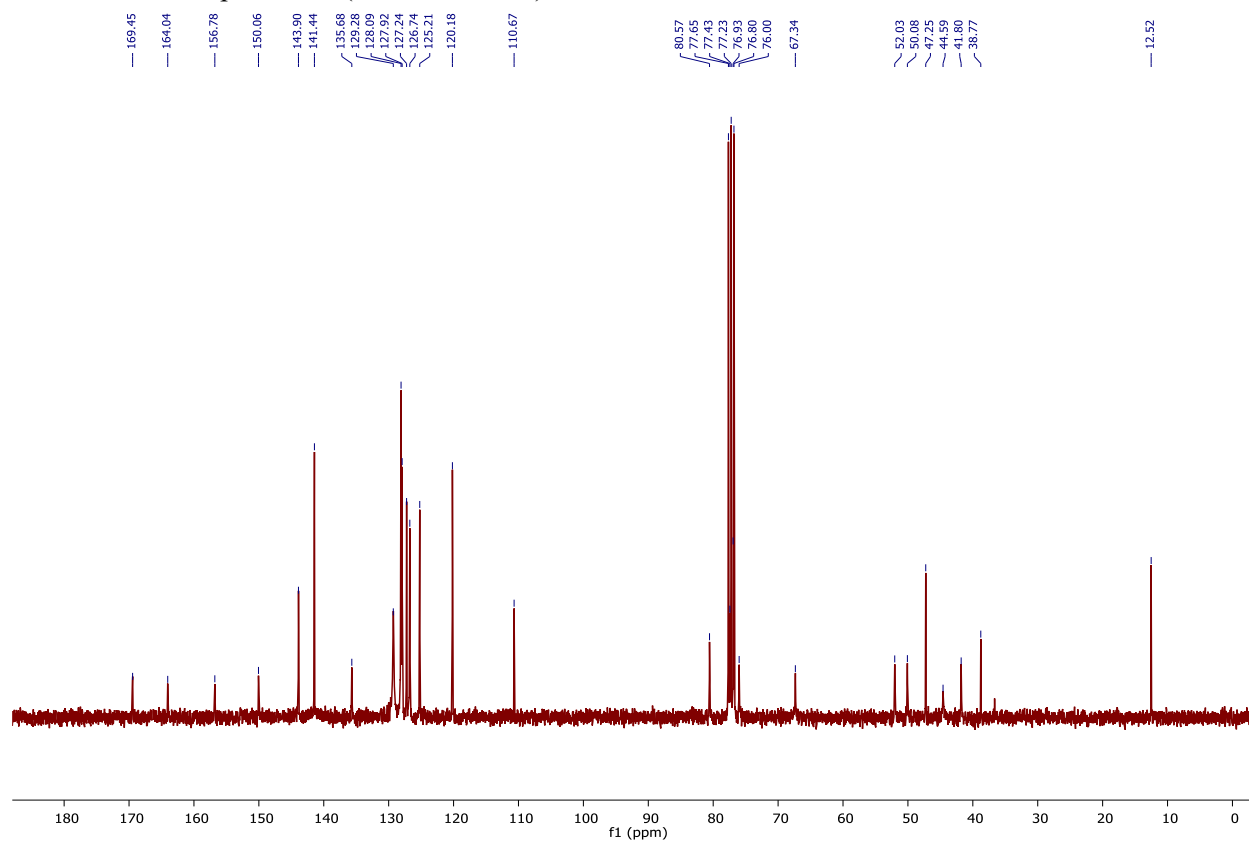
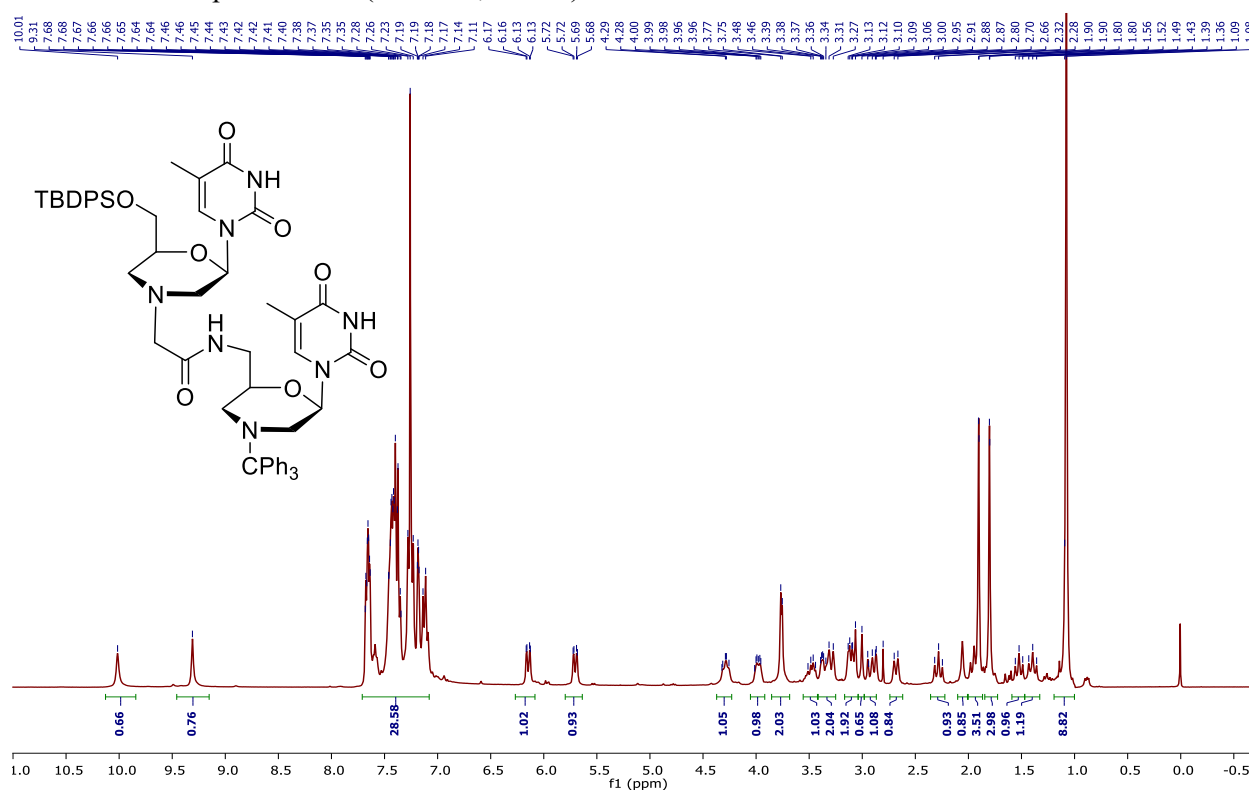
^1H NMR of Compound **5A^PG** (300MHz, CDCl_3) ^{13}C NMR of Compound **5A^PG** (300MHz, CDCl_3)

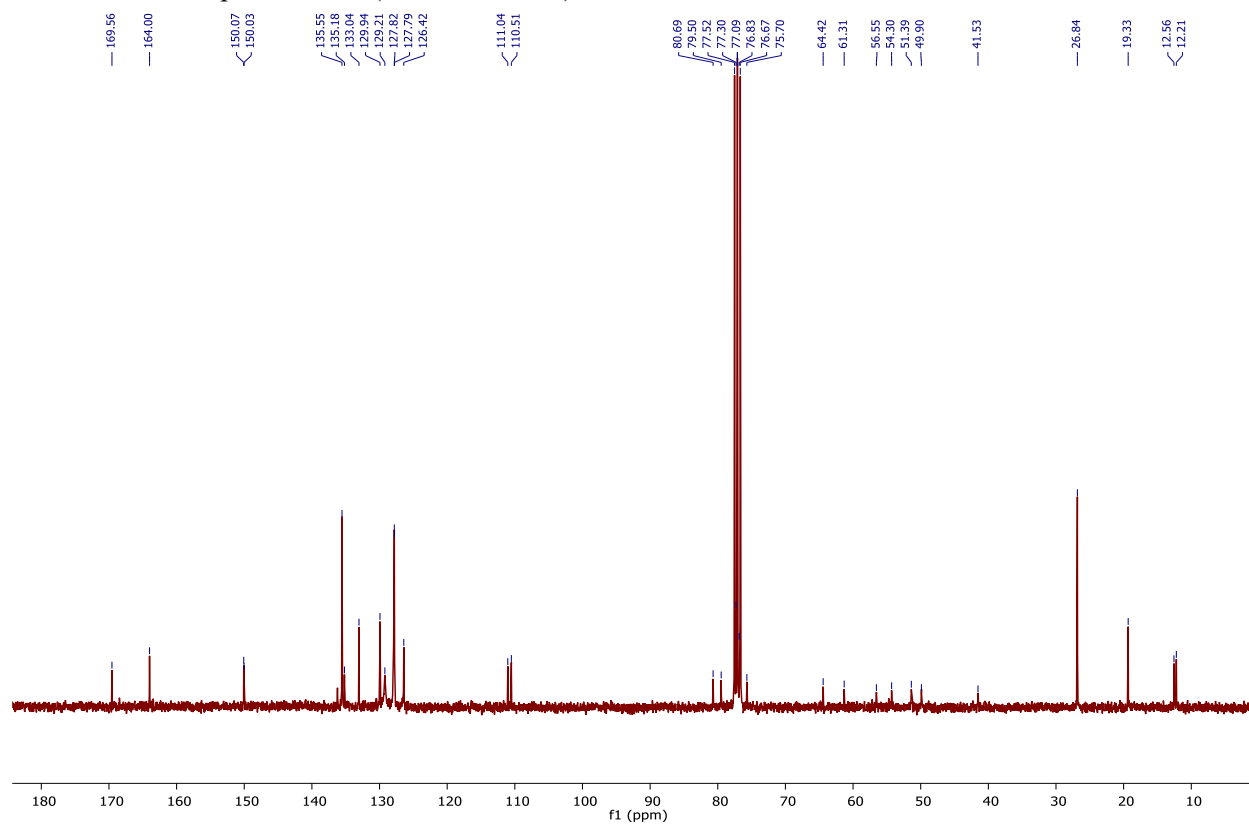
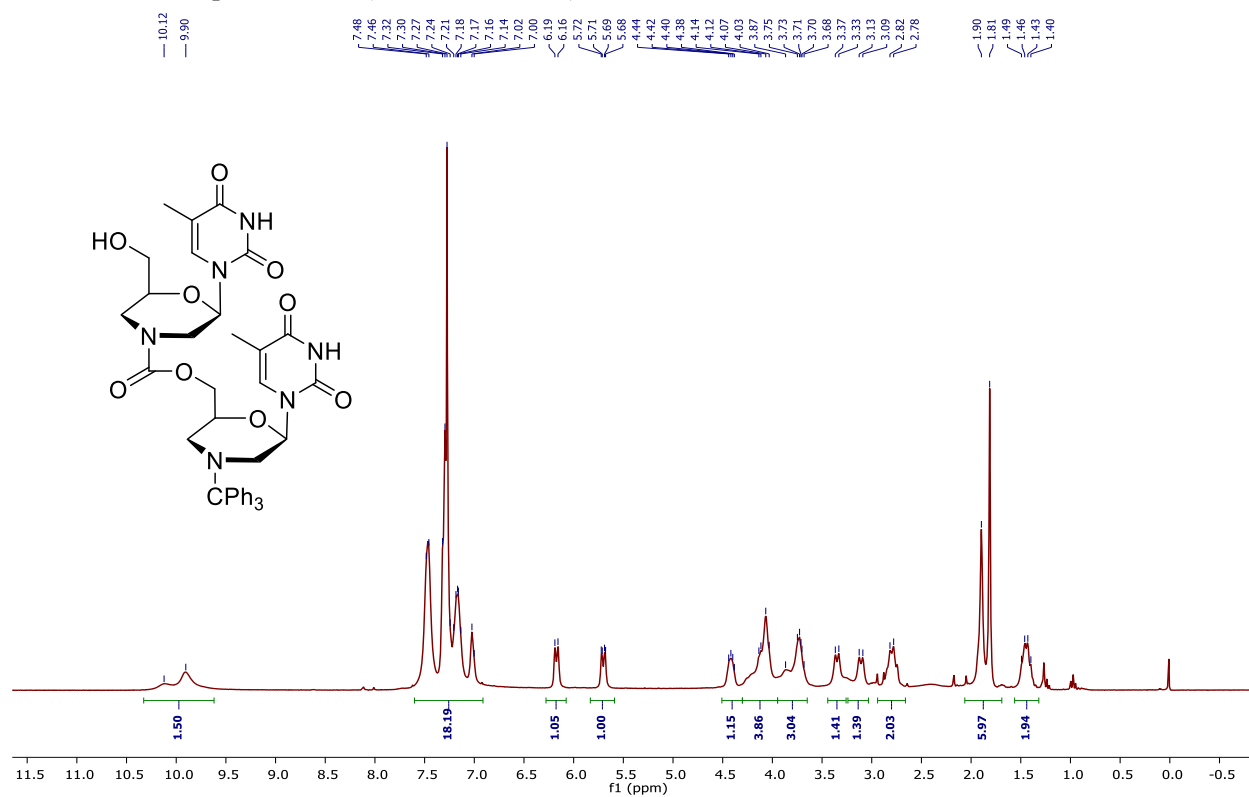
³¹P NMR of Compound **5A^pG** (121MHz, CDCl₃)¹H NMR of Compound **6T^cT** (300MHz, CDCl₃)

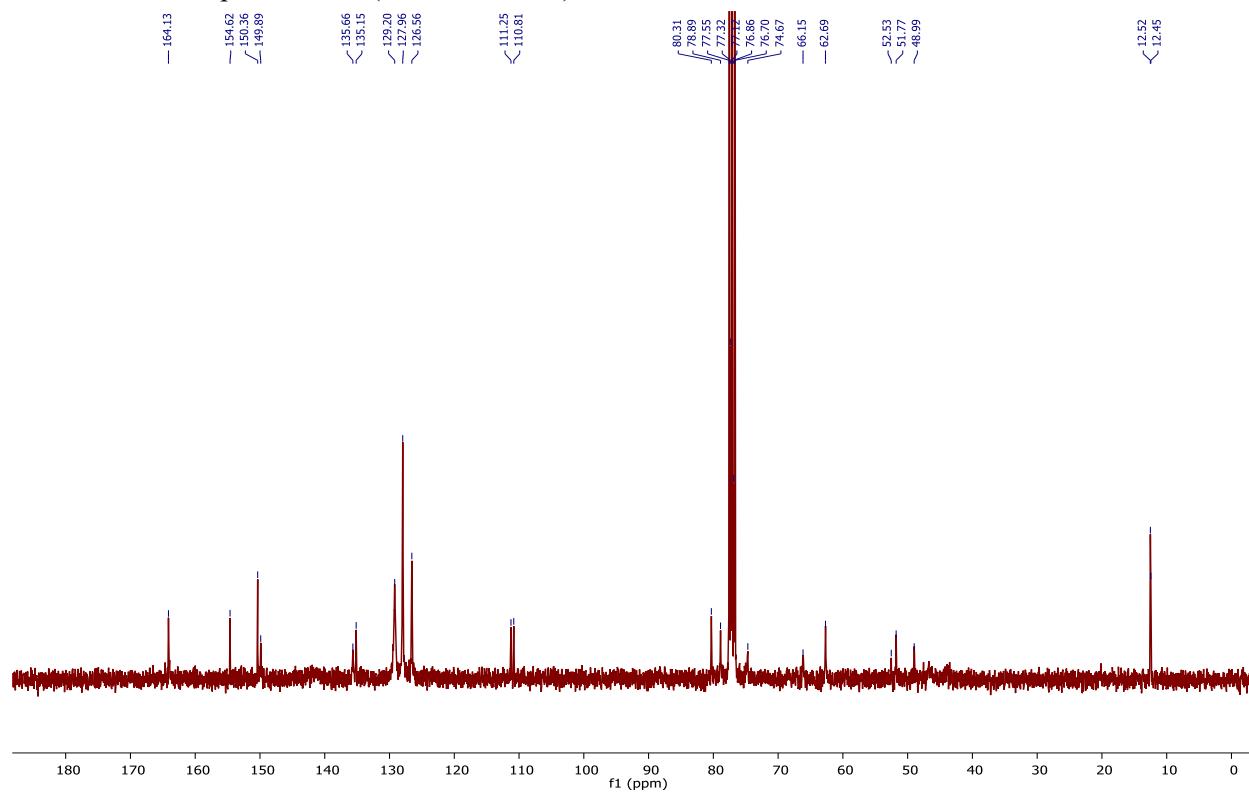
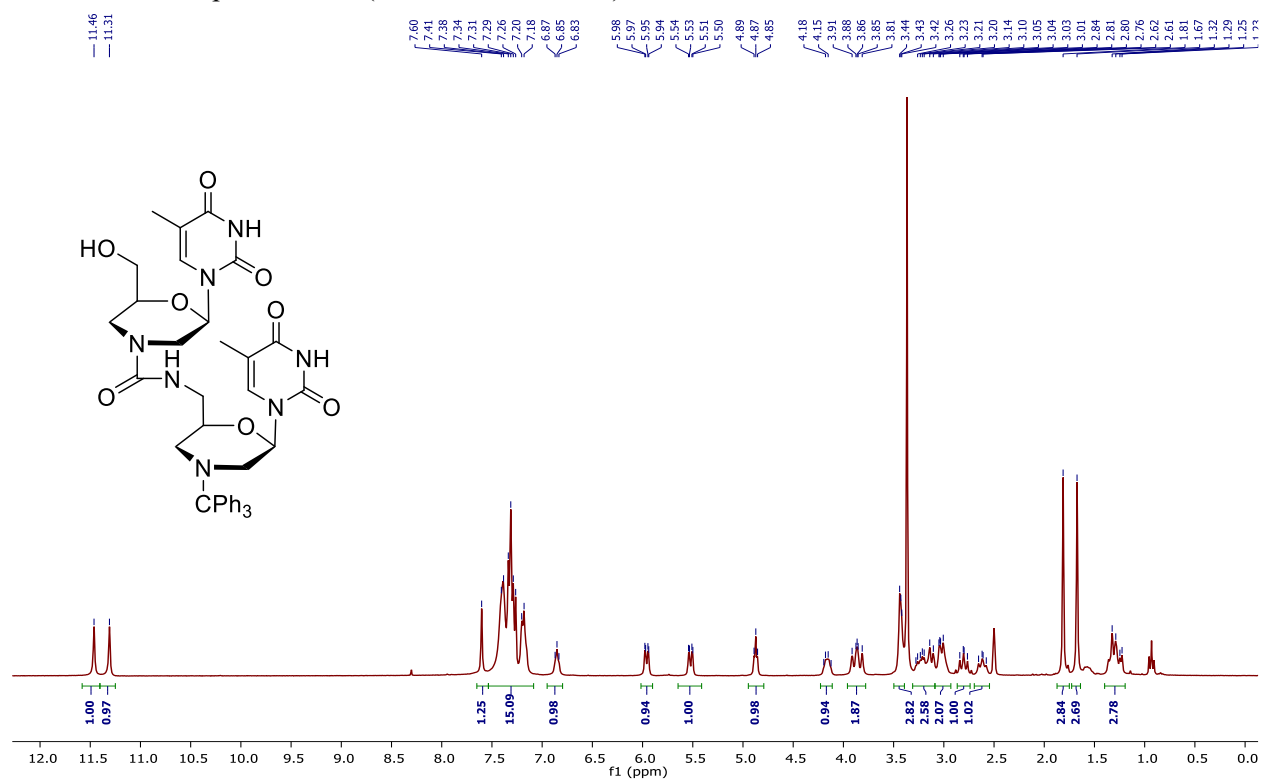
^{13}C NMR of Compound **6T^cT** (75MHz, CDCl_3) ^1H NMR of Compound **6T^uT** (300MHz, CDCl_3)

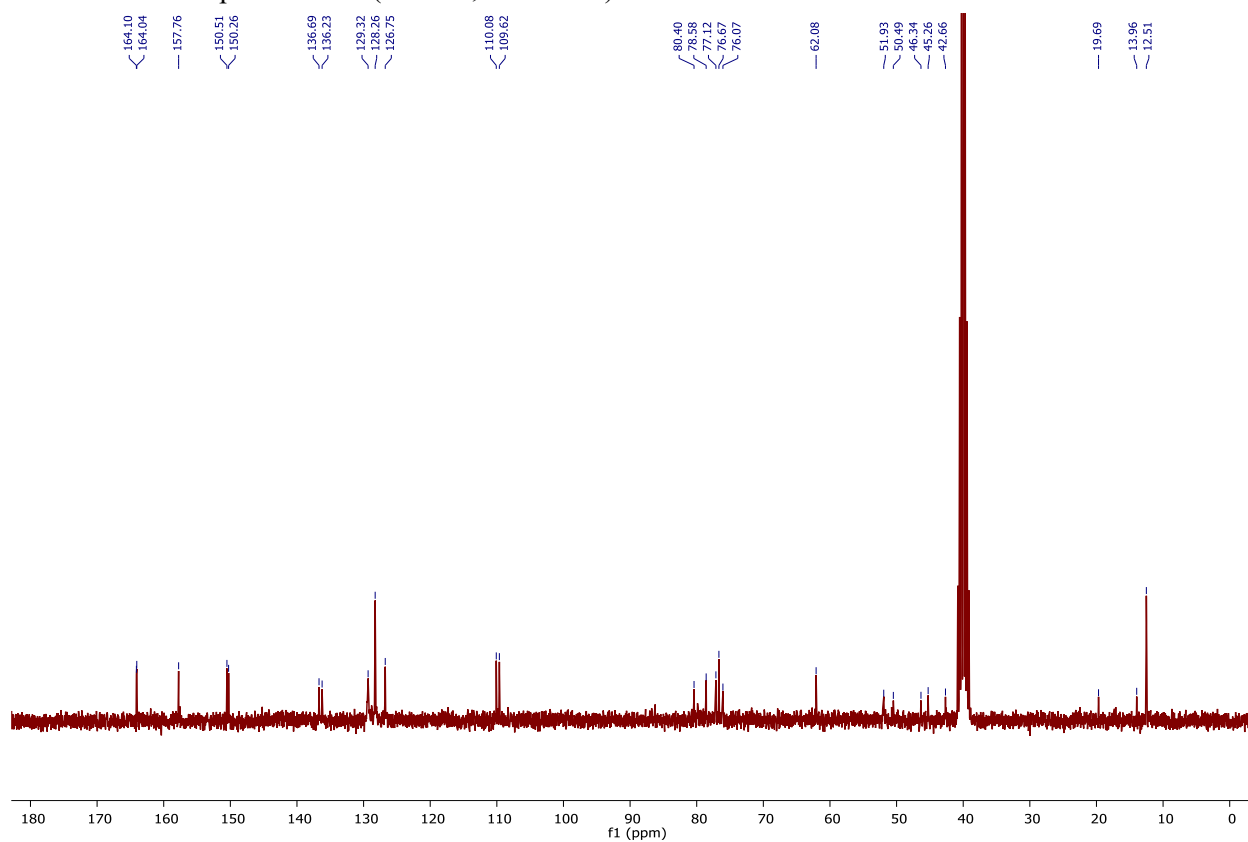
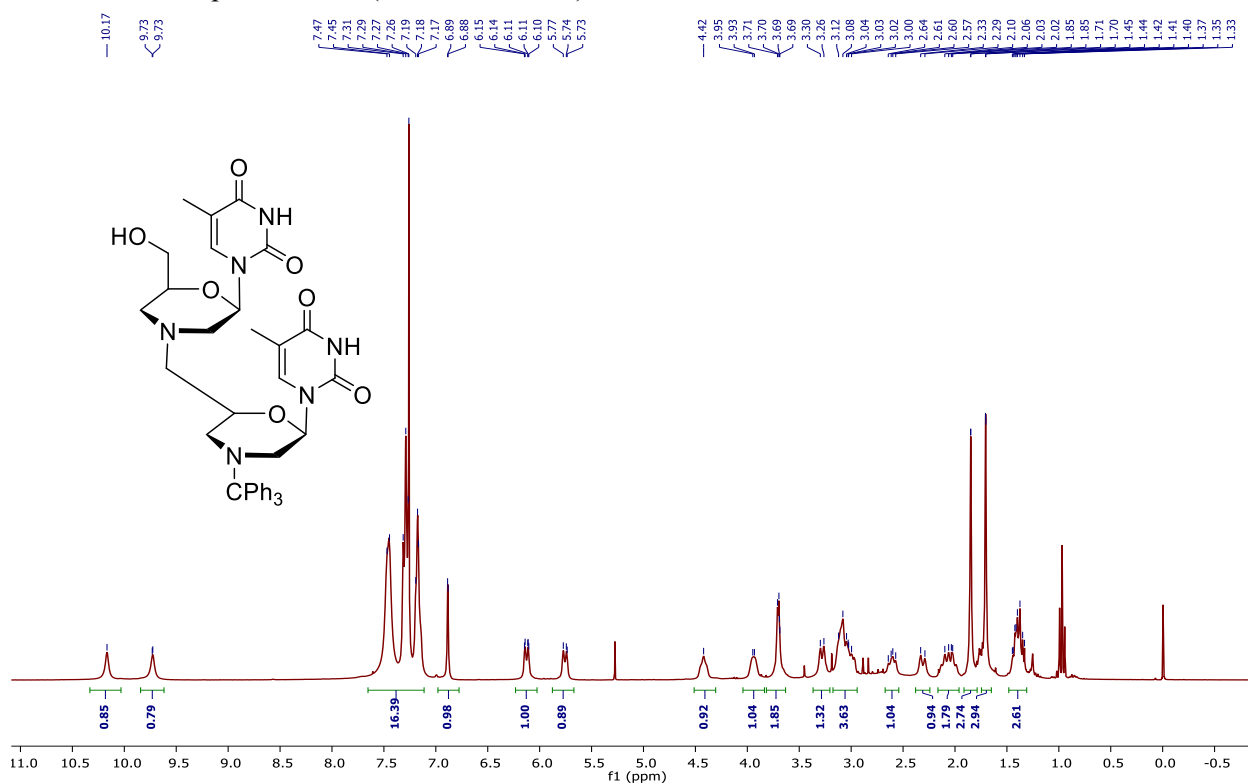
^{13}C NMR of Compound **6T^mT** (75MHz, CDCl_3)

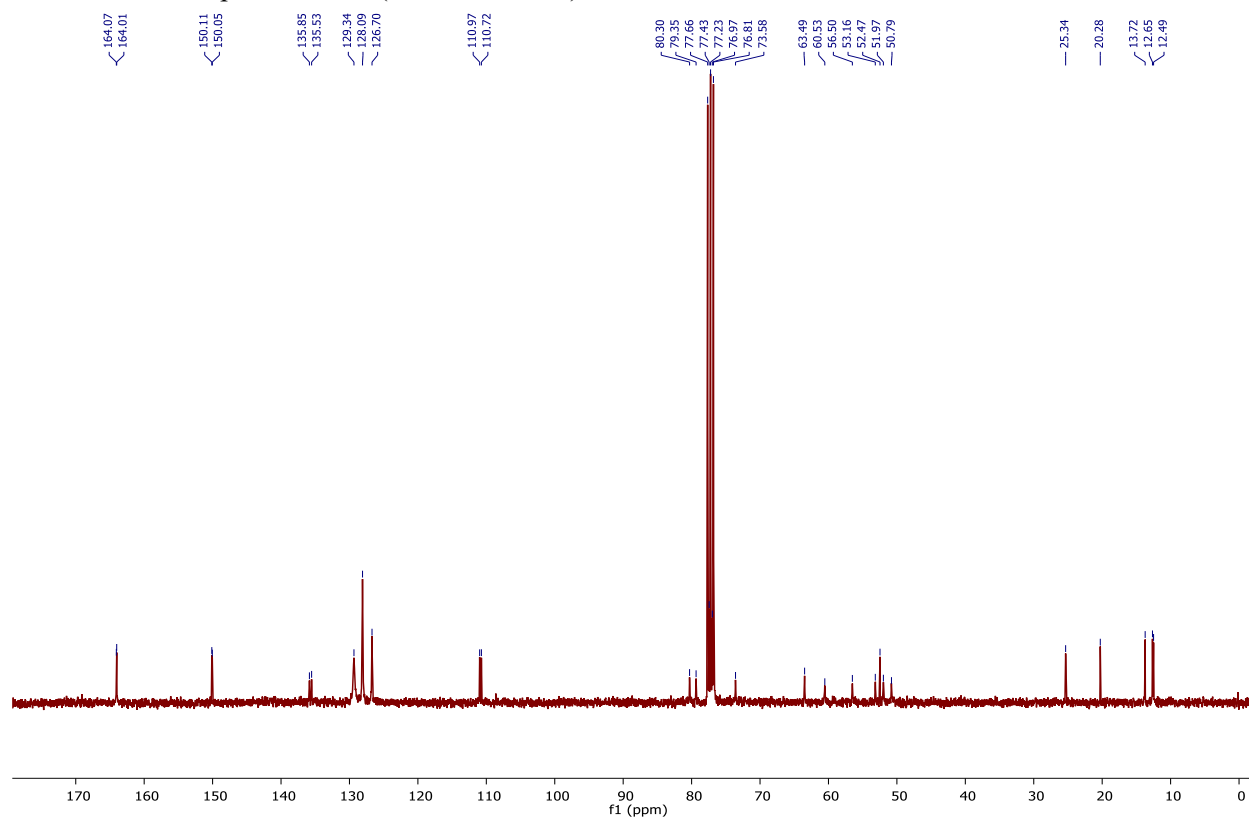
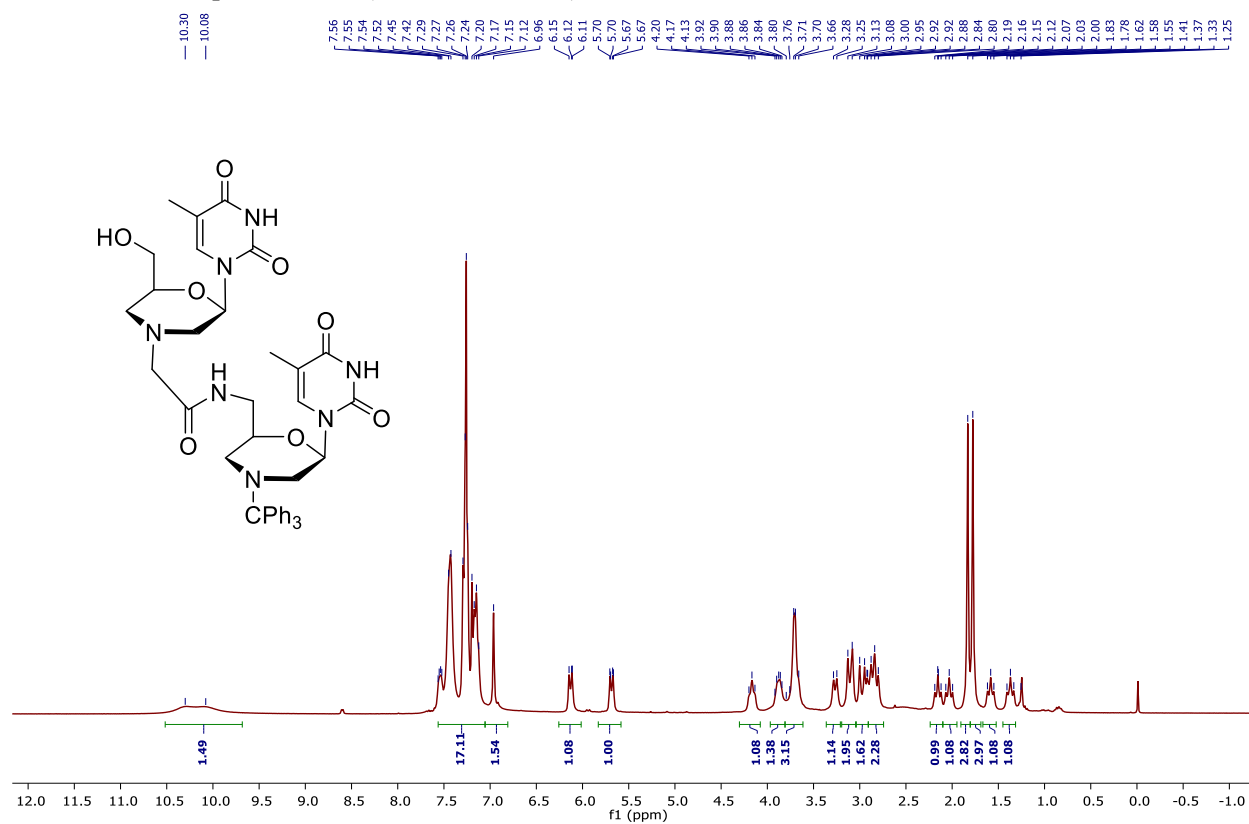
^{13}C NMR of Compound **6T^mT** (75MHz, CDCl_3) ^1H NMR of Compound **6T** (300MHz, CDCl_3)

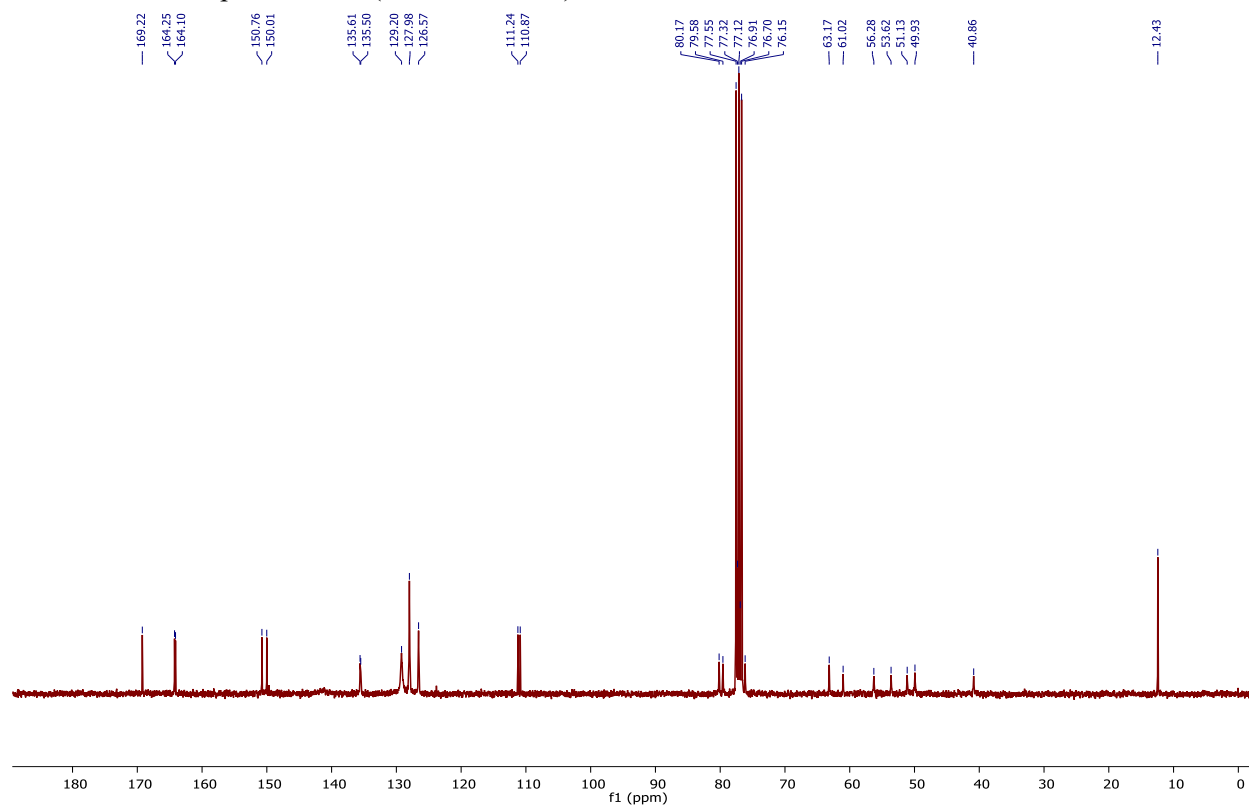
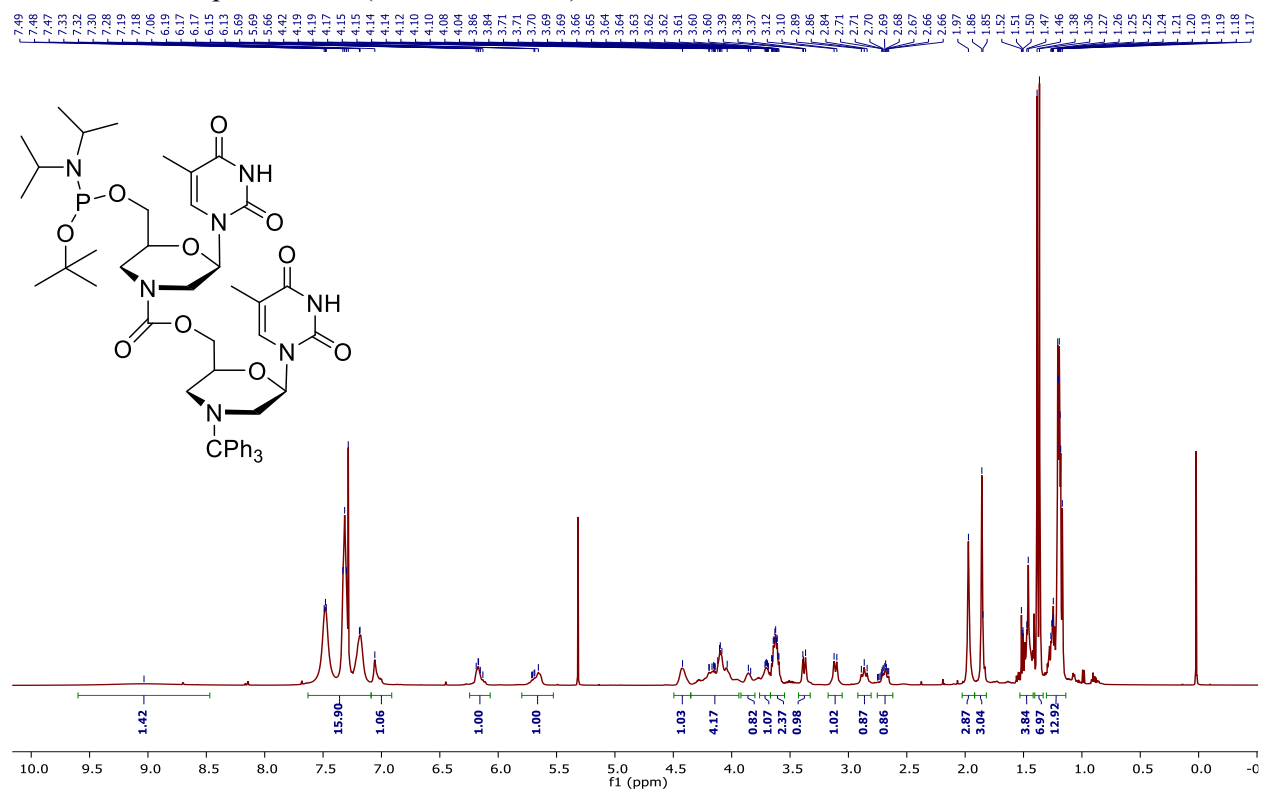
^{13}C NMR of Compound **6T** (75MHz, CDCl_3) ^1H NMR of Compound **6T** (300MHz, CDCl_3)

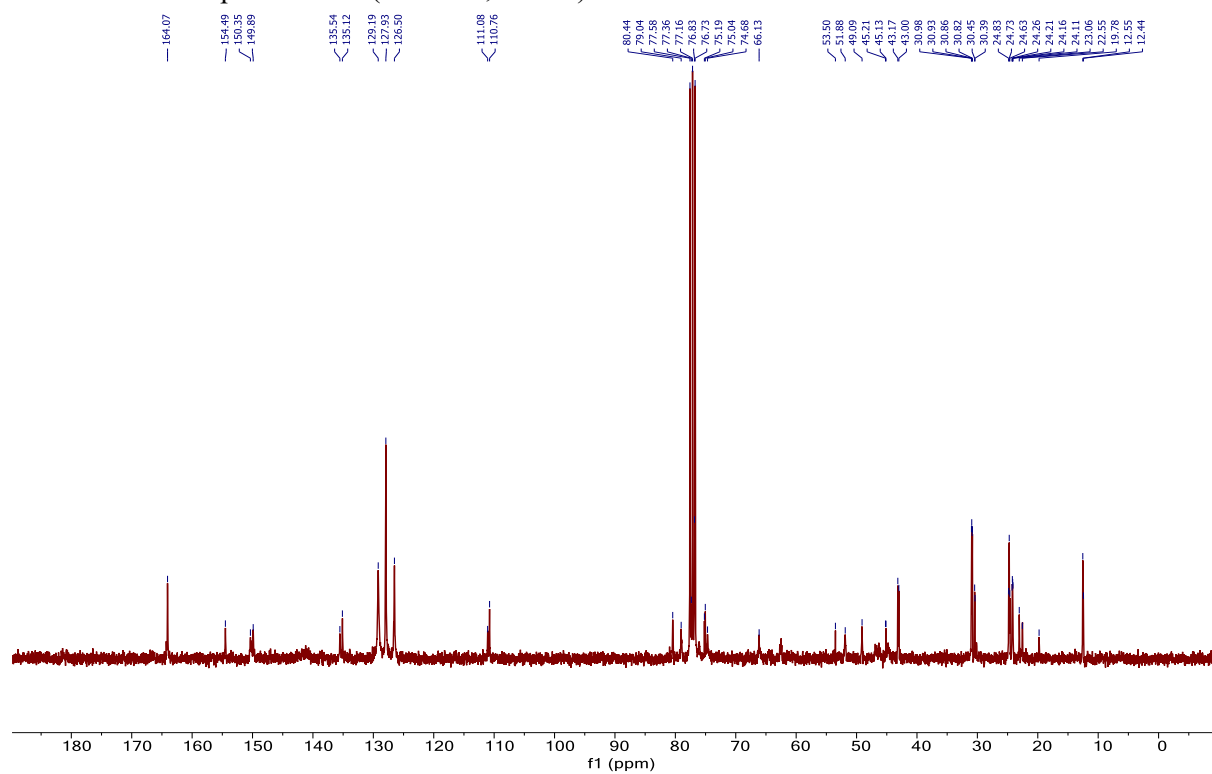
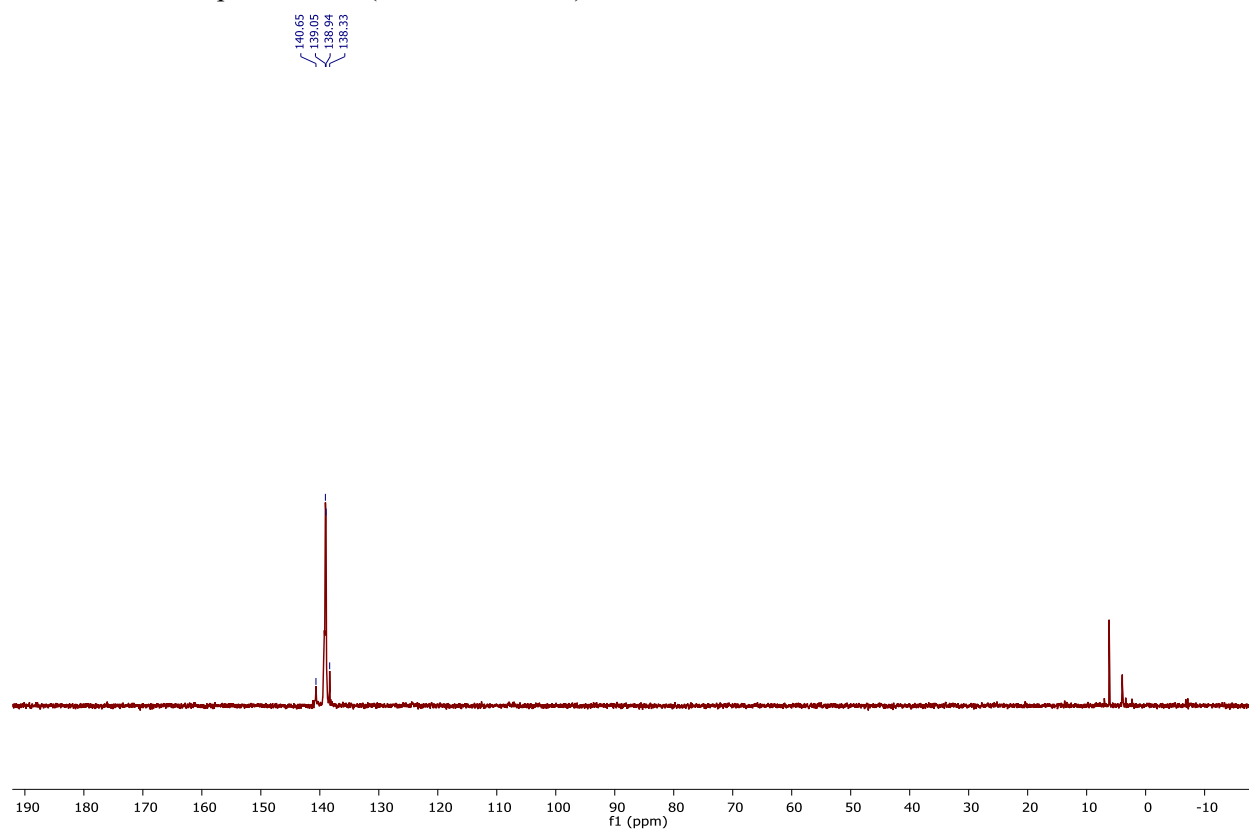
^{13}C NMR of Compound **6T^gT** (75MHz, CDCl_3) ^1H NMR of Compound **7T^gT** (300MHz, CDCl_3)

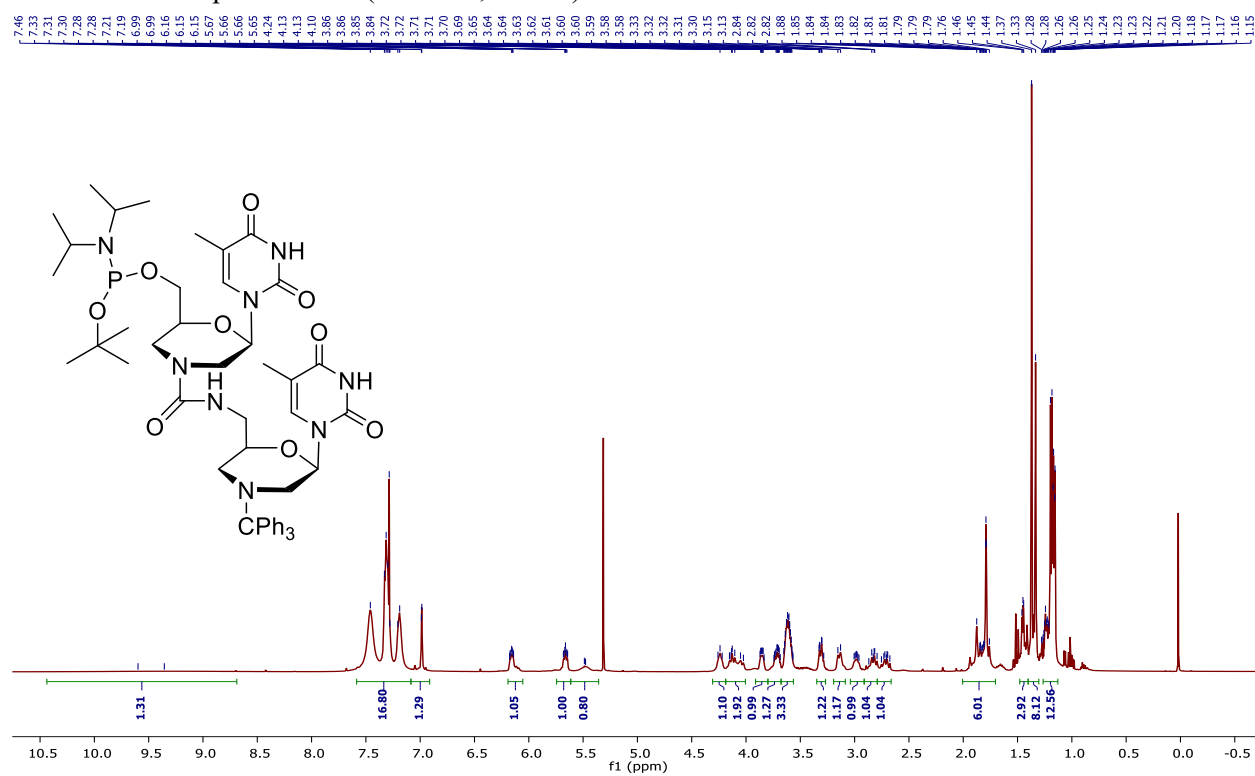
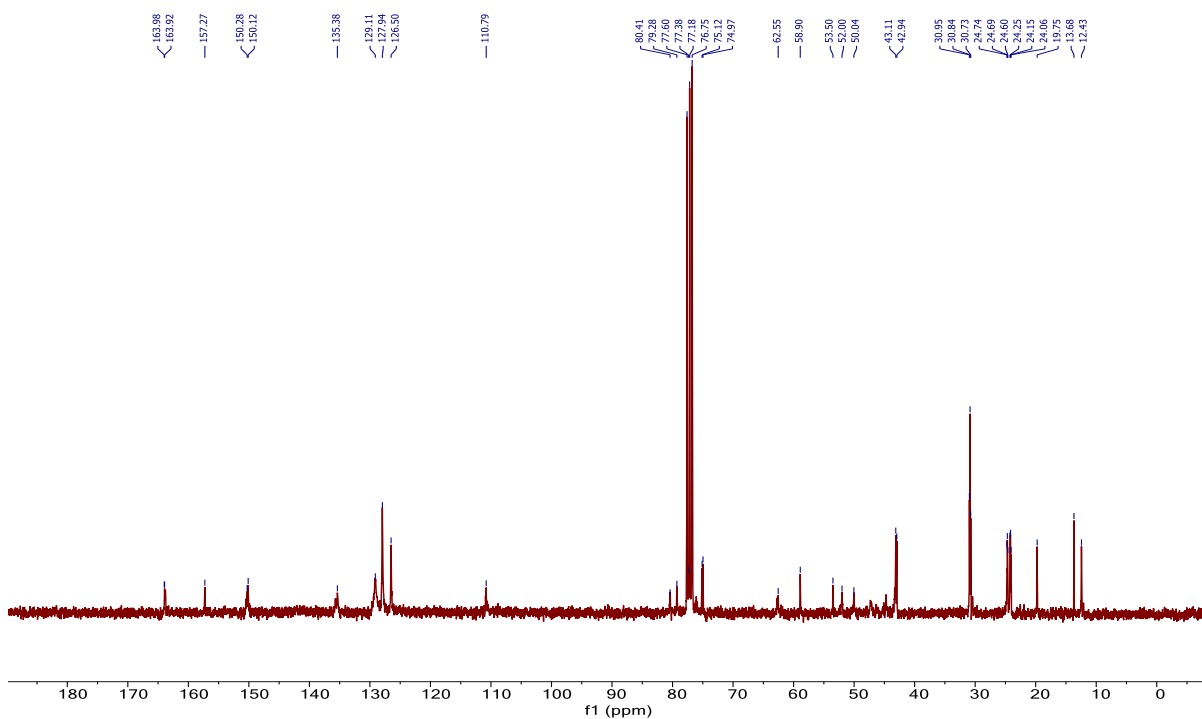
^{13}C NMR of Compound 7T^oT (75MHz, CDCl_3) ^1H NMR of Compound 7T^oT (300MHz, DMSO-d_6)

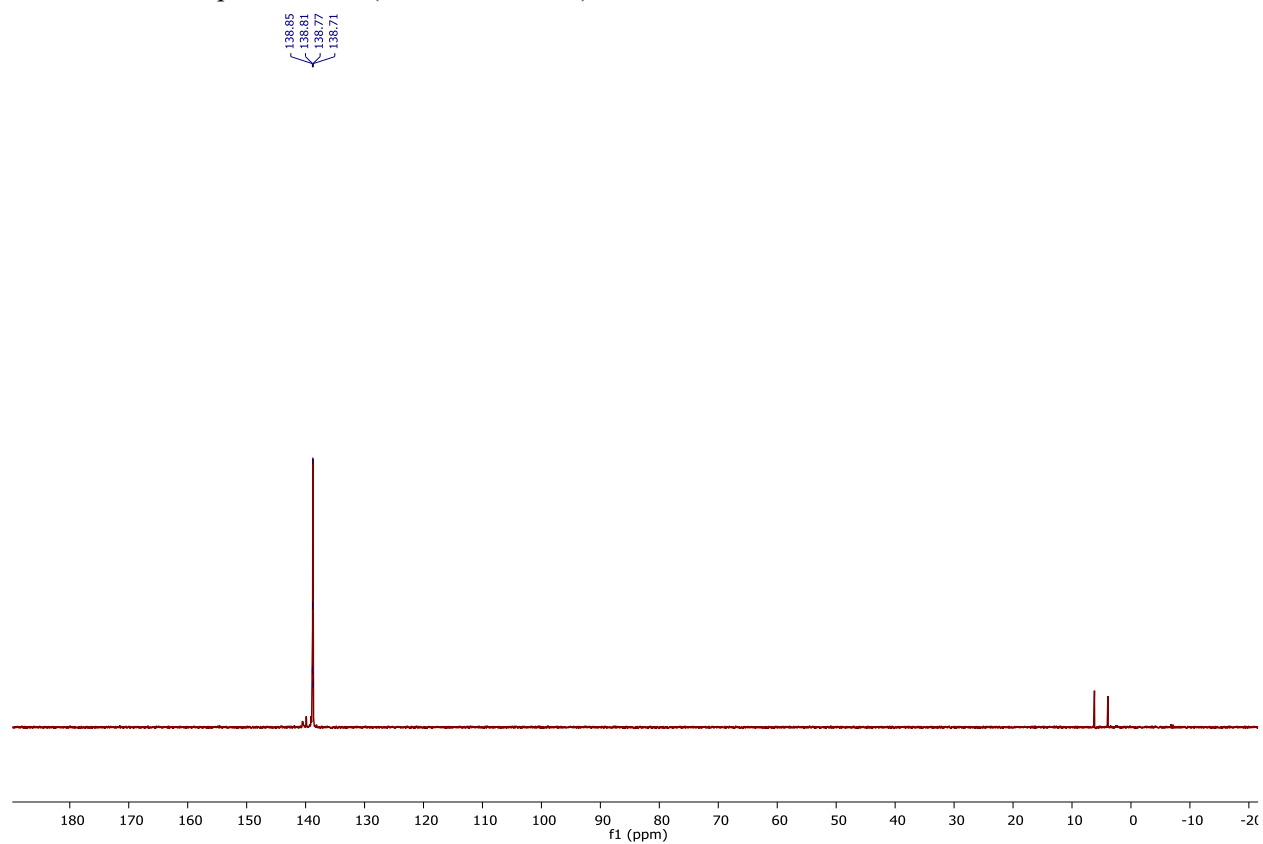
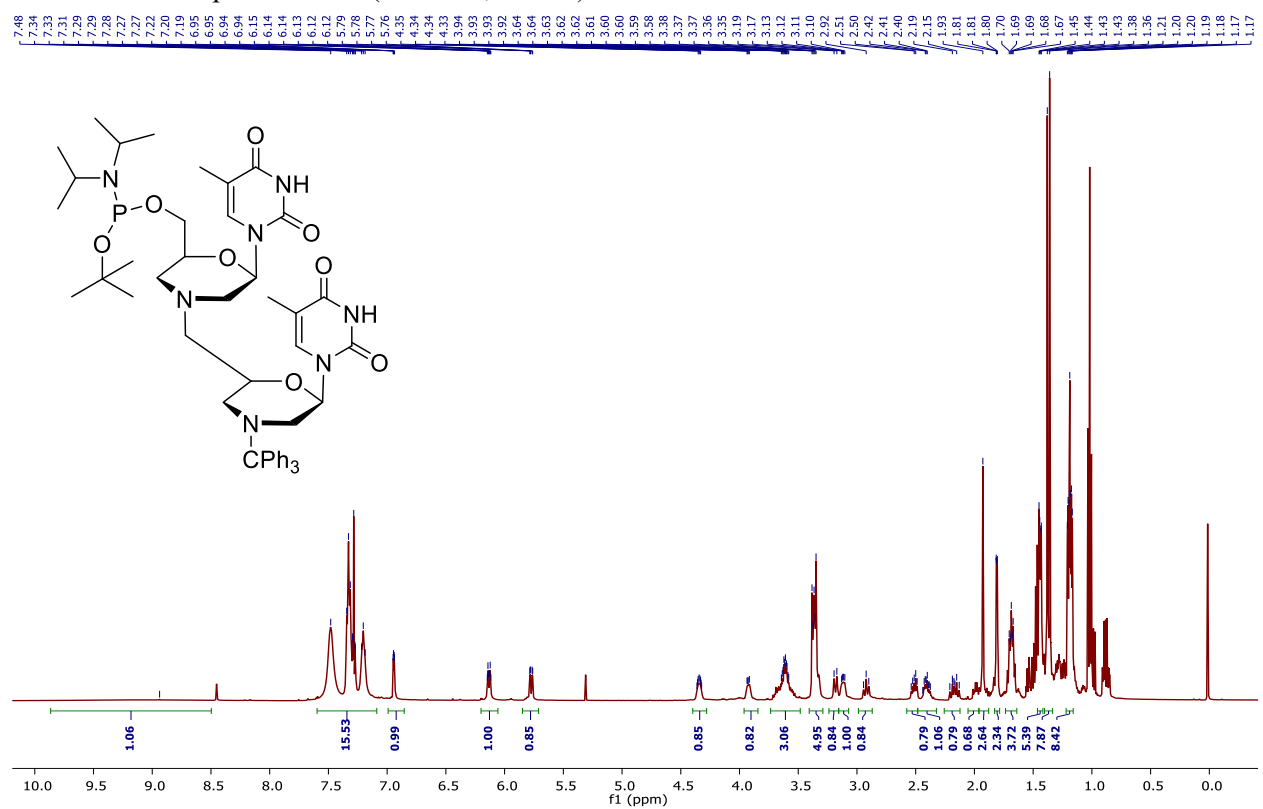
^{13}C NMR of Compound **7T^mT** (75MHz, DMSO- d_6) ^1H NMR of Compound **7T^mT** (300MHz, CDCl_3)

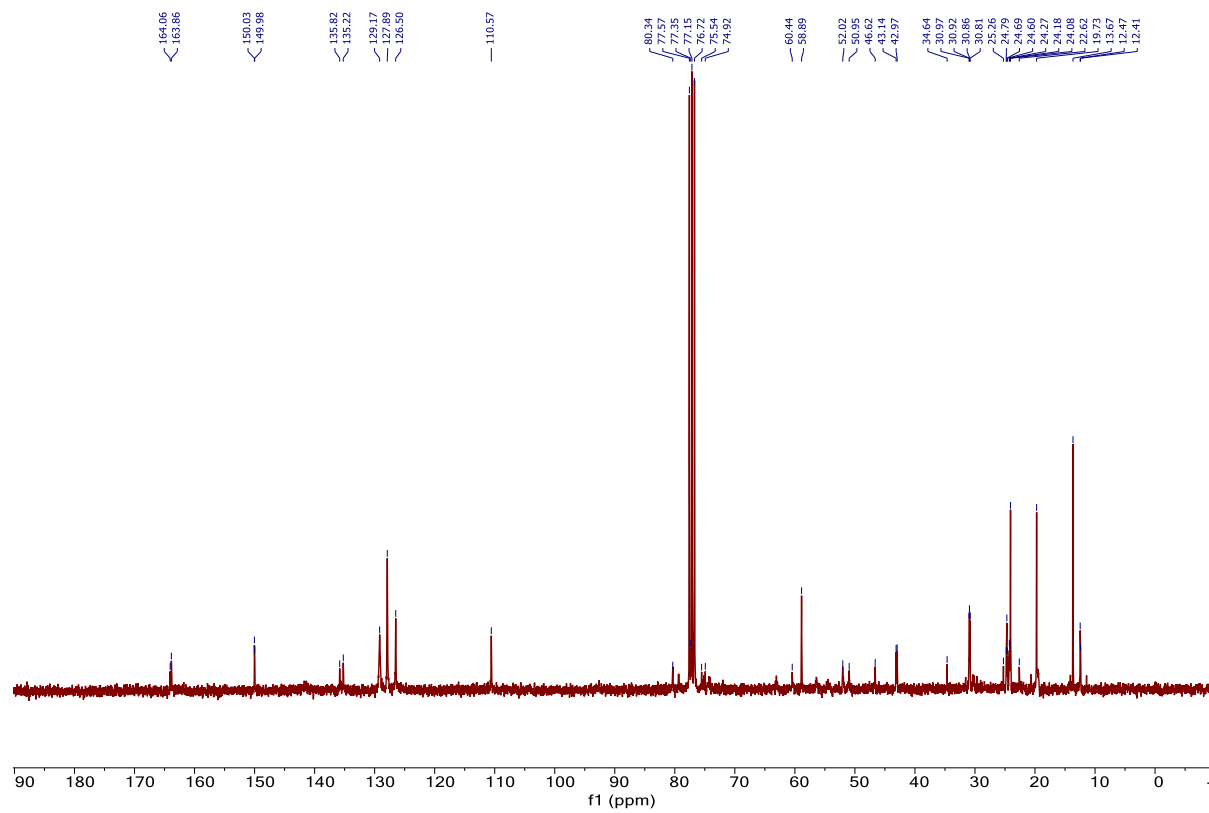
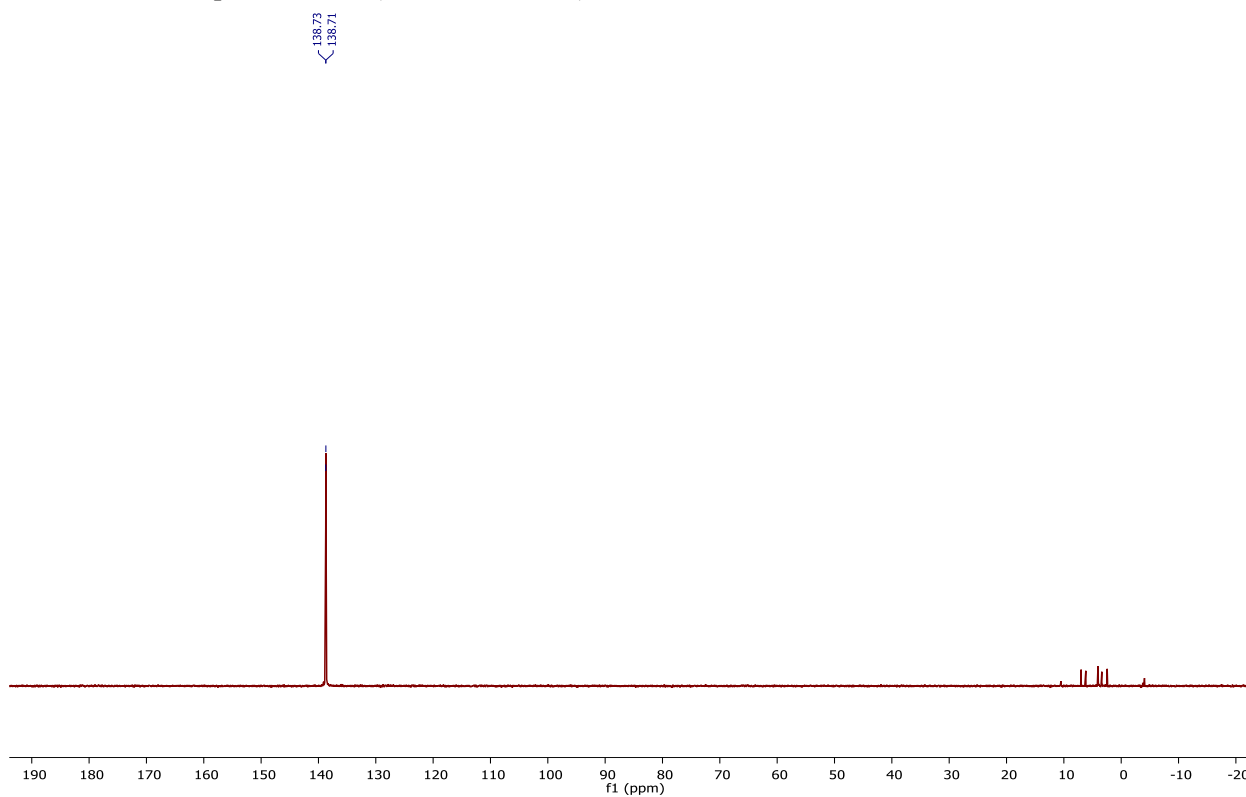
^{13}C NMR of Compound **7T^mT** (75MHz, CDCl_3) ^1H NMR of Compound **7T^gT** (300MHz, CDCl_3)

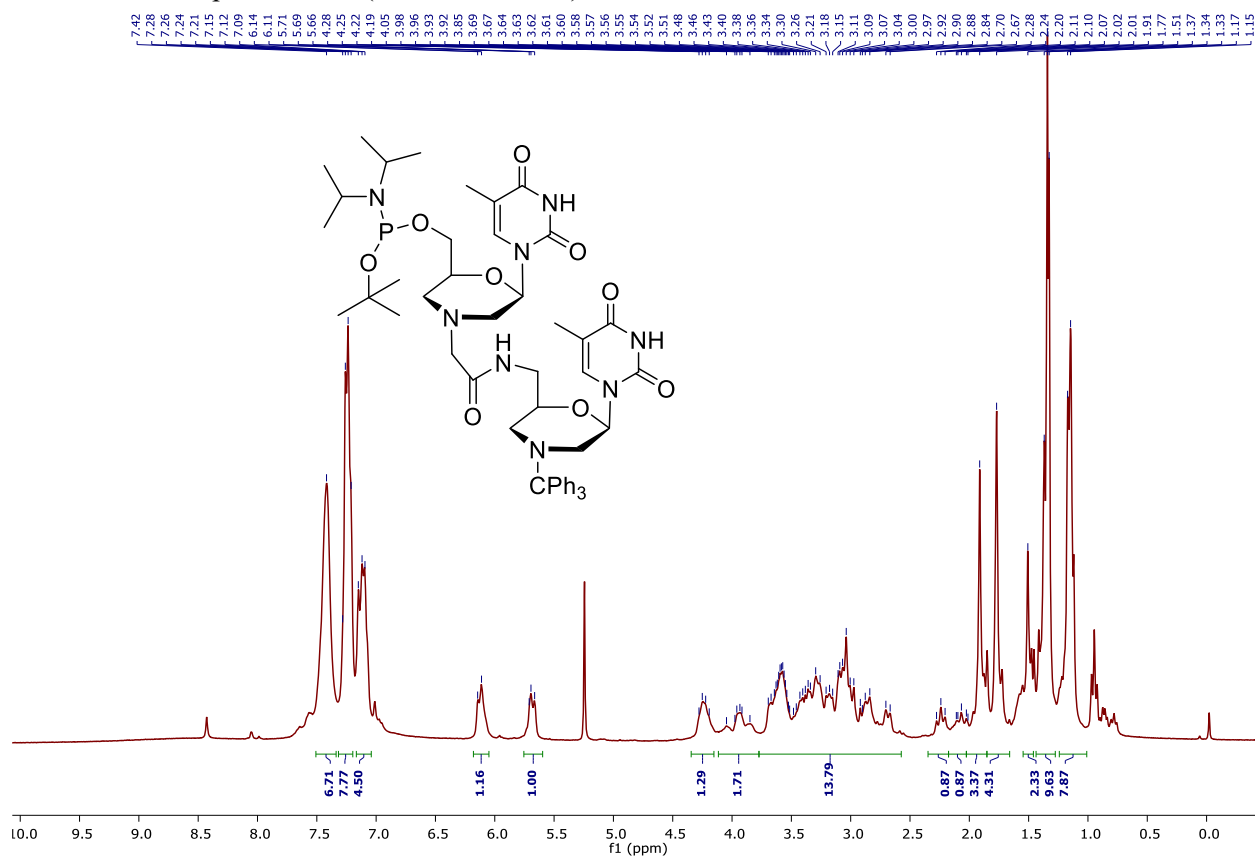
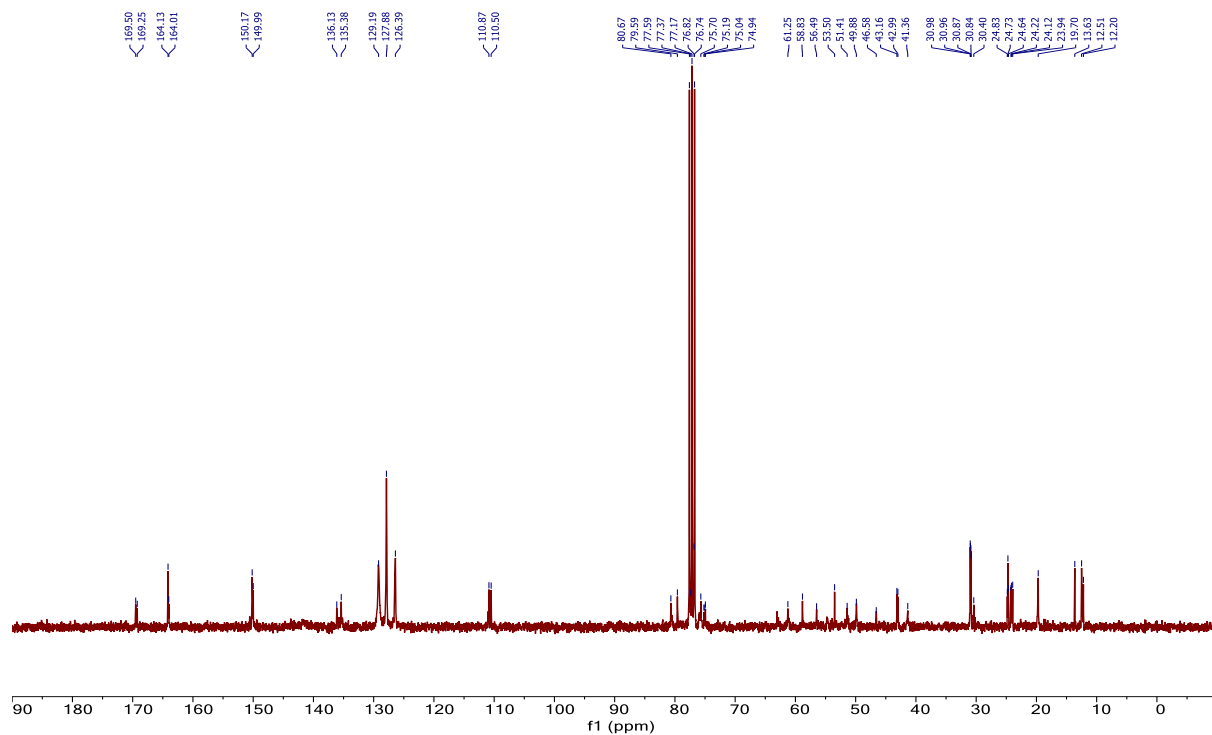
^{13}C NMR of Compound **7T^gT** (75MHz, CDCl_3) ^1H NMR of Compound **8T^gT** (500MHz, CDCl_3)

^{13}C NMR of Compound **8T^cT** (500MHz, CDCl_3) ^{31}P NMR of Compound **8T^cT** (202MHz, CDCl_3)

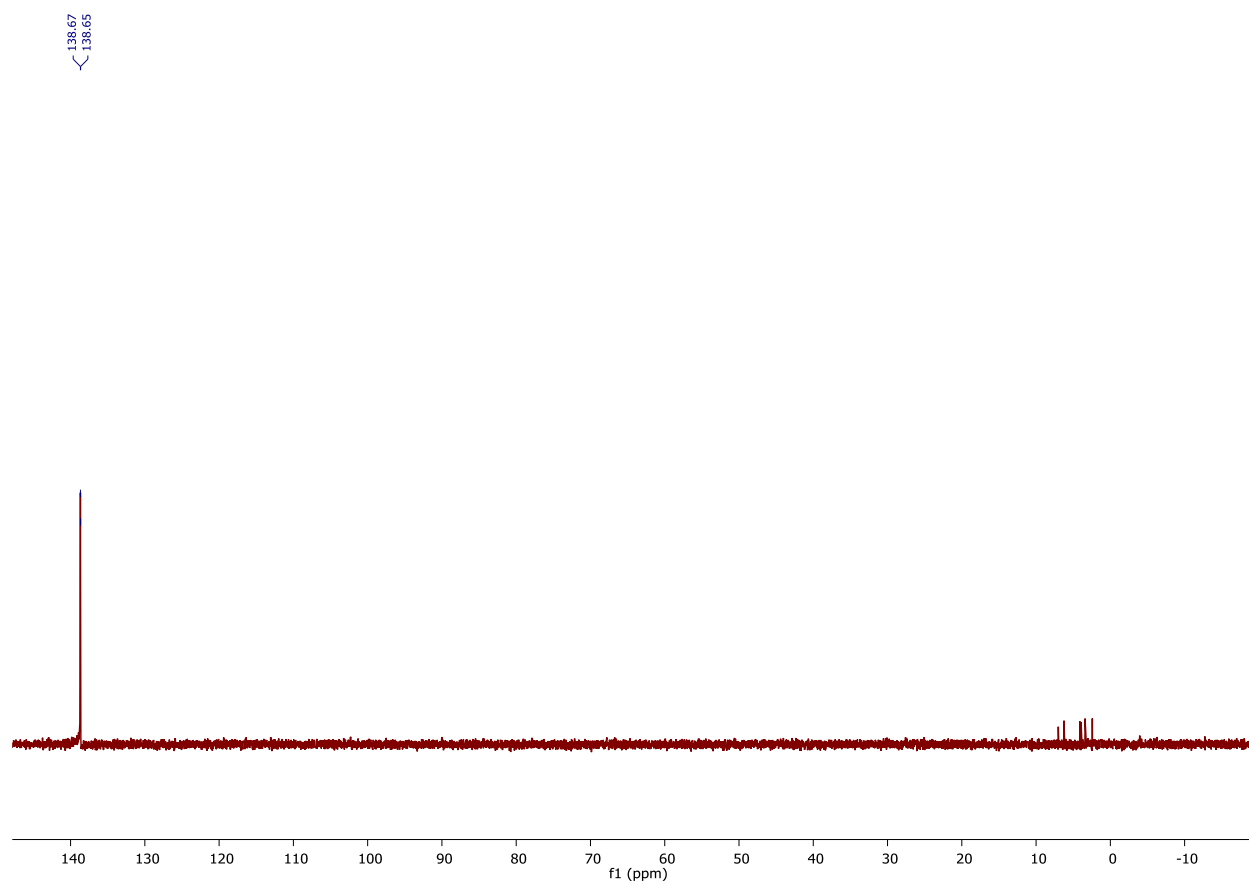
¹H NMR of Compound **8T^uT** (500MHz, CDCl₃)¹³C NMR of Compound **8T^uT** (500MHz, CDCl₃)

^{31}P NMR of Compound **8T^mT** (202MHz, CDCl_3) ^1H NMR of Compound **8T^mT** (500MHz, CDCl_3)

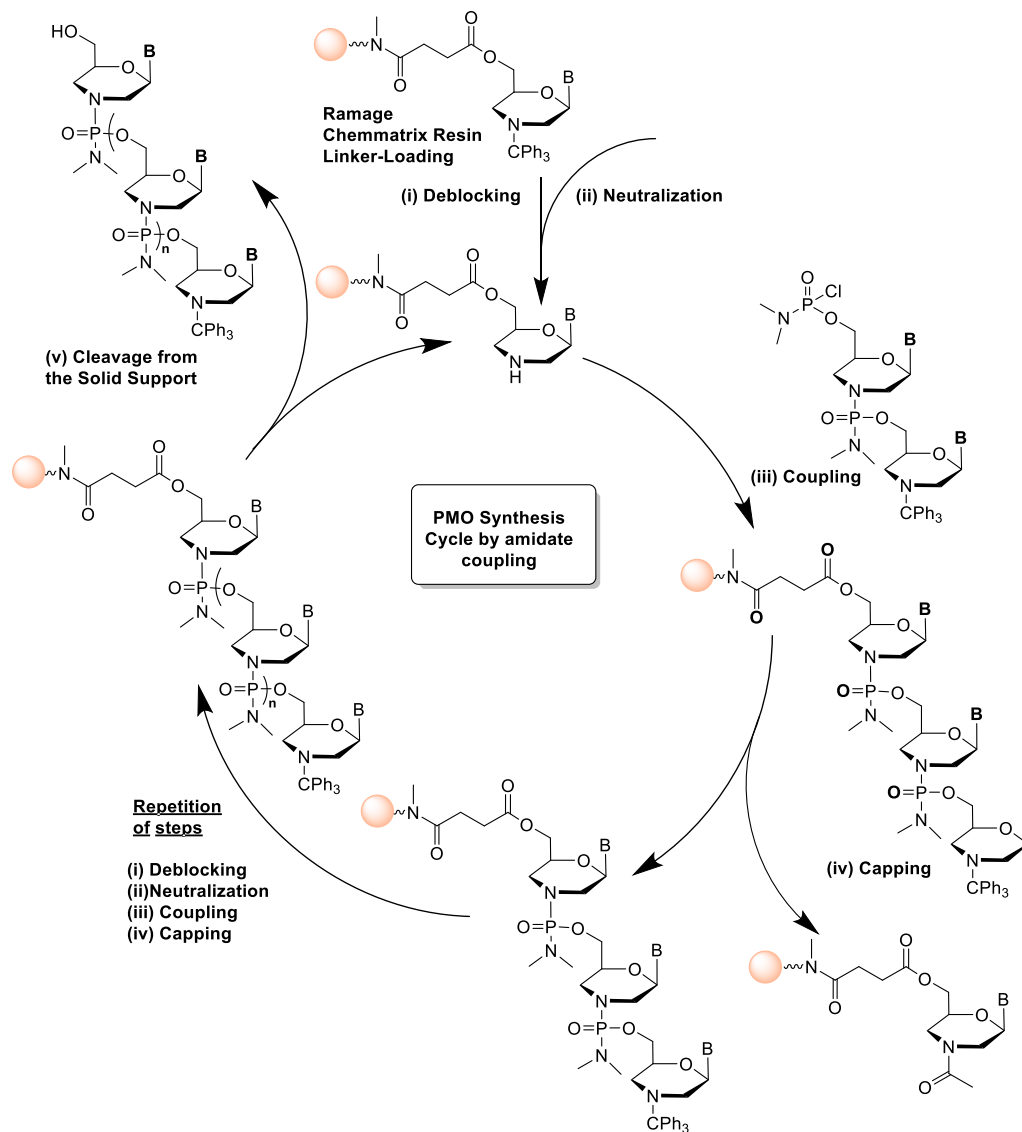
^{13}C NMR of Compound **8T^mT** (202MHz, CDCl_3) ^{31}P NMR of Compound **8T^mT** (202MHz, CDCl_3)

^1H NMR of Compound **8T^oT** (500MHz, CDCl_3) ^{13}C NMR of Compound **8T^oT** (500MHz, CDCl_3)

^{31}P NMR of Compound **8T^gT** (202MHz, CDCl_3)



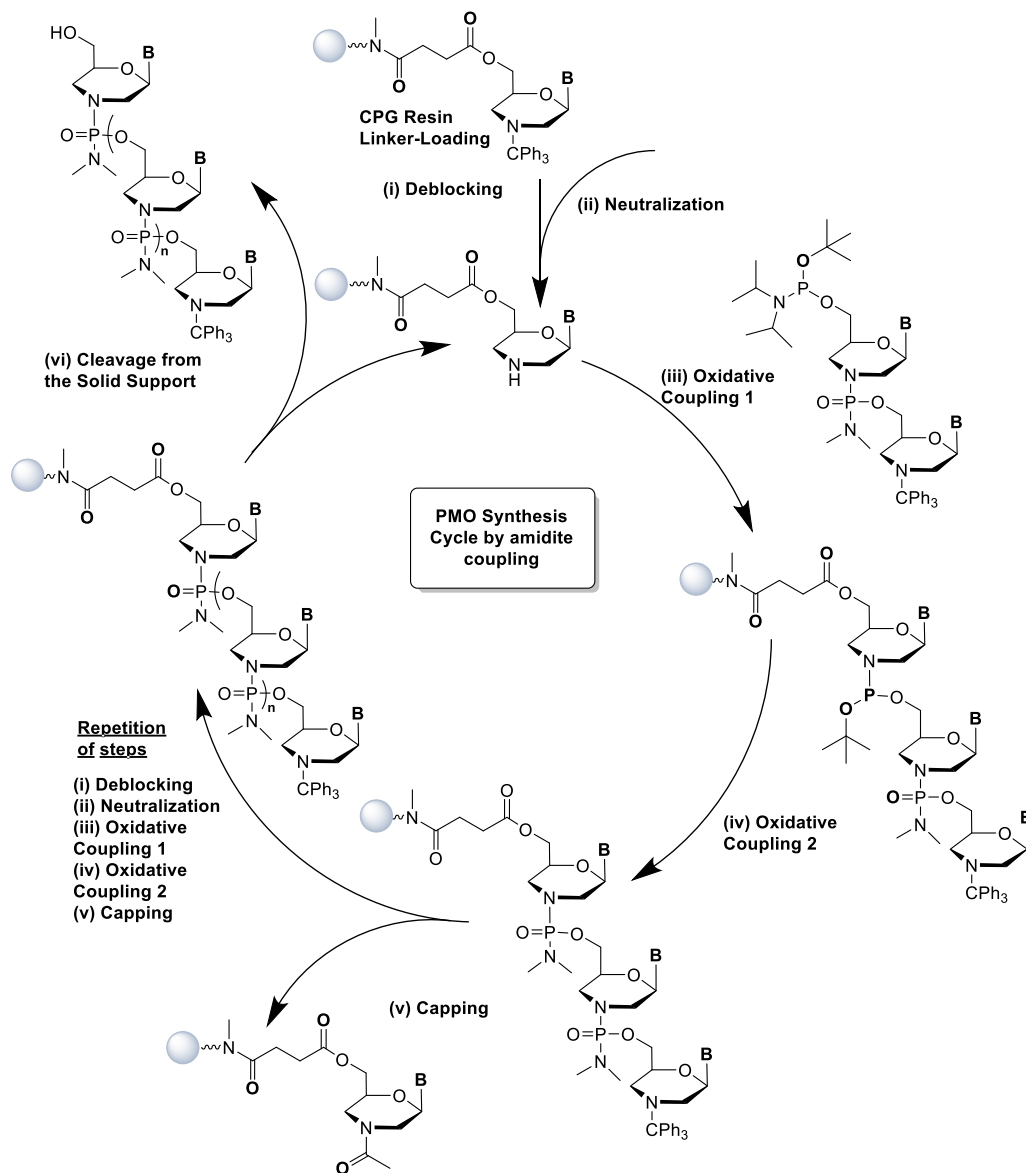
5. PMO synthesis cycle using chlorophosphoramidate dimer



Scheme S1: Synthesis of PMOs using dimer chlorophosphoramidates (Manual synthesis).

Reagents and Conditions: **(i) Deblocking:** CYPMSA (3-cyano pyridine, methanesulphonic acid, methanol, DCM) (4 times x 1 min = 4 min) ; **(ii) Neutralization:** 20% DIPEA-NMP (3times x 1min = 3 min) **(iii) Coupling:** Chlorophosphoramidate morpholino dimer (0.2M), ETT (5-ethylthio tetrazole, 0.075M) and NEM (4-ethyl morpholine, 5equiv.), DMI solvent, (30mins x 3 times = 1.5h) **(iv) Capping:** (1:1)- 10% Ac₂O-NMP and 10% DIPEA-NMP (3times x 1min = 3 min); **(v) Cleavage from the solid support:** 30% aq NH₃, 55°C, 16 h.

6. PMO synthesis cycle using phosphoramidite dimer



Scheme S2: Synthesis of PMOs using dimer phosphoramidite in automated oligosynthesizer

Reagents and Conditions: **(i) Deblocking:** CYPMSA (3-cyano pyridine, methanesulphonic acid, methanol, DCM) (4 times x 1 min = 4 min) ; **(ii) Neutralization:** 20% DIPEA-THF (3times x 1min = 3 min) **(iii) Oxidative Coupling 1:** coupling by phosphoramidite morpholino dimer (0.2M), ETT (5-ethylthio tetrazole, 0.07M) and NMI (N-methyl imidazole, 0.05M.), DCM-I solvent (1:1), (2times x 6mins = 12mins) and then oxidation by I₂(0.5M)-NHMe₂ (1M) in THF (4 min x 2 times = 8min) **(iv) Oxidative Coupling 2:** coupling by phosphoramidite morpholino dimer (0.2M), ETT (5-ethylthio tetrazole, 0.07M) and NMI (N-methyl imidazole, 0.05M.), DCM-I solvent (1:1), (1time x 6mins = 6mins) and then oxidation by I₂(0.5M)-NHMe₂ (1M) in THF (4 min x 1 times = 4min) **(v) Capping:** (1:1)- 10% Ac₂O-THF and 10% DIPEA-THF (3times x 1min = 3 mins); **(vi) Cleavage from the solid support:** 30% aq NH₃, 55°C, 16 h.

7. Comparative HPLC chromatogram in chlorophosphoramidate and phosphoramidite methods

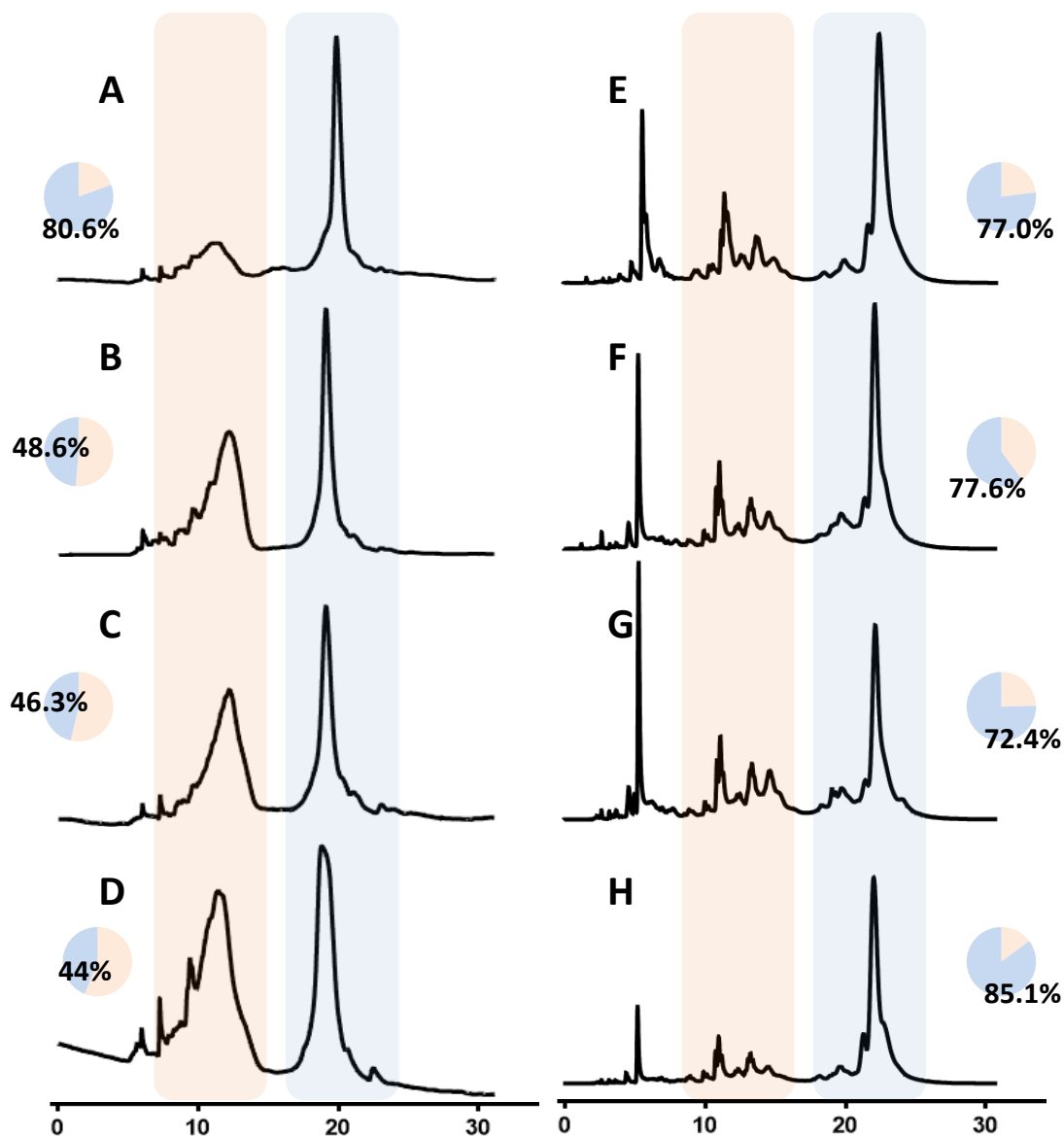


Figure S1: HPLC chromatogram of PMOs (A) **PMO1** with 0.2M Compound **4TT**, 0.075M ETT and 5equiv NEM (B) **PMO2** with 0.2M Compound **4TT**, 0.075M ETT and 5equiv NEM (C) **PMO2** with 0.4M Compound **4TT**, 0.075M ETT and 5equiv NEM (D) **PMO2** with 0.2M Compound **4TT**, 0.075M ETT and 5equiv NEM at 50°C (E) **PMO1** with 0.2M Compound **5TT**, 0.07M ETT (F) **PMO1** with 0.2M Compound **5TT**, 0.07M BTT (G) **PMO1** with 0.2M Compound **5TT**, 0.07M DCI (H) **PMO1** with 0.2M Compound **5TT**, 0.07M ETT containing 0.05M NMI.

8. Table for HPLC retention time and MALDI-TOF mass of oligonucleotides

Sl. No.	Sequence (5'→3')	Molecular Formula	Calculated Mass	Obtained mass	R _t (min)
PMO1	TTTTTTTTT(Synthesized by chlorophosphoramidate)	C ₁₀₆ H ₁₆₇ N ₃₅ O ₄₄ P ₈ K	2920.944	2916.741	19.7
PMO2	TTTTTTTTT (synthesized by phosphoramidite coupling)	C ₁₀₆ H ₁₆₇ N ₃₅ O ₄₄ P ₈ Na	2906.483	2907.678	18.9
PMO2	TTTTTTTTTTTTTTTT	C ₁₇₈ H ₂₈₁ N ₅₉ O ₇₄ P ₁₄ Na ₂	4911.157	4910.529	22.1
PMO3	TTTTTTTTTTTTTT	C ₁₄₂ H ₂₂₄ N ₄₇ O ₅₉ P ₁₁ Na ₂	3918.288	3919.707	13.1
PMO4	TTTTTT ^c TTTTTT	C ₁₄₁ H ₂₁₈ N ₄₆ O ₅₉ P ₁₀ Na ₂	3855.264	3855.634	12.8
PMO5	TTTTTT ^u TTTTTT	C ₁₄₁ H ₂₁₉ N ₄₇ O ₅₈ P ₁₀ Na ₂	3854.956	3854.280	12.9
PMO6	TTTTTT ^m TTTTTT	C ₁₄₀ H ₂₁₈ N ₄₆ O ₅₇ P ₁₀ Na ₂	3811.275	3811.188	13.0
PMO7	TTTTTT ^g TTTTTT	C ₁₄₂ H ₂₂₁ N ₄₇ O ₅₈ P ₁₀ Na ₂	3870.318	3870.129	12.8
PMO8	TGGAGTTCCTGT	C ₁₄₀ H ₂₁₇ N ₆₄ O ₅₁ P ₁₁ Na ₂	3997.326	3998.814	8.1
PMO9	TGGAGT ^c TCCTGT	C ₁₃₉ H ₂₁₁ N ₆₃ O ₅₁ P ₁₀ Na ₂	3934.303	3935.466	8.1
PMO10	TGGAGT ^u TCCTGT	C ₁₃₉ H ₂₁₂ N ₆₄ O ₅₀ P ₁₀ Na ₂	3933.319	3935.008	8.0
PMO11	TGGAGT ^m TCCTGT	C ₁₃₈ H ₂₁₁ N ₆₃ O ₄₉ P ₁₀ Na ₂	3890.313	3891.014	7.9
PMO12	TGGAGT ^g TCCTGT	C ₁₄₀ H ₂₁₄ N ₆₄ O ₅₀ P ₁₀ Na ₂	3947.334	3949.881	8.1

Table S1: HPLC retention time and MALDI-TOF mass of oligonucleotides

9. HPLC profiles of PMOs using dimer chlorophosphoramidate under different coupling conditions

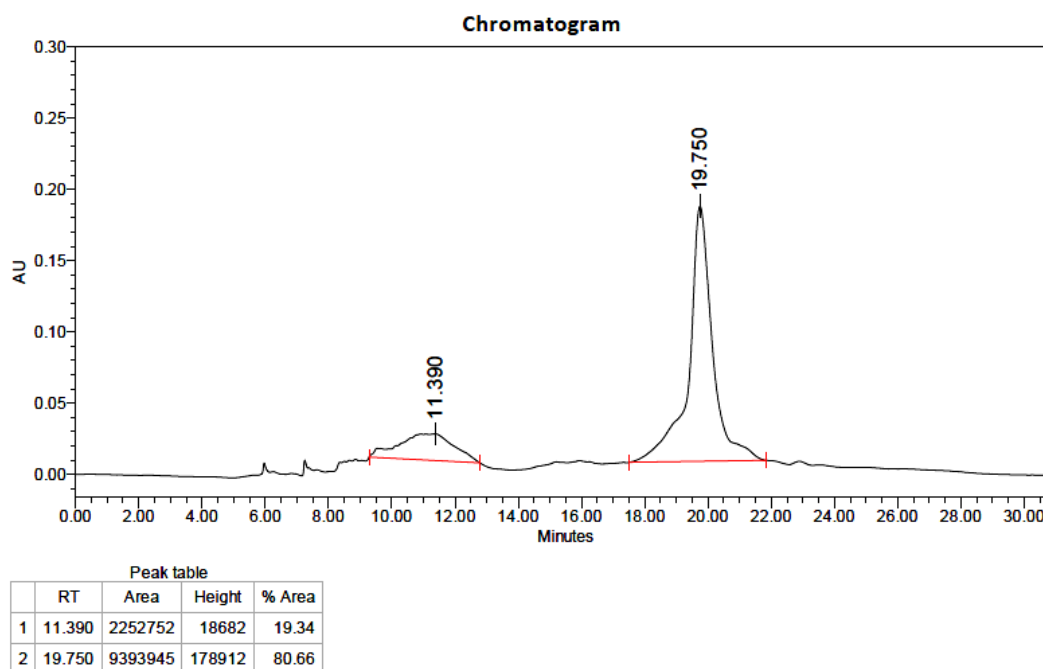


Figure S2: HPLC chromatogram of PMO1 (HPLC condition 1)

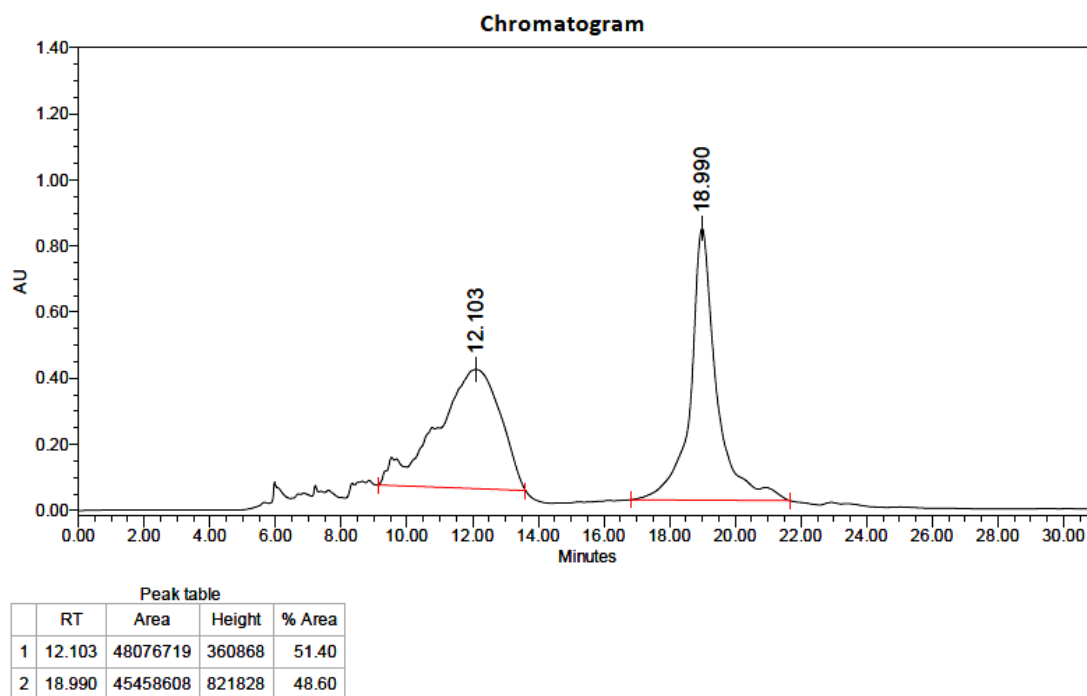


Figure S3: HPLC chromatogram of **PMO2** (HPLC condition 1)

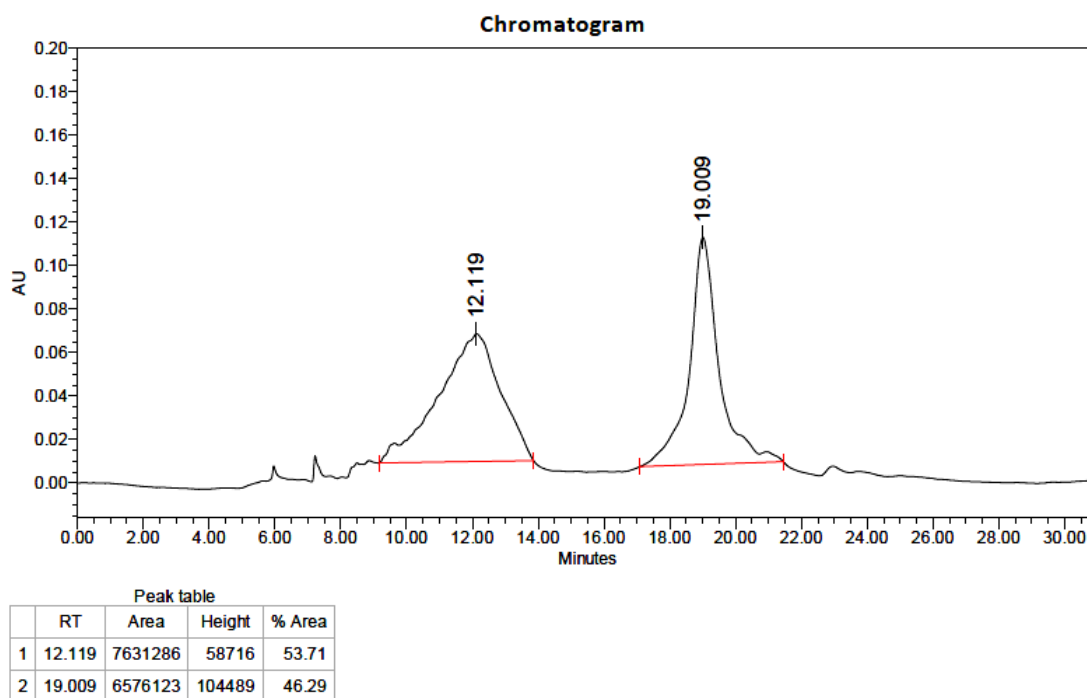


Figure S4: HPLC chromatogram of **PMO2** (HPLC condition 1, 0.4M dimer chlorophosphoramidate was used).

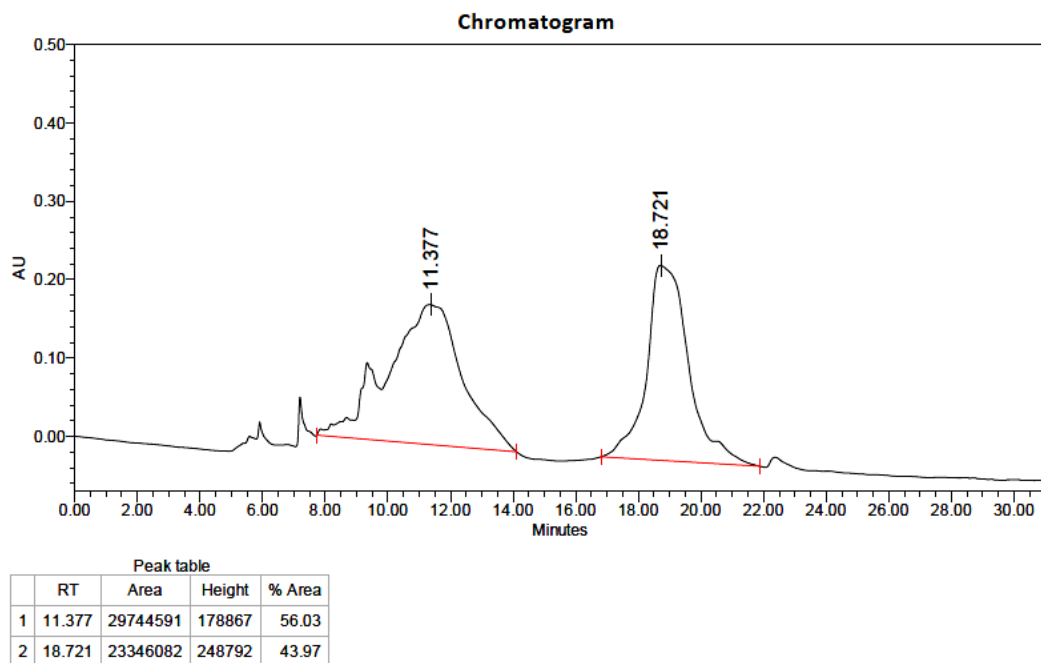


Figure S5: HPLC chromatogram of **PMO2** (HPLC condition 1, 0.2M dimer chlorophosphoramidate was used at 50°C).

10. HPLC profiles of PMOs using dimer phosphoramidite with different activators

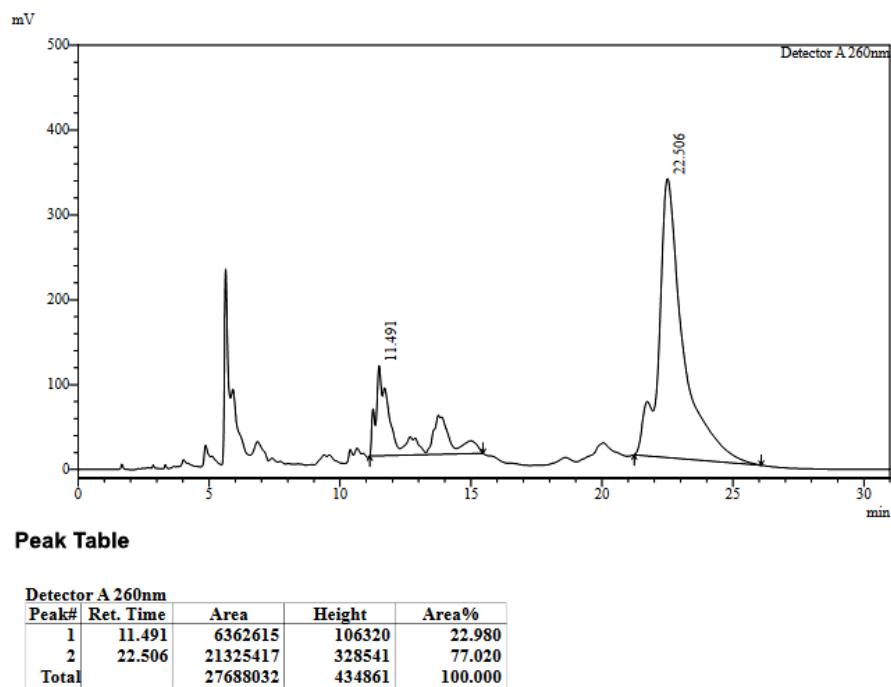
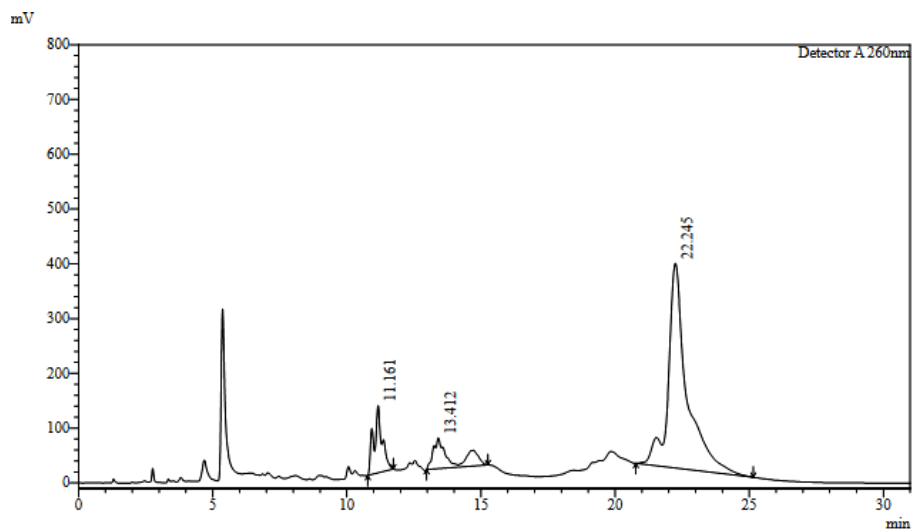
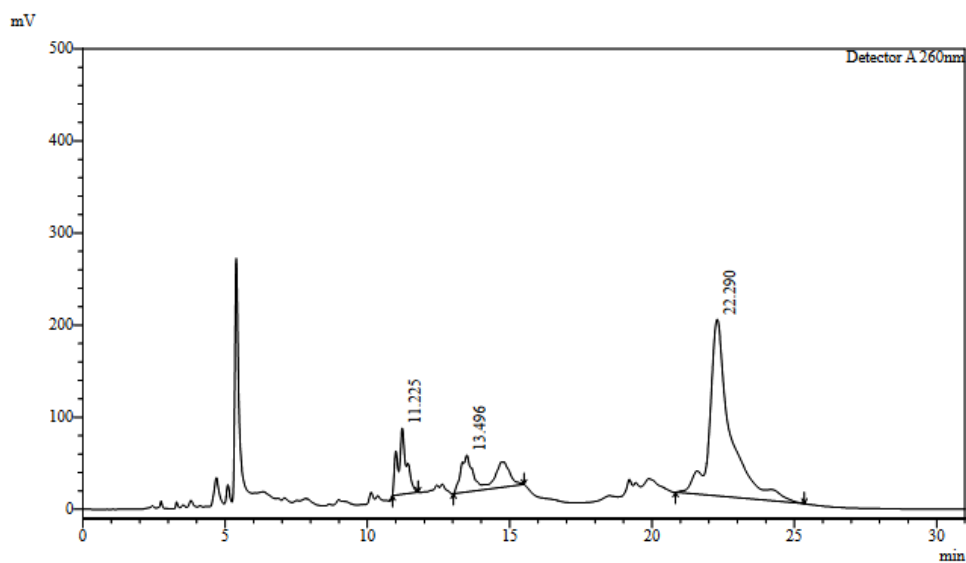


Figure S6: HPLC chromatogram of **PMO1** (HPLC condition 2, ETT activator was used).



Peak Table

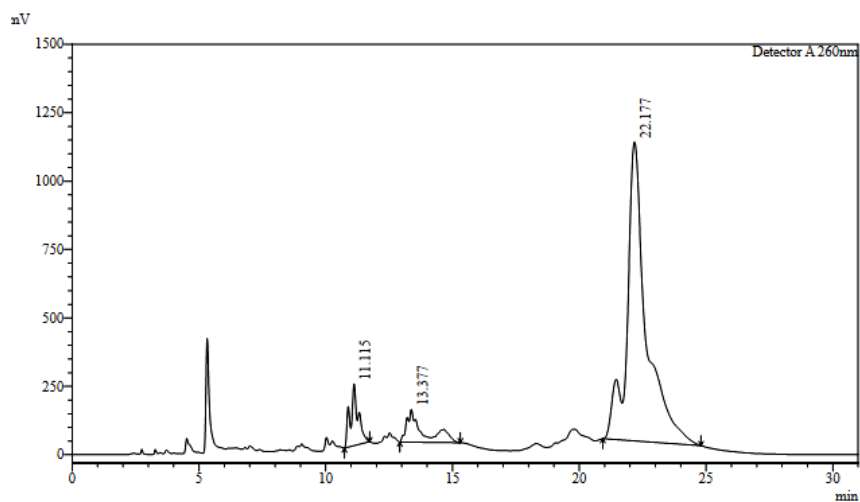
Detector A 260nm				
Peak#	Ret. Time	Area	Height	Area%
1	11.161	2640116	123028	11.318
2	13.412	2576170	55973	11.044
3	22.245	18110151	373721	77.638
Total		23326437	552722	100.000

Figure S7: HPLC chromatogram of **PMO1** (HPLC condition 2, BTT activator was used).

Peak Table

Detector A 260nm				
Peak#	Ret. Time	Area	Height	Area%
1	11.225	1519038	71846	11.358
2	13.496	2173309	39956	16.249
3	22.290	9682407	191613	72.393
Total		13374754	303415	100.000

Figure S8: HPLC chromatogram of **PMO1** (HPLC condition 2, DCI activator was used).



Peak Table

Detector A 260nm				
Peak#	Ret. Time	Area	Height	Area%
1	11.115	4806076	225602	7.090
2	13.377	5281694	120114	7.792
3	22.177	57695019	1092144	85.118
Total		67782789	1437861	100.000

Figure S9: HPLC chromatogram of **PMO1** (HPLC condition 2, ETT activator was used along with NMI).

11. MALDI-TOF mass of PMOs synthesized using dimer amidate and amidite

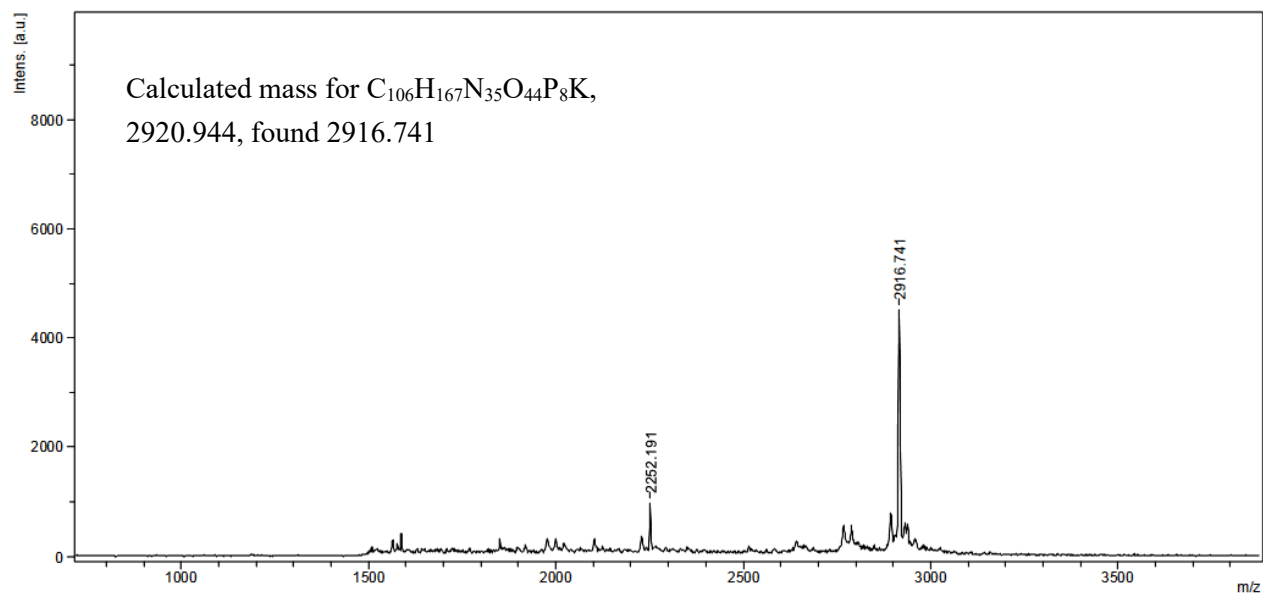


Figure S10: MALDI-TOF mass of **PMO1 (9mer-T)** synthesized by chlorophosphoramidate dimer).

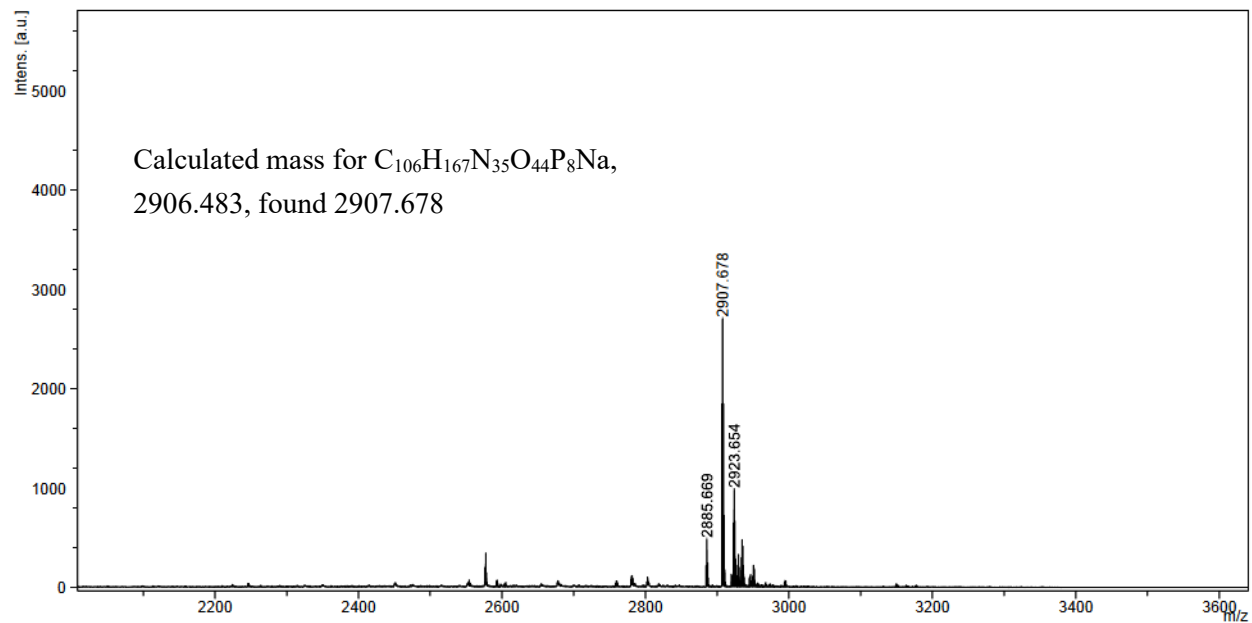


Figure S11: MALDI-TOF mass of PMO1 (**9mer-T** synthesized by phosphoramidite dimer).

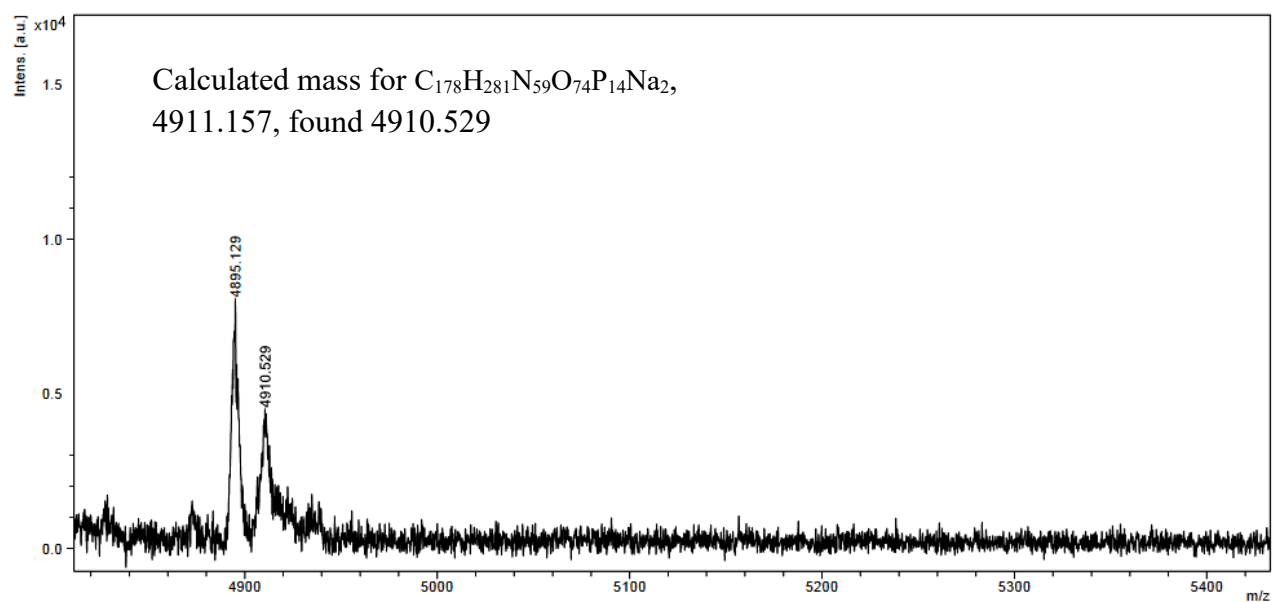


Figure S12: MALDI-TOF mass of PMO2 (**15mer-T** synthesized by chlorophosphoramidate dimer).

12. HPLC and MALDI-TOF mass of modified 12mer PMOs

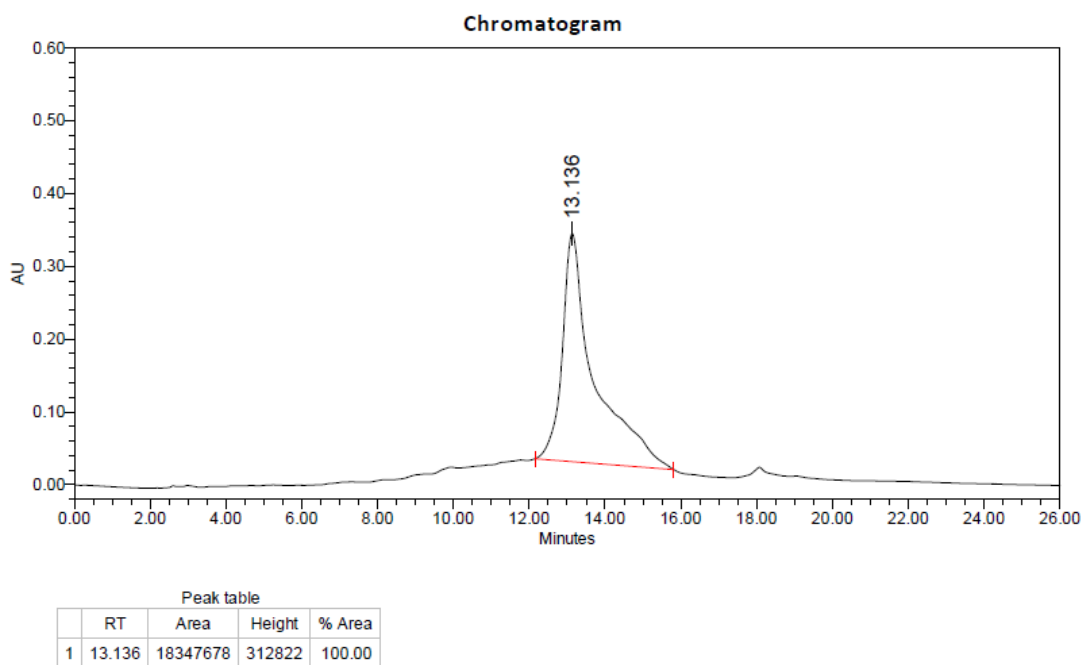


Figure S13: HPLC chromatogram of **PMO3** (HPLC condition 3).

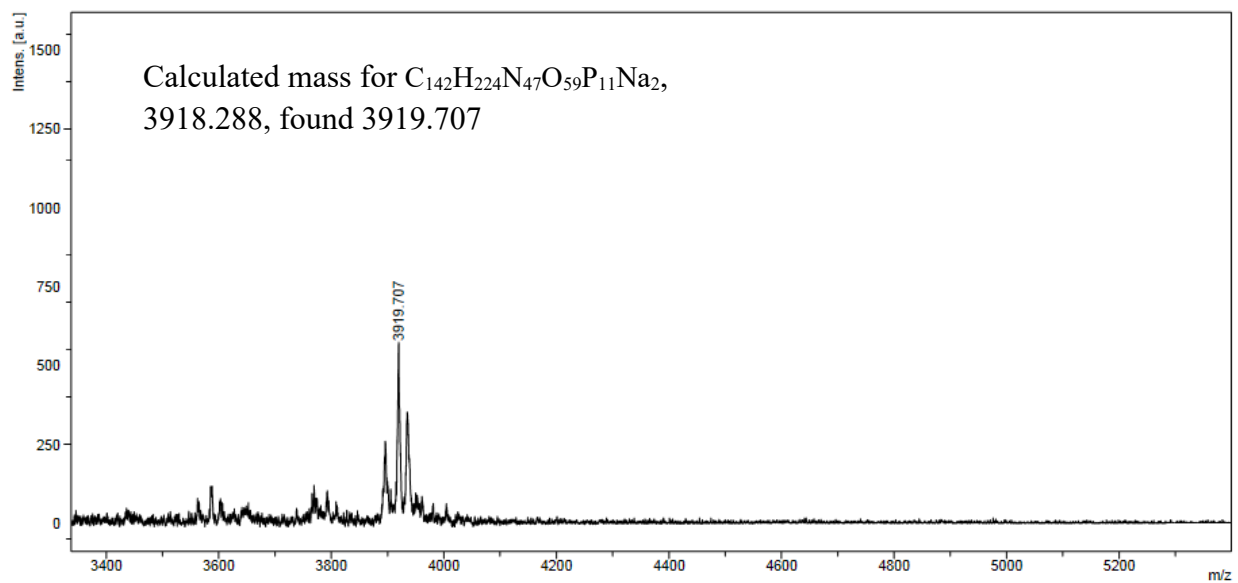
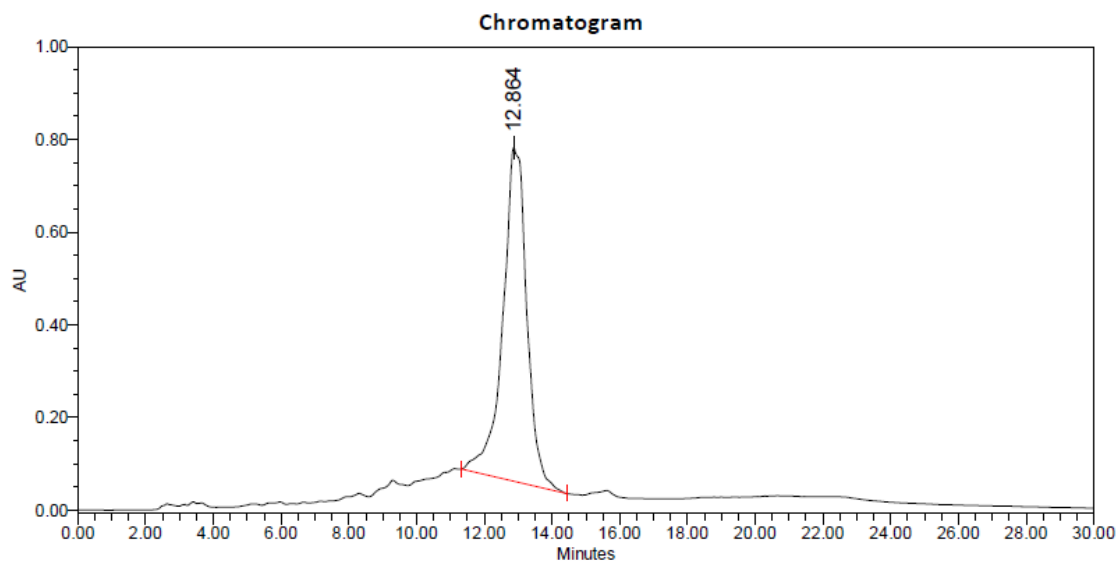


Figure S14: MALDI-TOF mass of **PMO3**.



Peak table

	RT	Area	Height	% Area
1	12.864	35693008	720027	100.00

Figure S15: HPLC chromatogram of **PMO4** (HPLC condition 3).

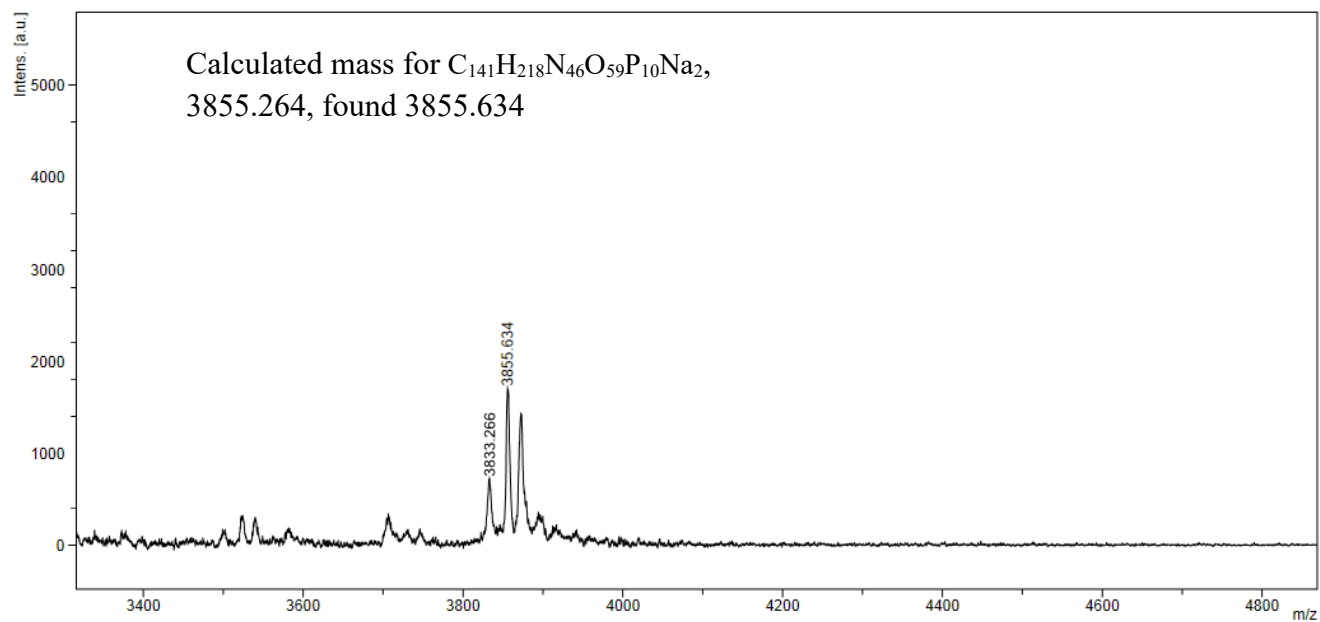


Figure S16: MALDI-TOF mass of **PMO4**.

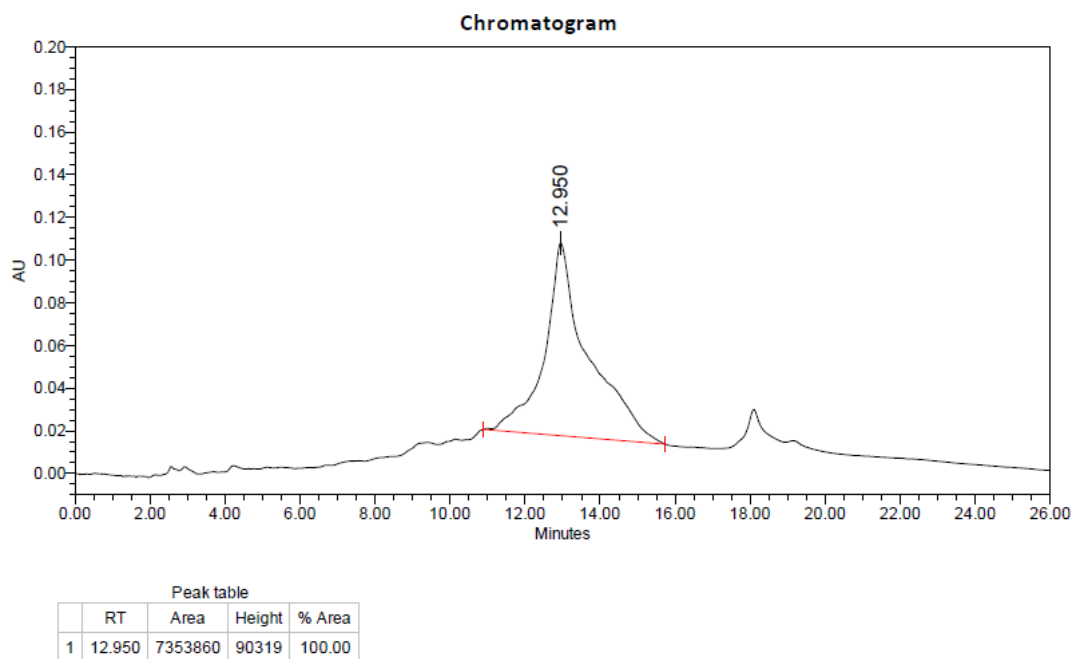


Figure S17: HPLC chromatogram of **PMO5** (HPLC condition 3).

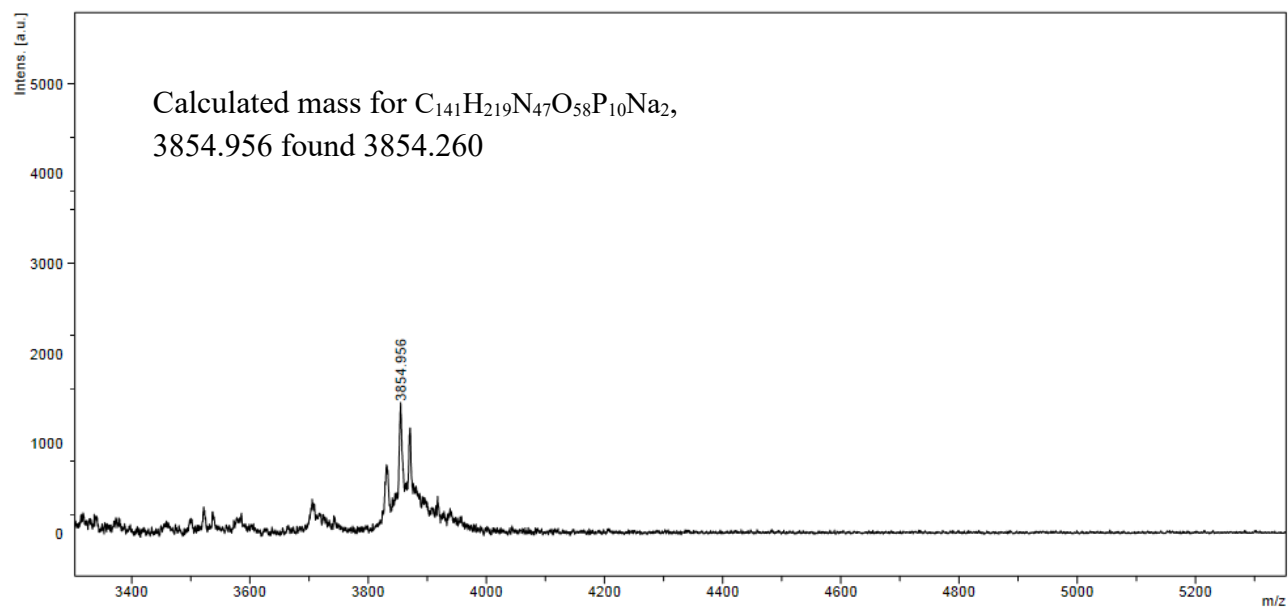
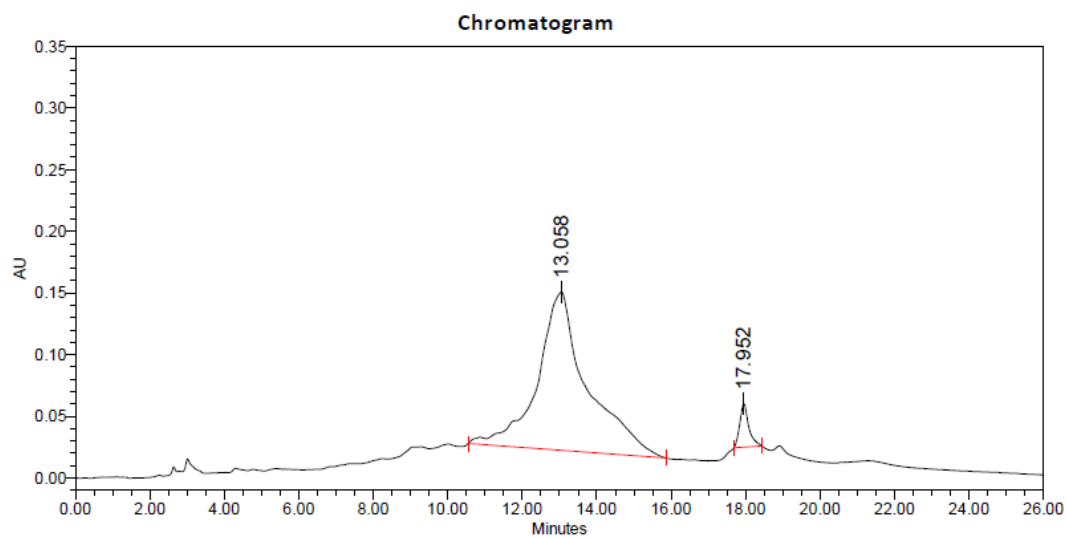


Figure S18: MALDI-TOF mass of **PMO5**.



Peak table

	RT	Area	Height	% Area
1	13.058	11858720	128320	95.26
2	17.952	590257	35165	4.74

Figure S19: HPLC chromatogram of **PMO6** (HPLC condition 3).

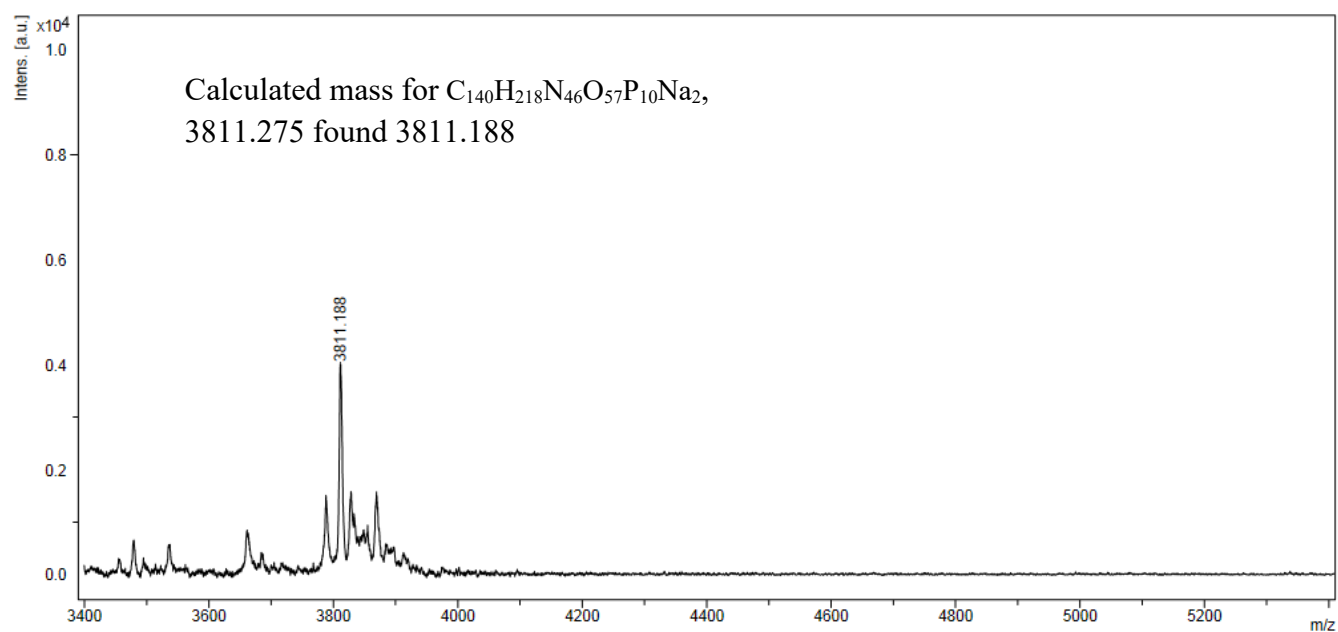


Figure S20: MALDI-TOF mass of **PMO6**.

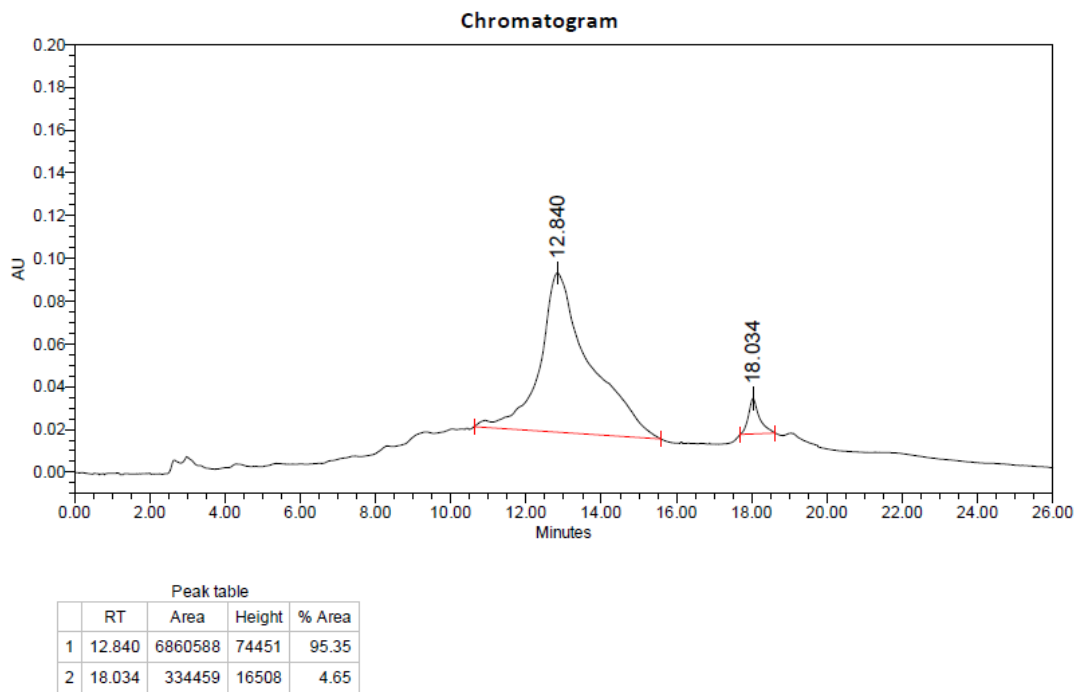


Figure S21: HPLC chromatogram of **PMO7** (HPLC condition 3).

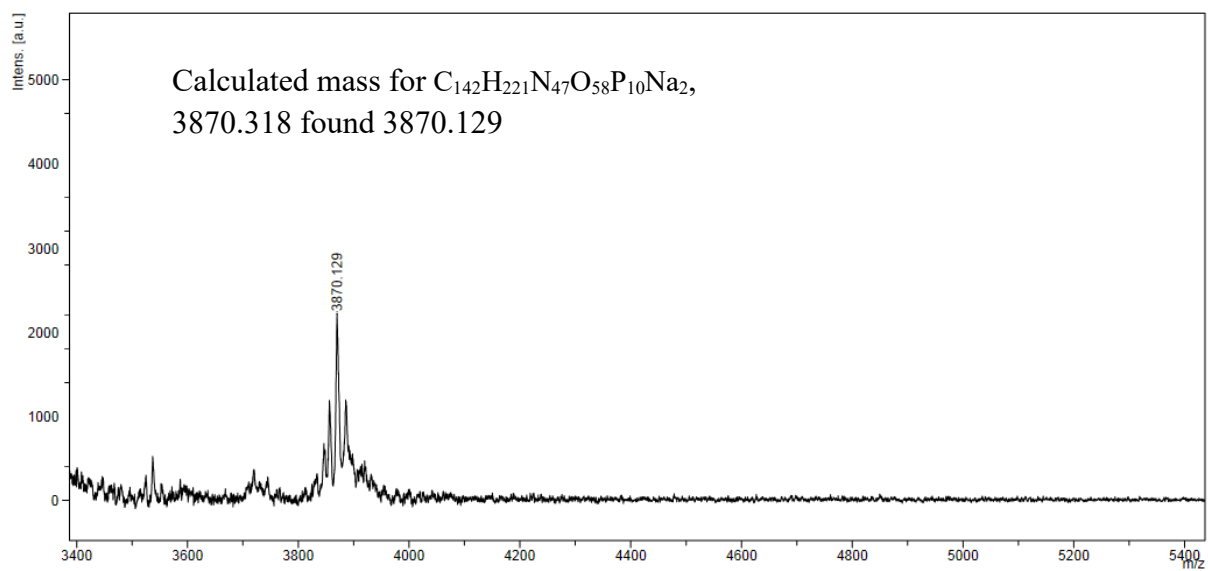
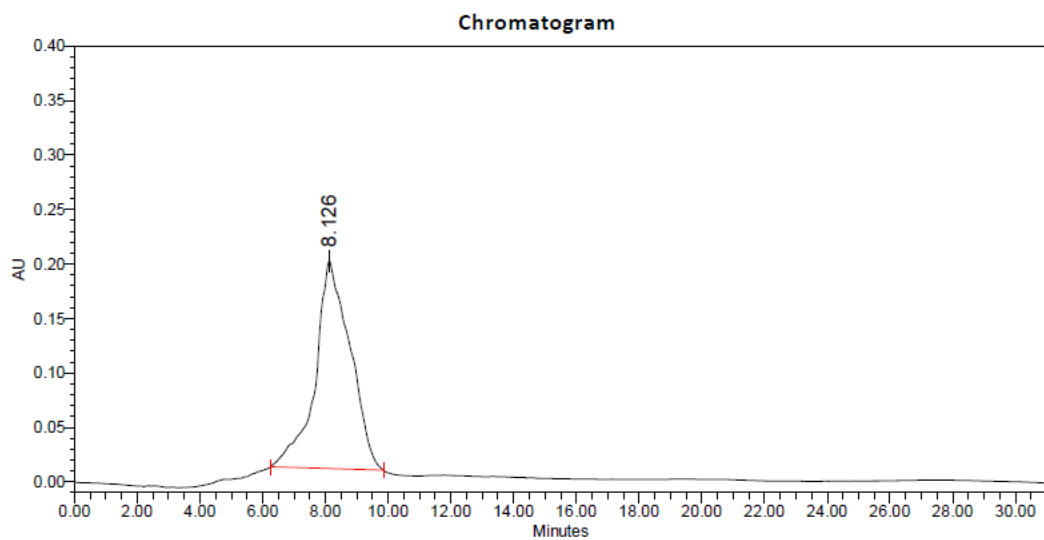


Figure S22: MALDI-TOF mass of **PMO7**.



Peak table

RT	Area	Height	% Area
1 8.126	14763303	190192	100.00

Figure S23: HPLC chromatogram of **PMO8** (HPLC condition 4).

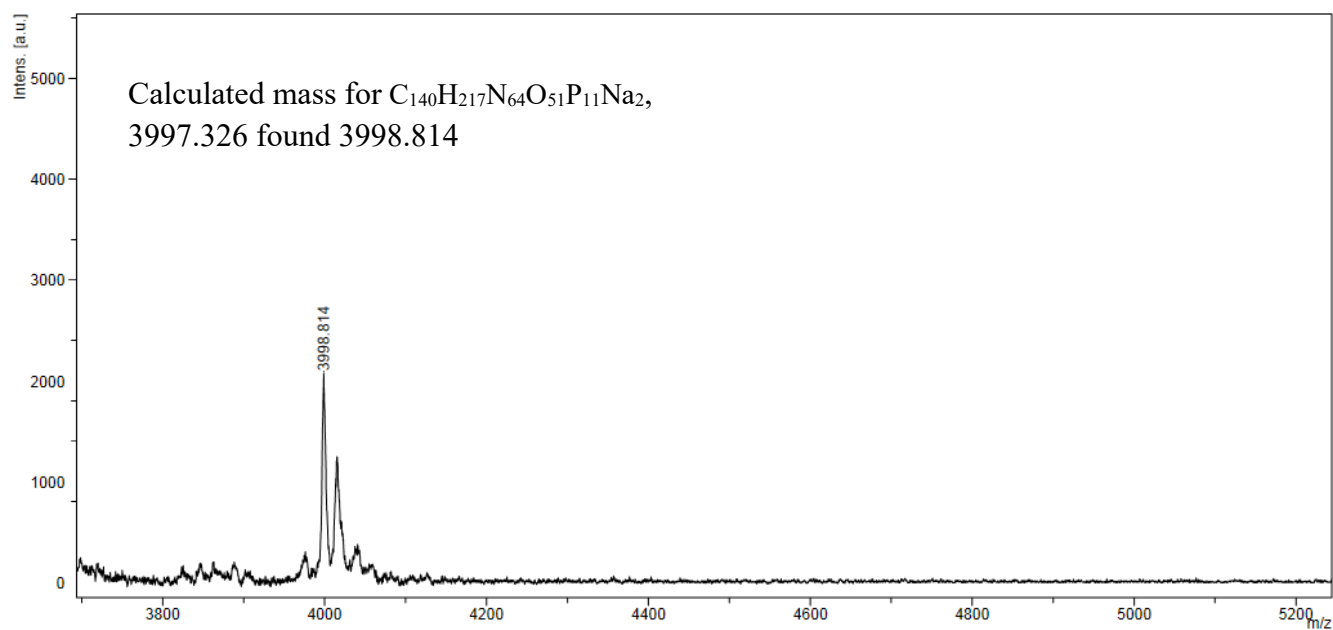
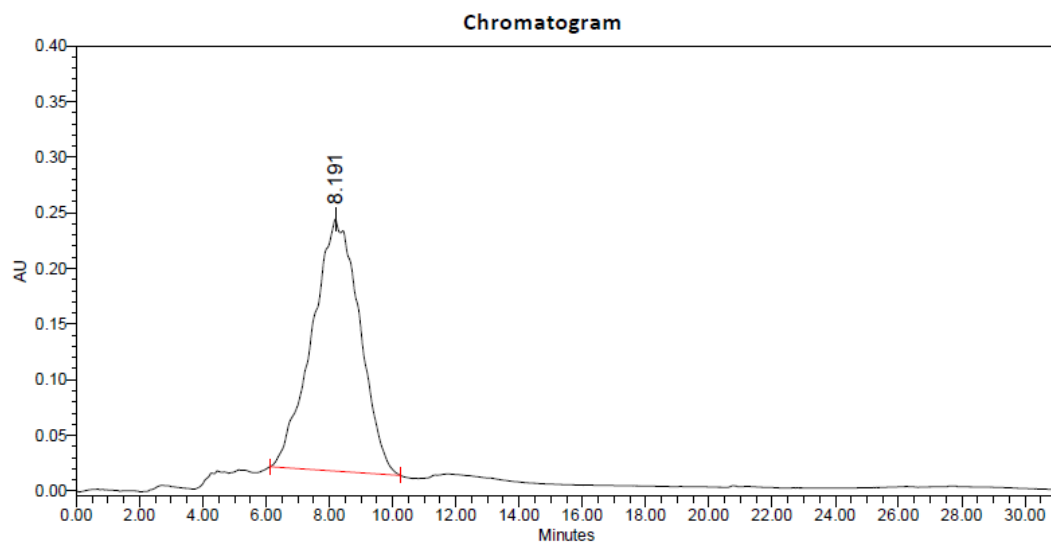


Figure S24: MALDI-TOF mass of **PMO8**.



Peak table

	RT	Area	Height	% Area
1	8.191	23855807	226507	100.00

Figure S25: HPLC chromatogram of **PMO9** (HPLC condition 4).

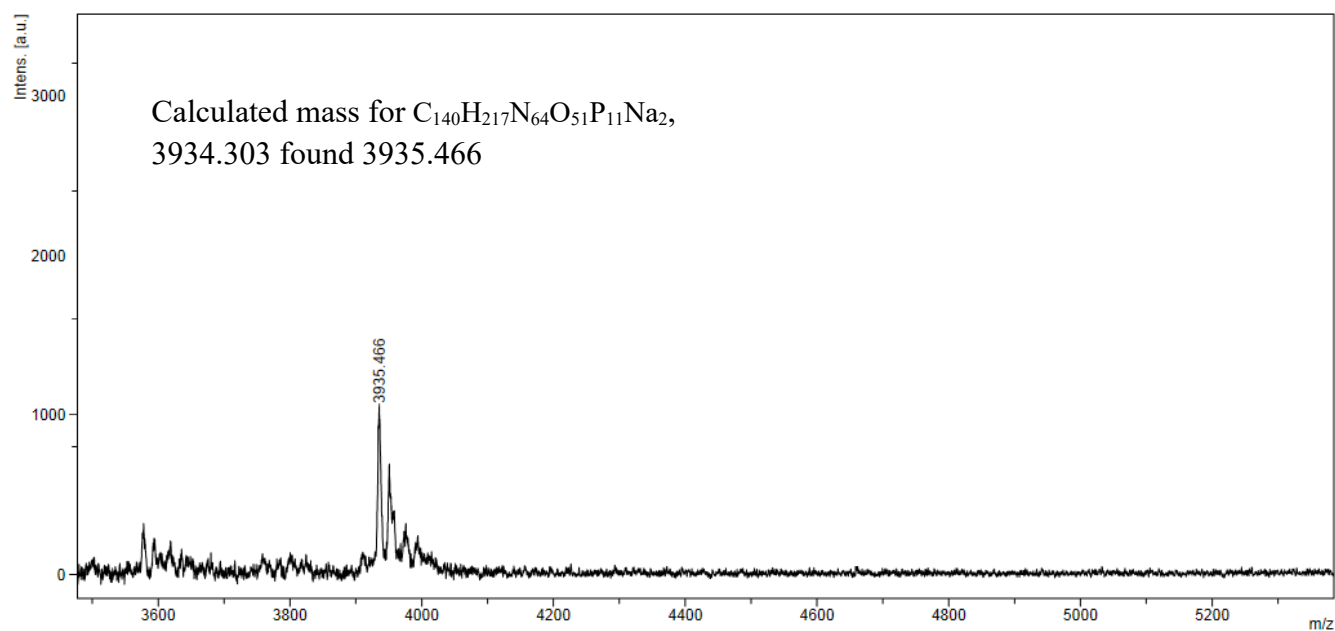


Figure S26: MALDI-TOF mass of **PMO9**.

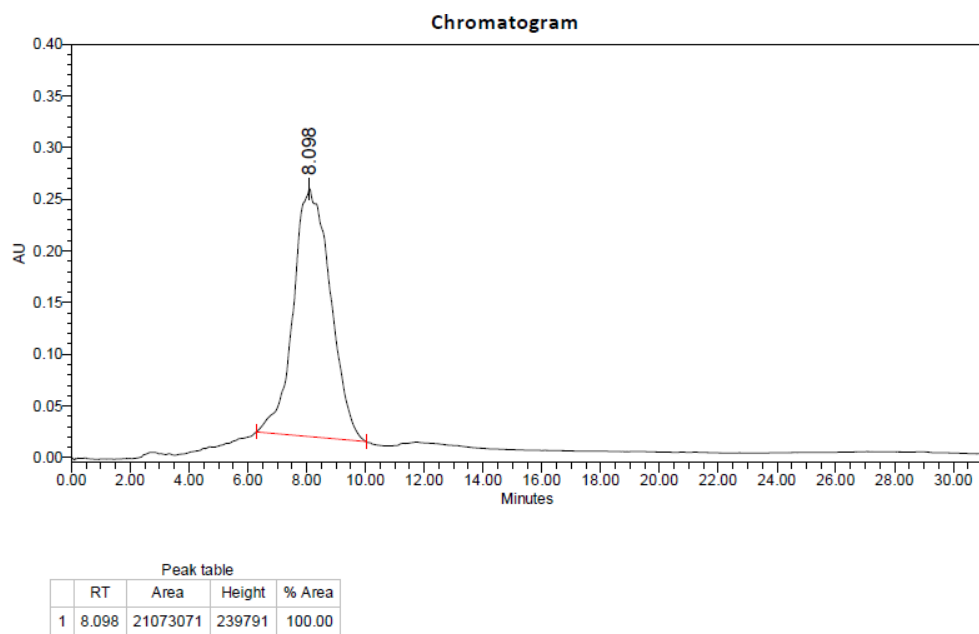


Figure S27: HPLC chromatogram of **PMO10** (HPLC condition 4).

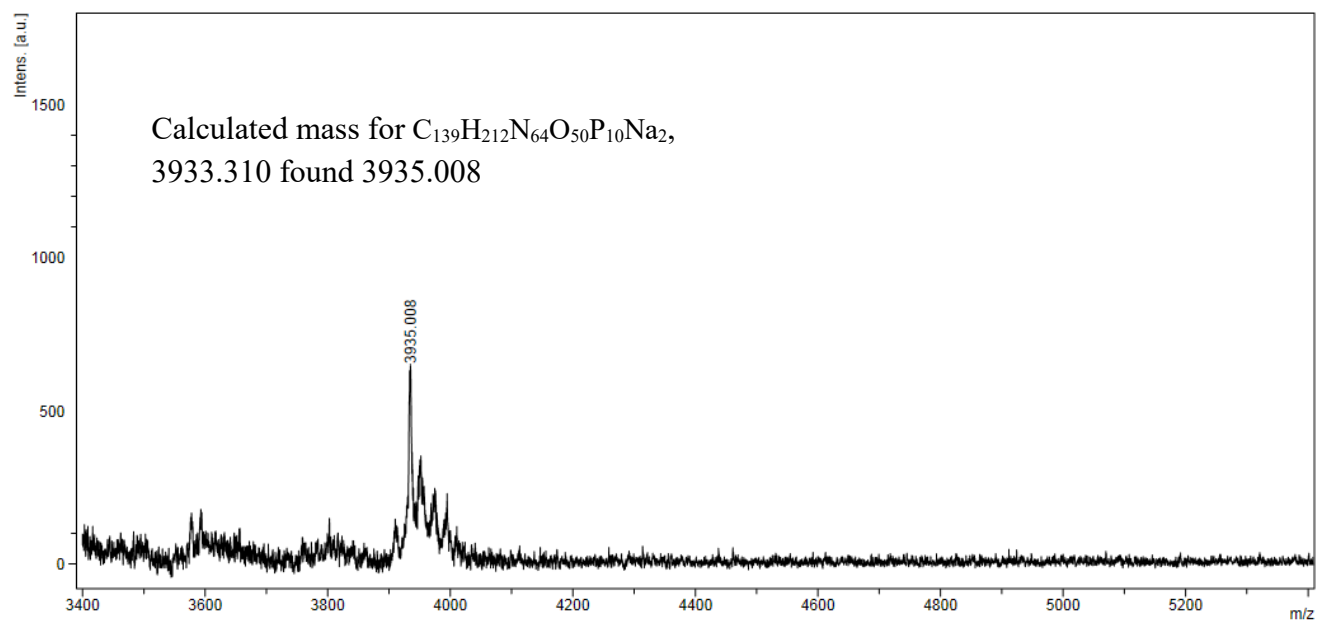


Figure S28: MALDI-TOF mass of **PMO10**.

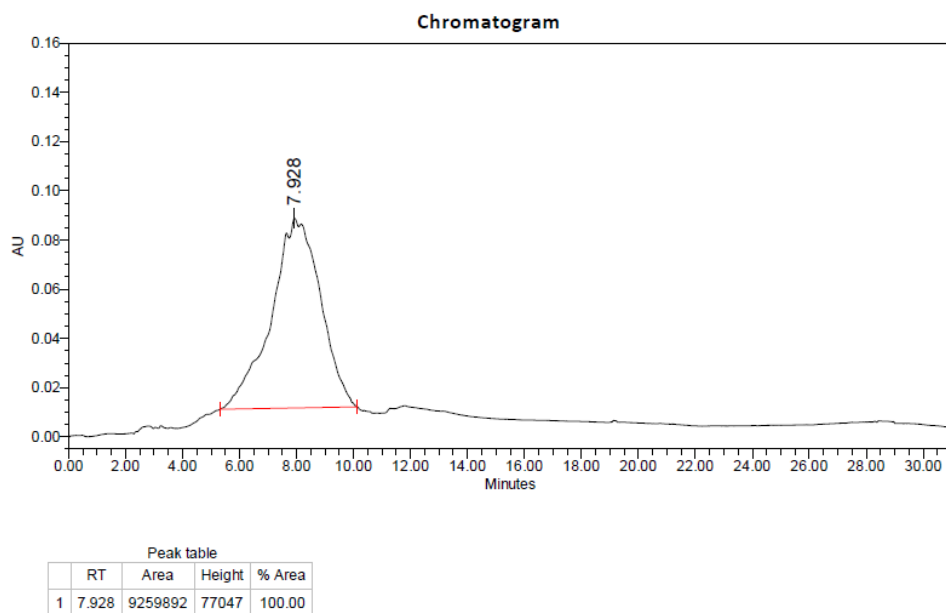


Figure S29: HPLC chromatogram of **PMO11** (HPLC condition 4).

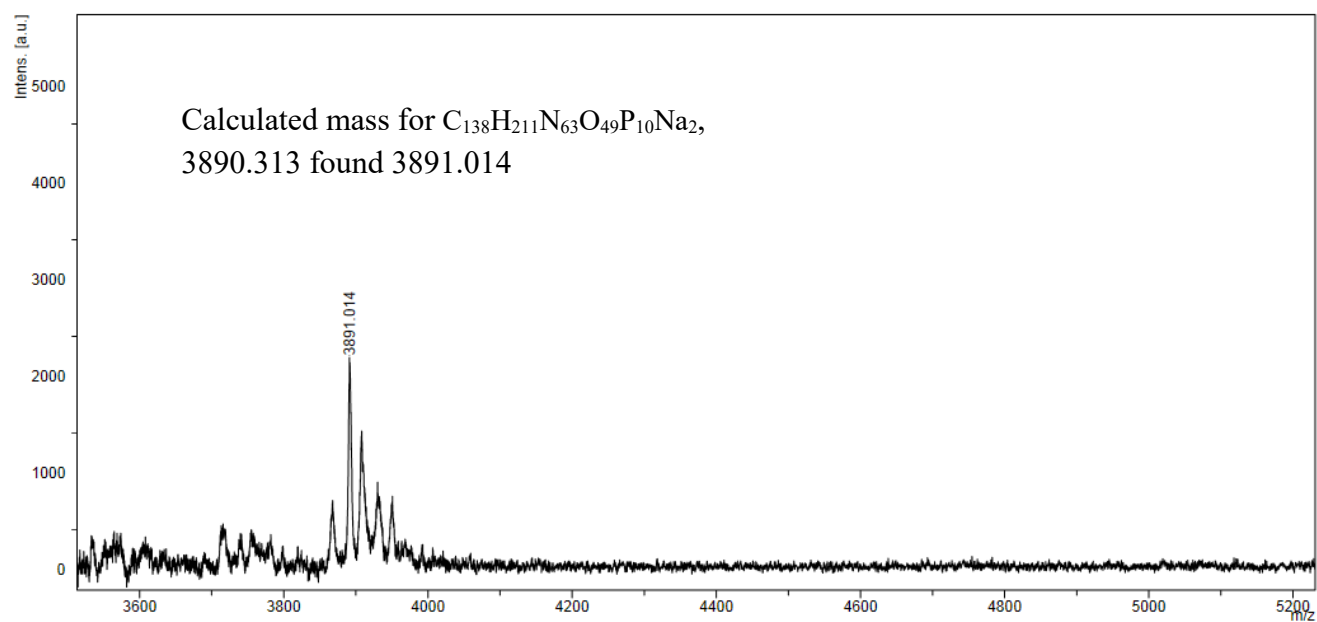
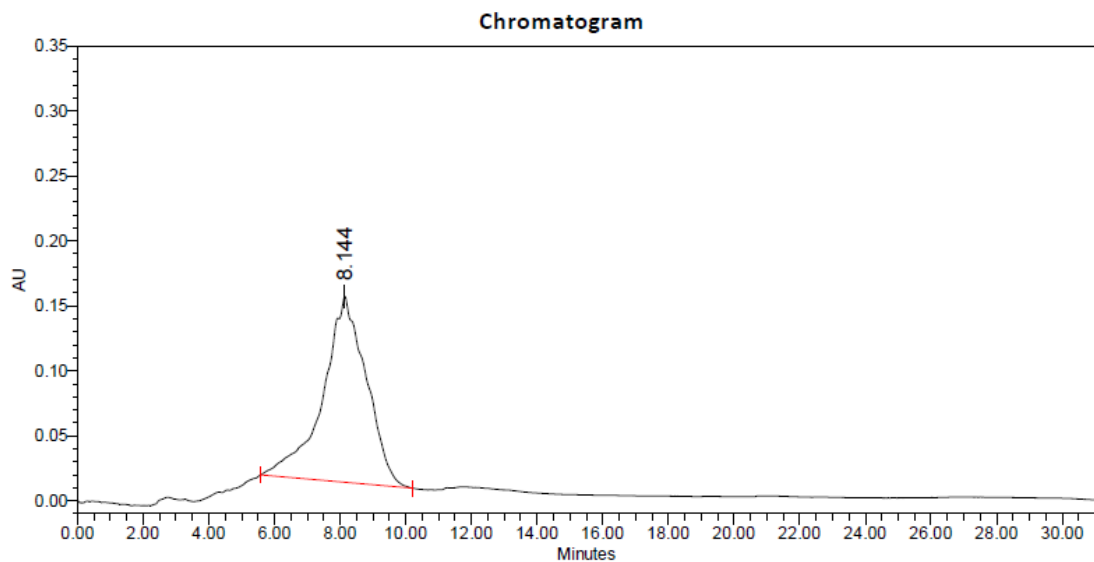


Figure S30: MALDI-TOF mass of **PMO11**.



Peak table

	RT	Area	Height	% Area
1	8.144	13192778	143040	100.00

Figure S31: HPLC chromatogram of **PMO12** (HPLC condition 4).

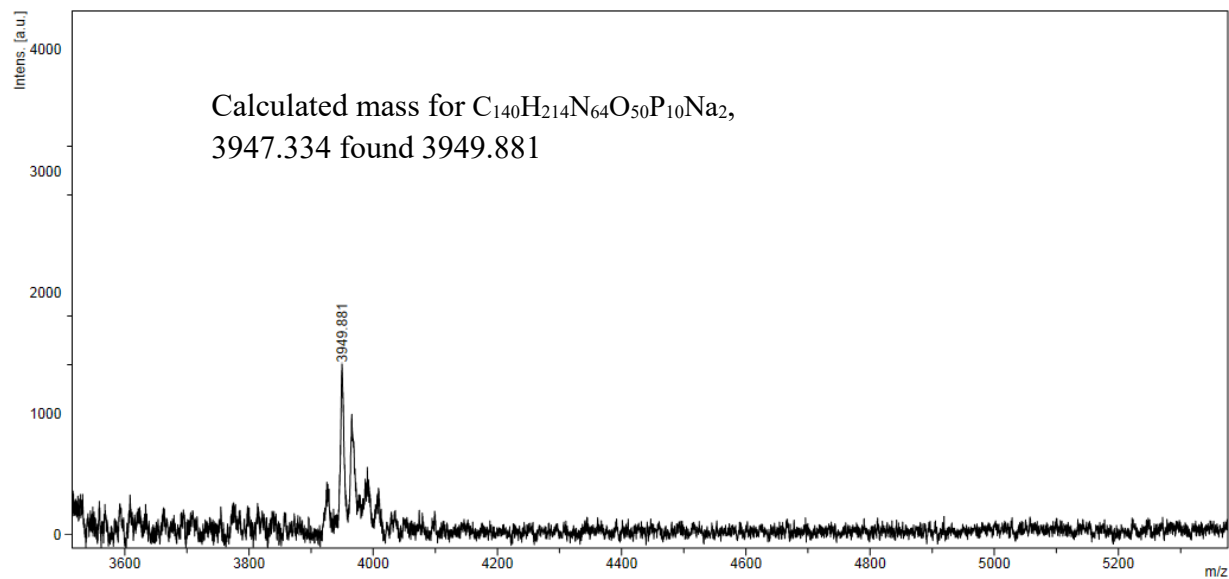


Figure S32: MALDI-TOF mass of **PMO12**.

13. Thermal melting analysis of modified 12mer PMOs with complementary DNA and RNA

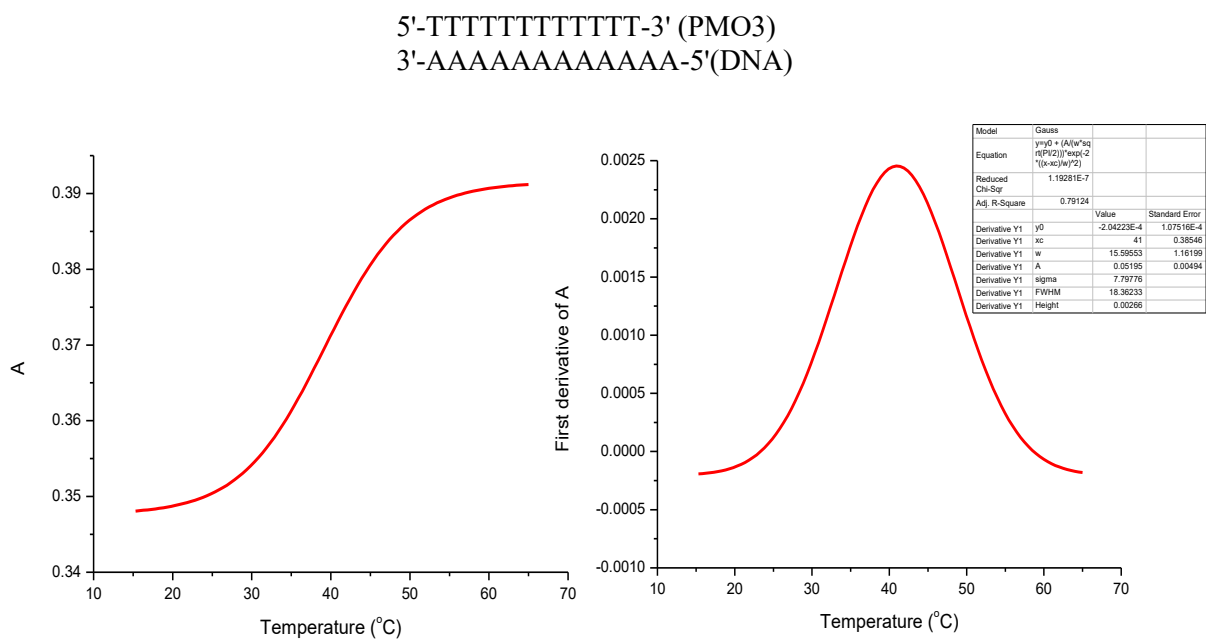


Figure S33: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO3-DNA duplex

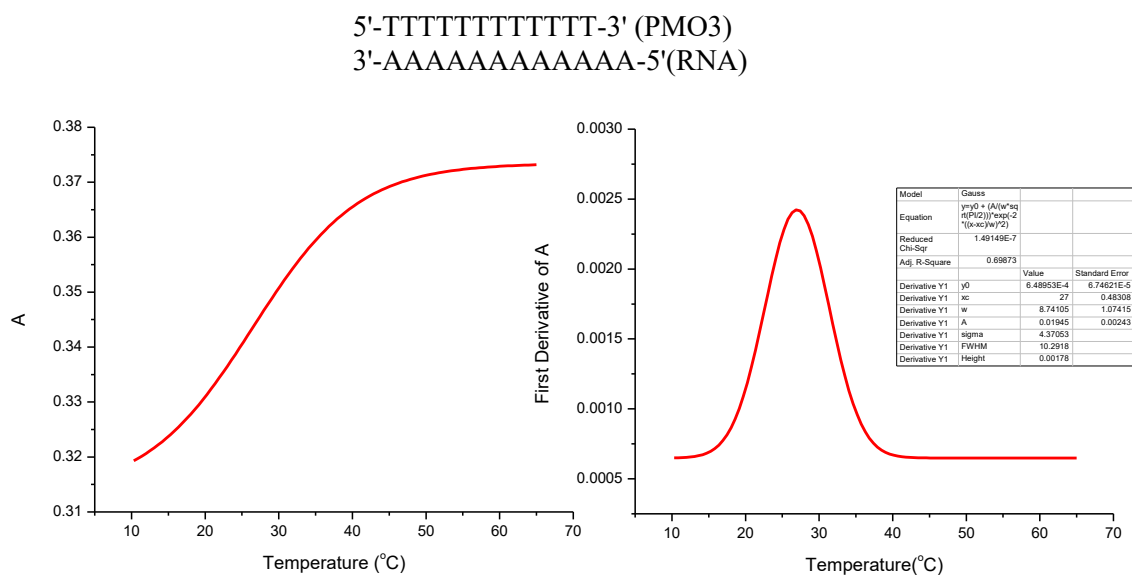


Figure S34: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO3-RNA duplex

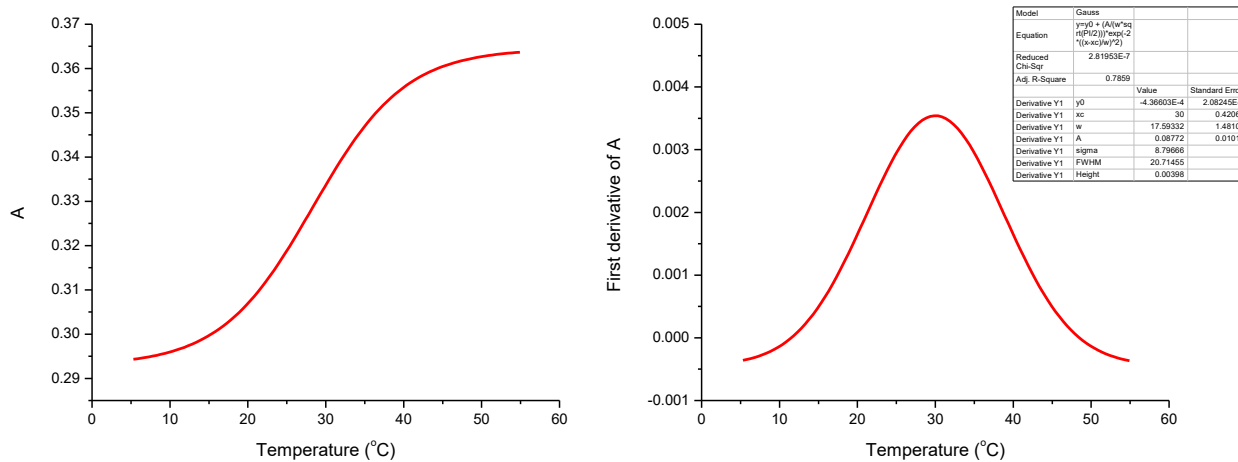


Figure S35: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO4-DNA duplex

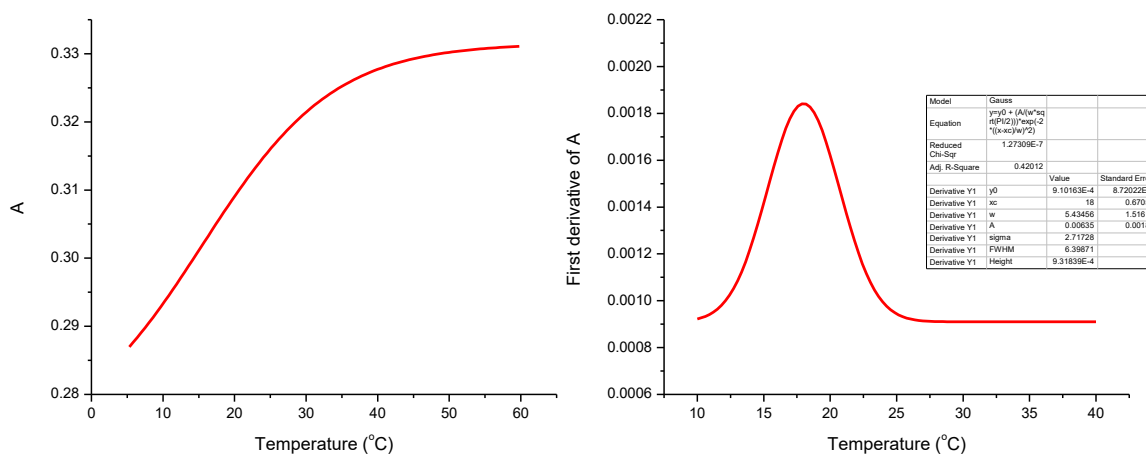


Figure S36: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO4-RNA duplex

5'-TTTTTT^uTTTTTT-3' (PMO5)
3'-AAAAAAAAAAAAA-5'(DNA)

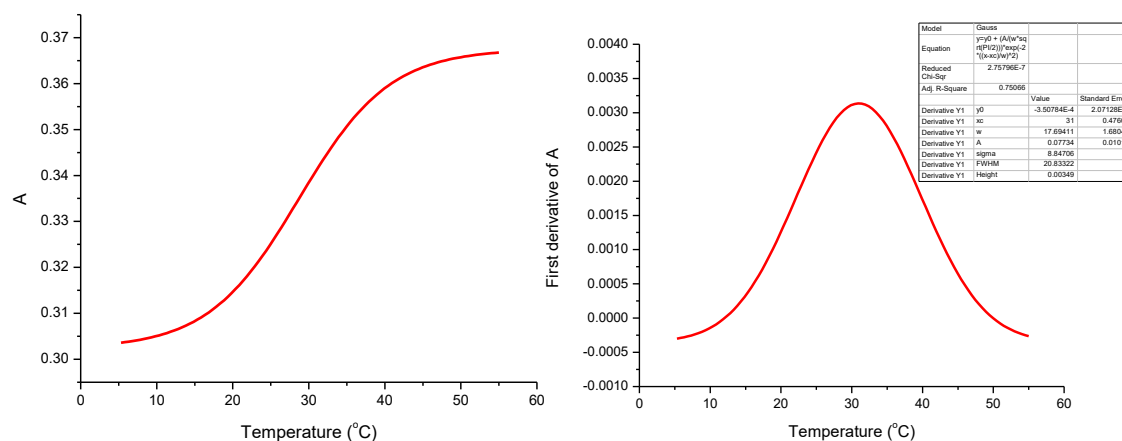


Figure S37: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO5-DNA duplex

5'-TTTTTT^uTTTTTT-3' (PMO5)
3'-AAAAAAAAAAAAA-5'(RNA)

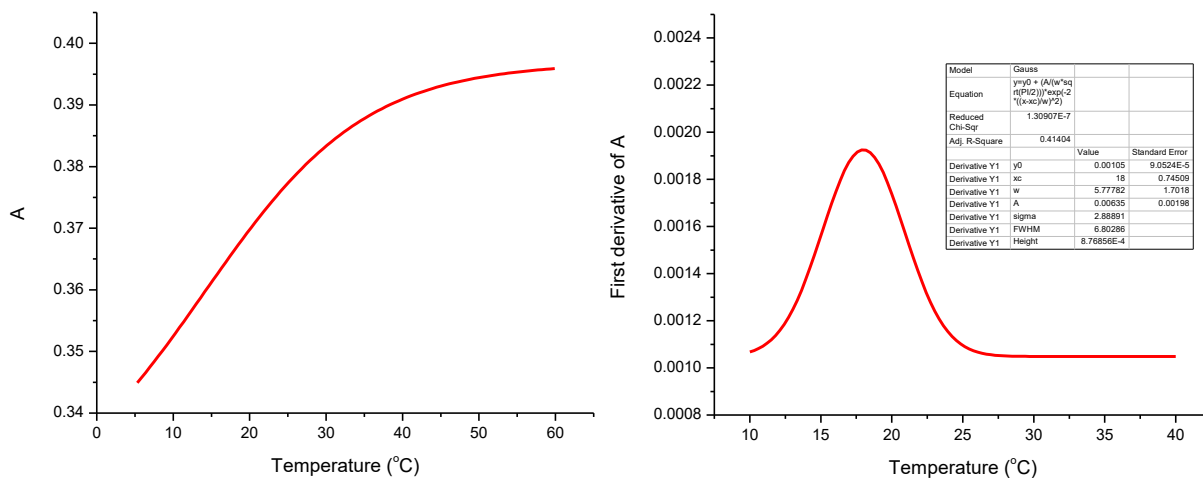


Figure S38: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO5-RNA duplex

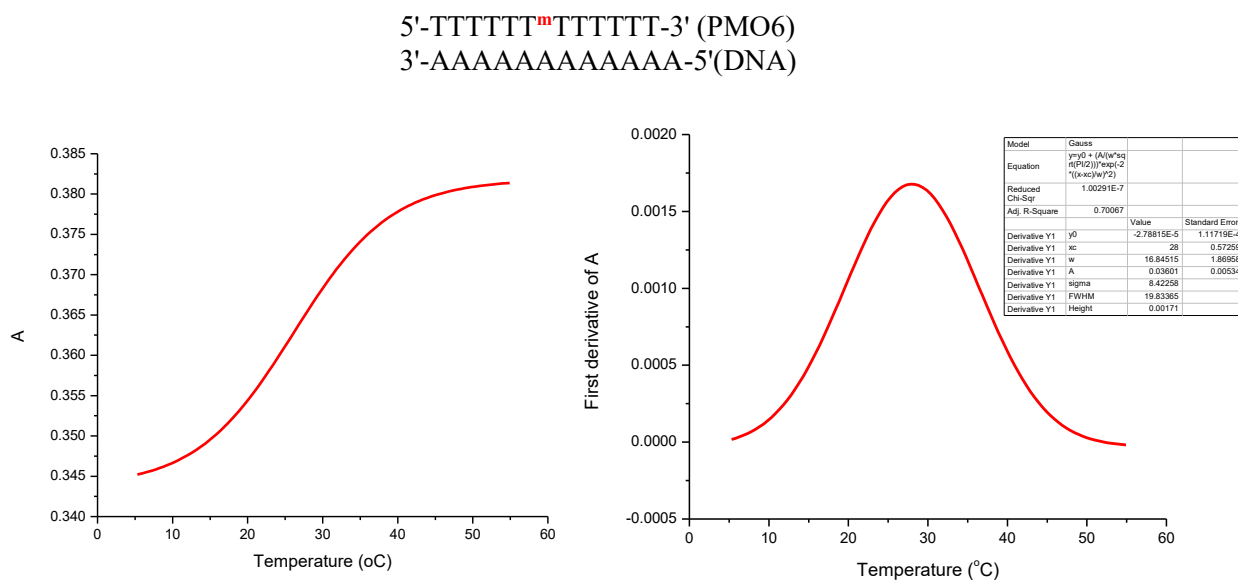


Figure S39: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO6-DNA duplex

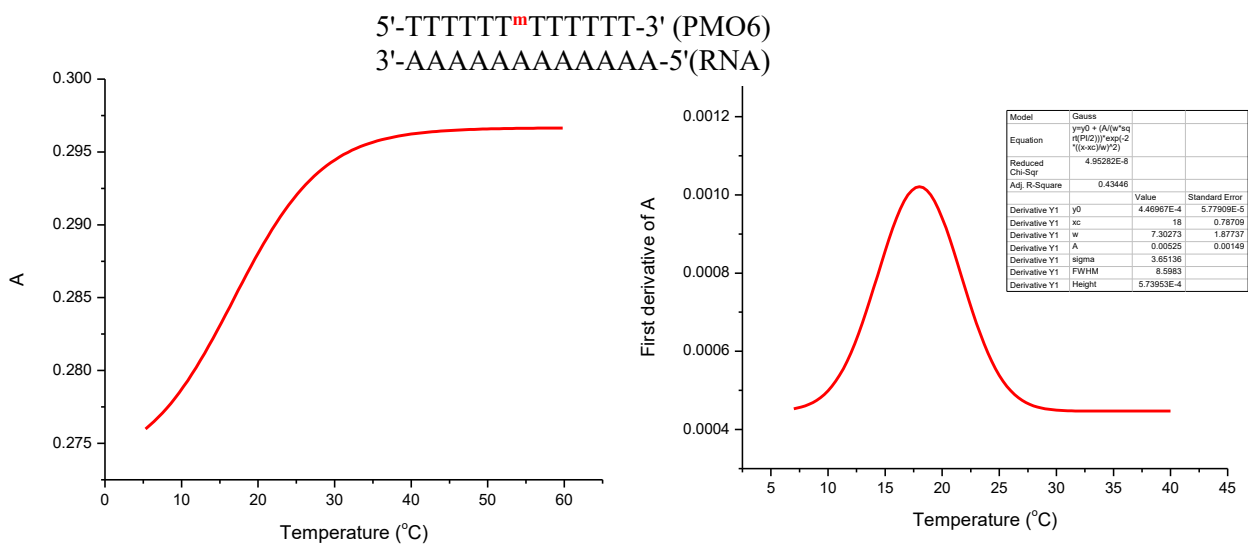


Figure S40: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO6-RNA duplex

5'-TTTTTTT^{*}TTTTTTT-3' (PMO7)
3'-AAAAAAAAAAAAA-5'(DNA)

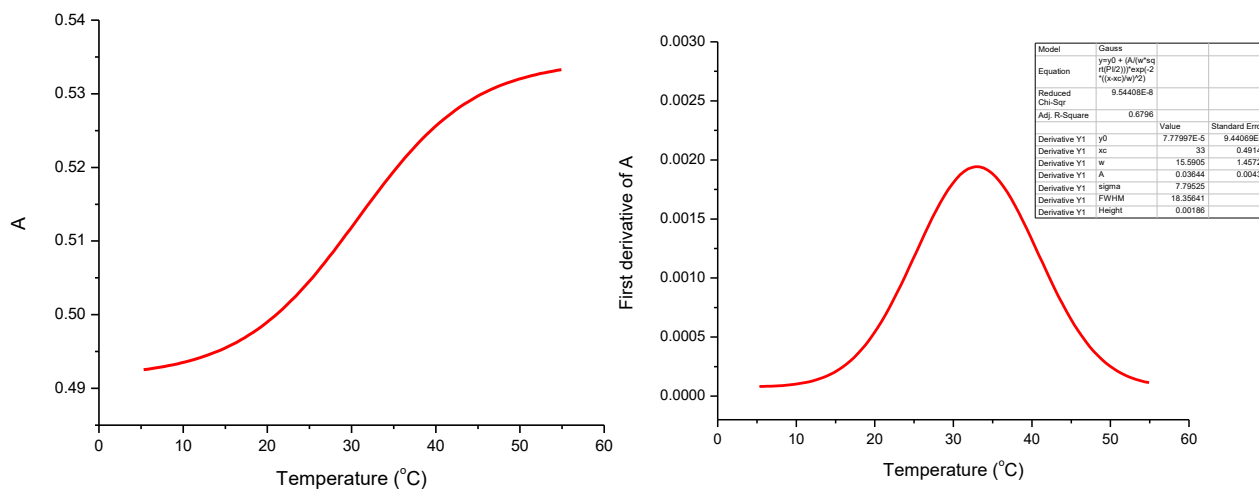


Figure S41: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO7-DNA duplex

5'-TTTTTTT^{*}TTTTTTT-3' (PMO7)
3'-AAAAAAAAAAAAA-5'(RNA)

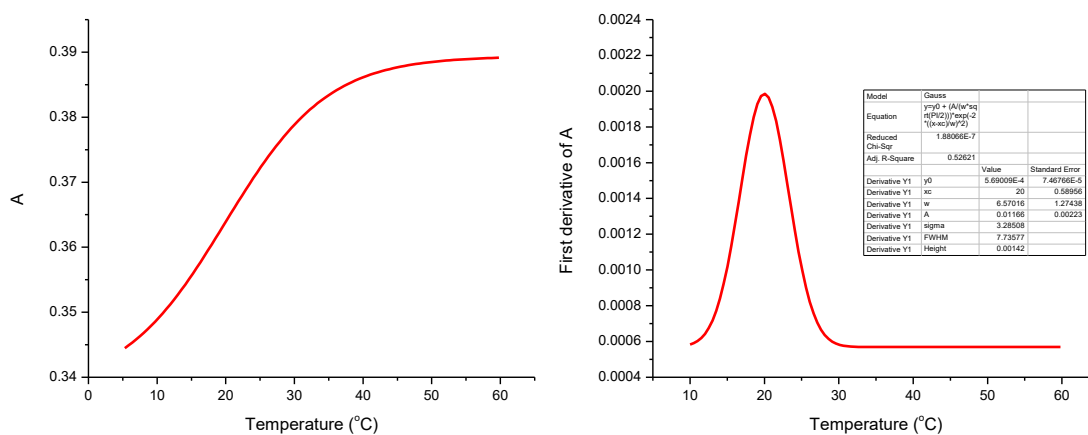


Figure S42: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO7-RNA duplex

5'-TGGAGTTCCTGT-3' (PMO8)
3'-ACCTCAAGGACA-5'(DNA)

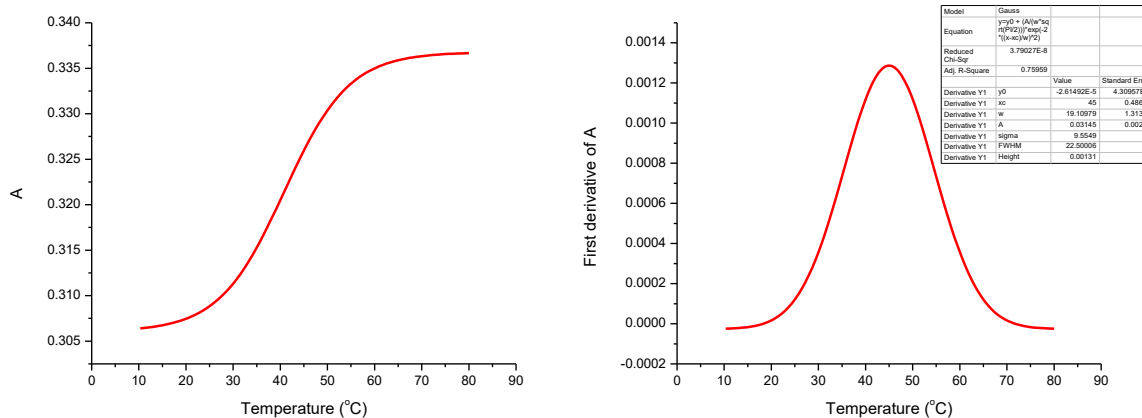


Figure S43: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO8-DNA duplex

5'-TGGAGTTCCTGT-3' (PMO8)
3'-ACCUCAAGGACA-5'(RNA)

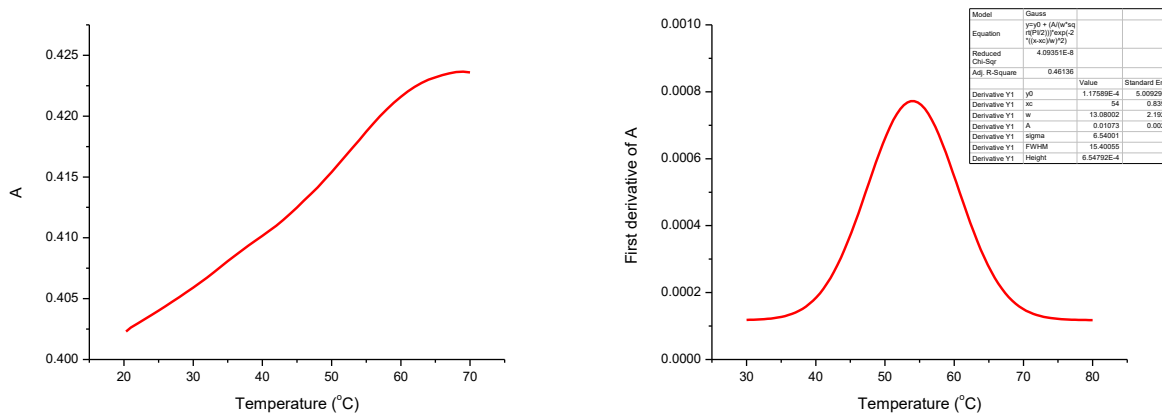


Figure S44: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO8-RNA duplex

5'-TGGAGT^cTCCTGT-3' (PMO9)
3'-ACCTCAAGGACA-5'(DNA)

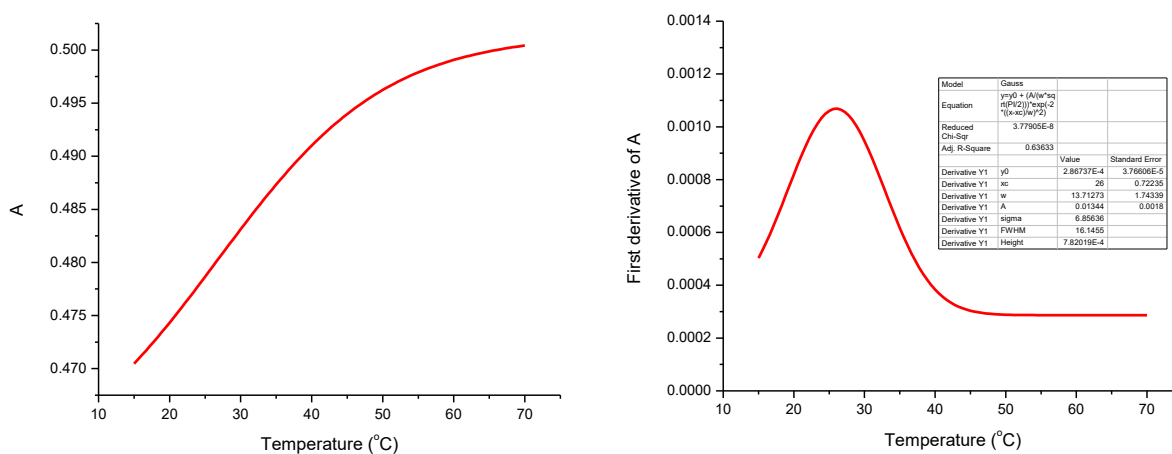


Figure S45: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO9-DNA duplex

5'-TGGAGT^cTCCTGT-3' (PMO9)
3'-ACCUCAAGGACA-5'(RNA)

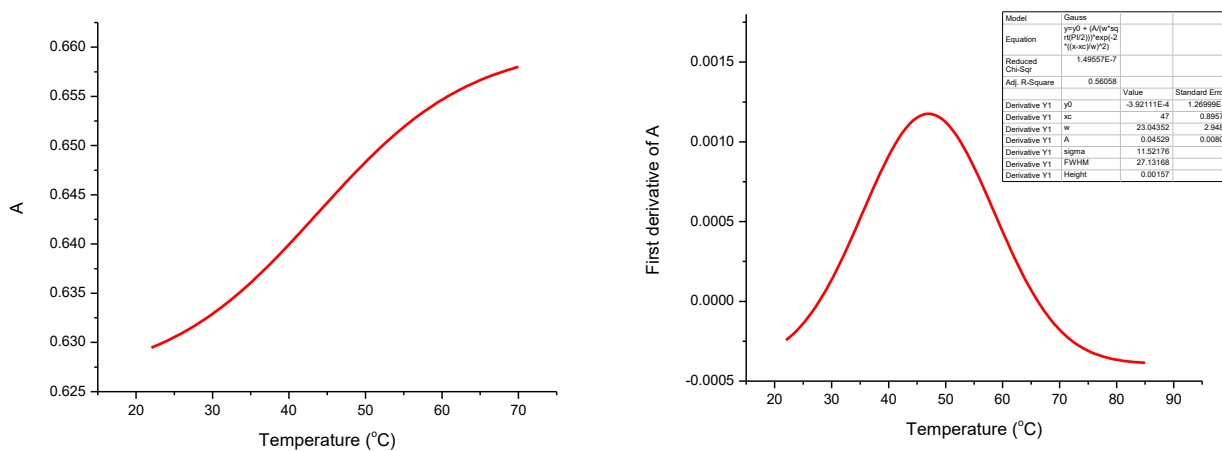


Figure S46: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO9-RNA duplex

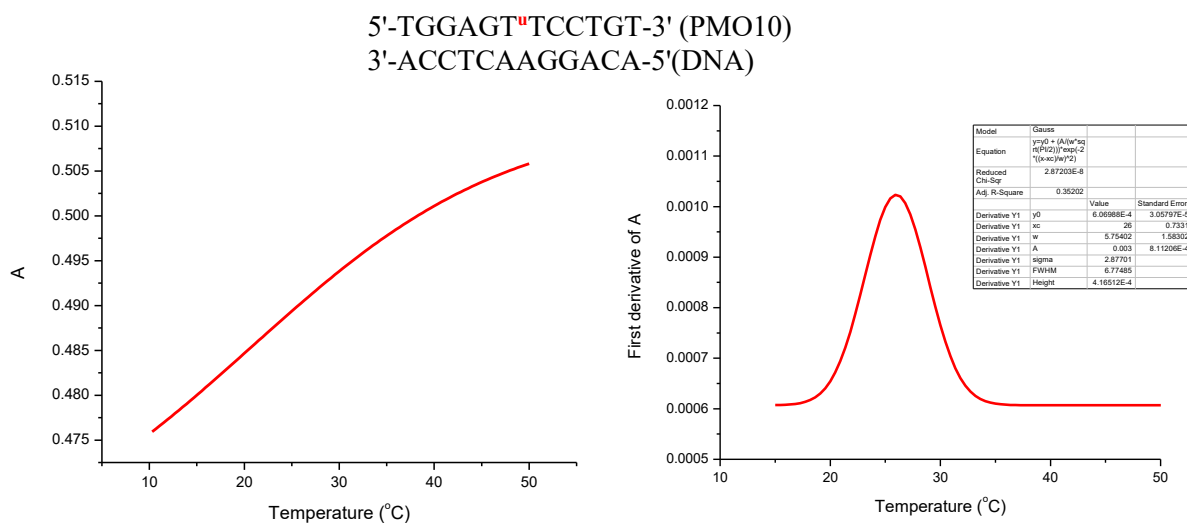


Figure S47: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO10-DNA duplex

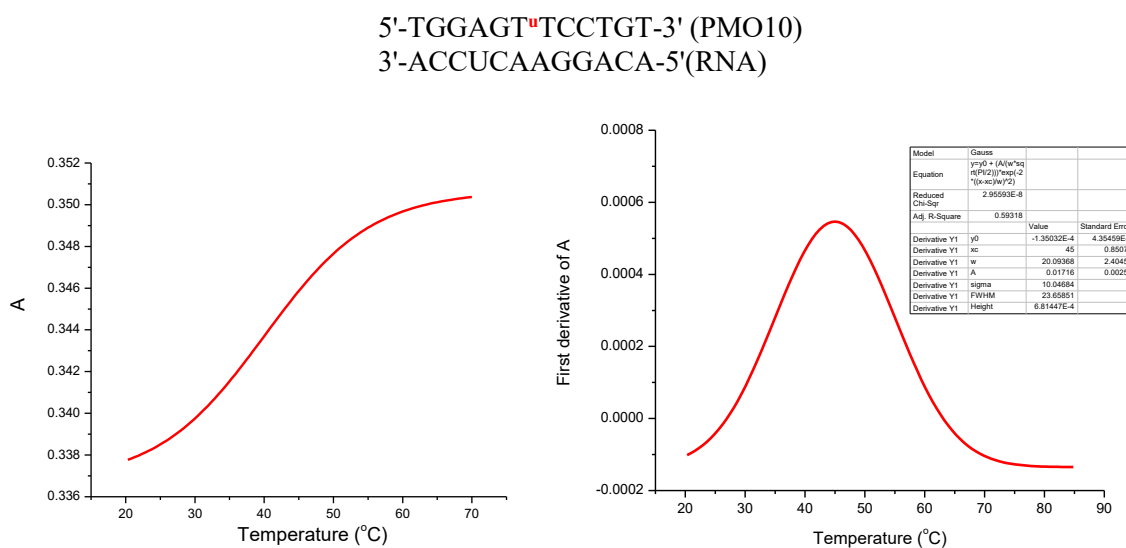


Figure S48: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO10-RNA duplex

5'-TGGAGT^mTCCTGT-3' (PMO11)
3'-ACCTCAAGGACA-5'(DNA)

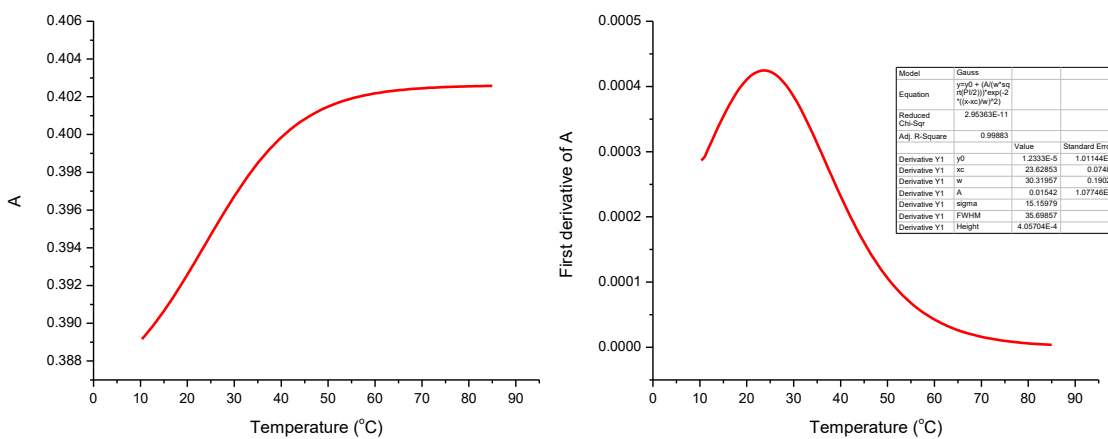


Figure S49: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO11-DNA duplex

5'-TGGAGT^mTCCTGT-3' (PMO11)
3'-ACCUCAAGGACA-5'(RNA)

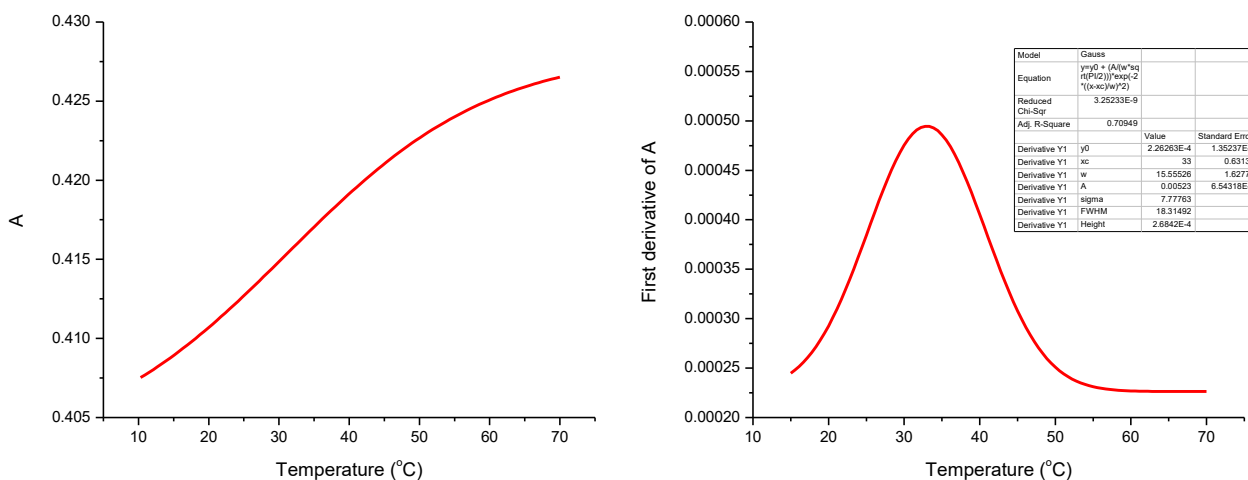


Figure S50: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO11-RNA duplex

5'-TGGAGT^gTCCTGT-3' (PMO12)
3'-ACCTCAAGGACA-5'(DNA)

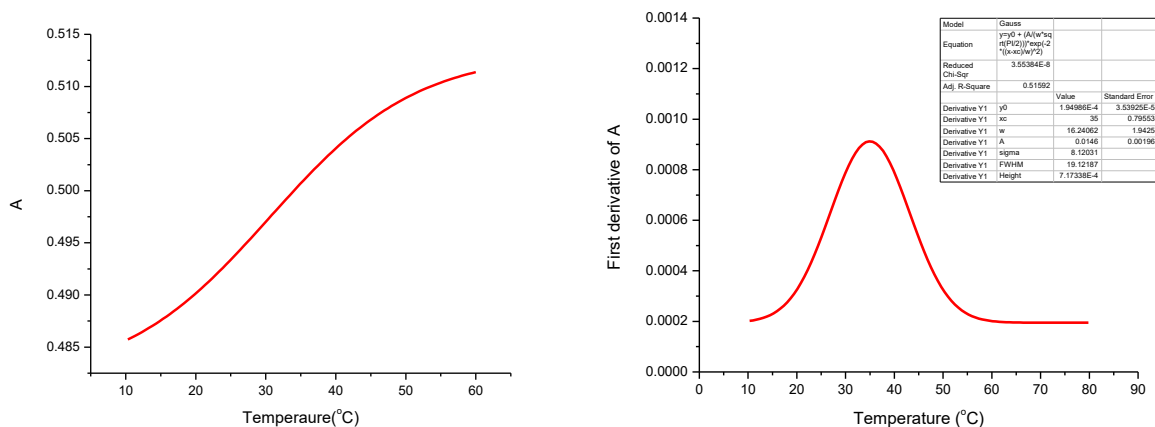


Figure S51: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO12-DNA duplex

5'-TGGAGT^gTCCTGT-3' (PMO12)
3'-ACCUCAAGGACA-5'(RNA)

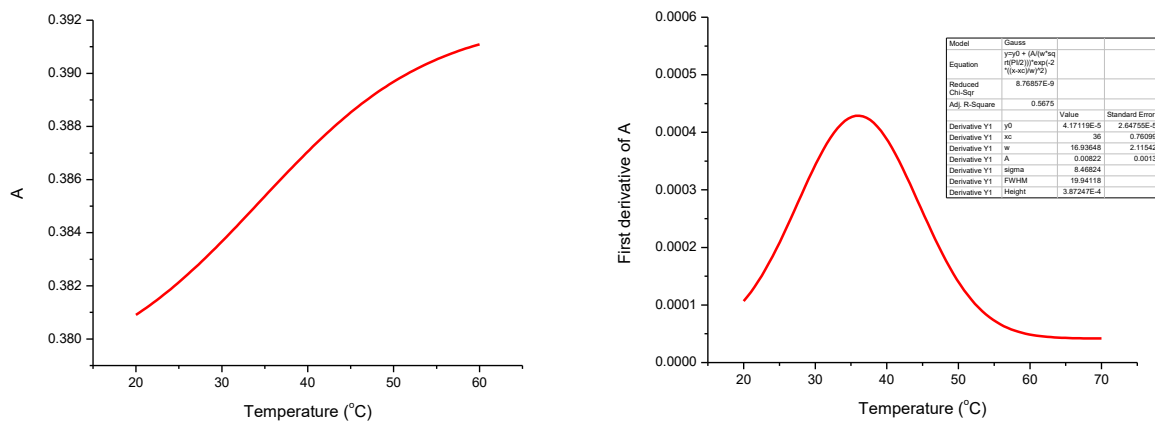


Figure S52: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO12-RNA duplex

14. Thermal melting analysis of modified 12mer PMOs with single mismatched DNA

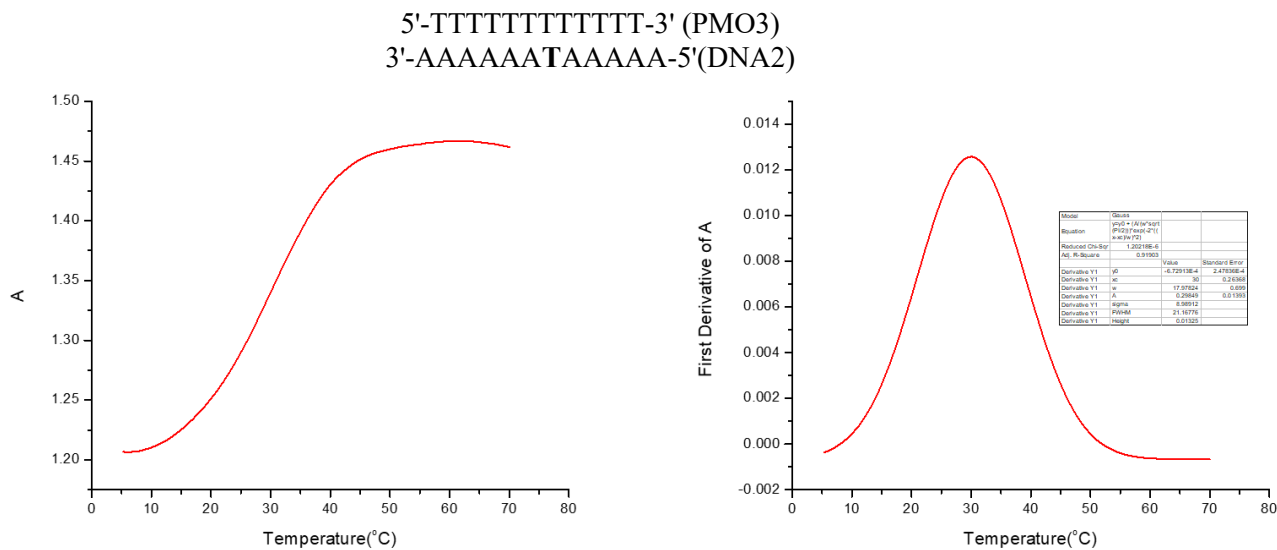


Figure S53: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO3-DNA2 duplex

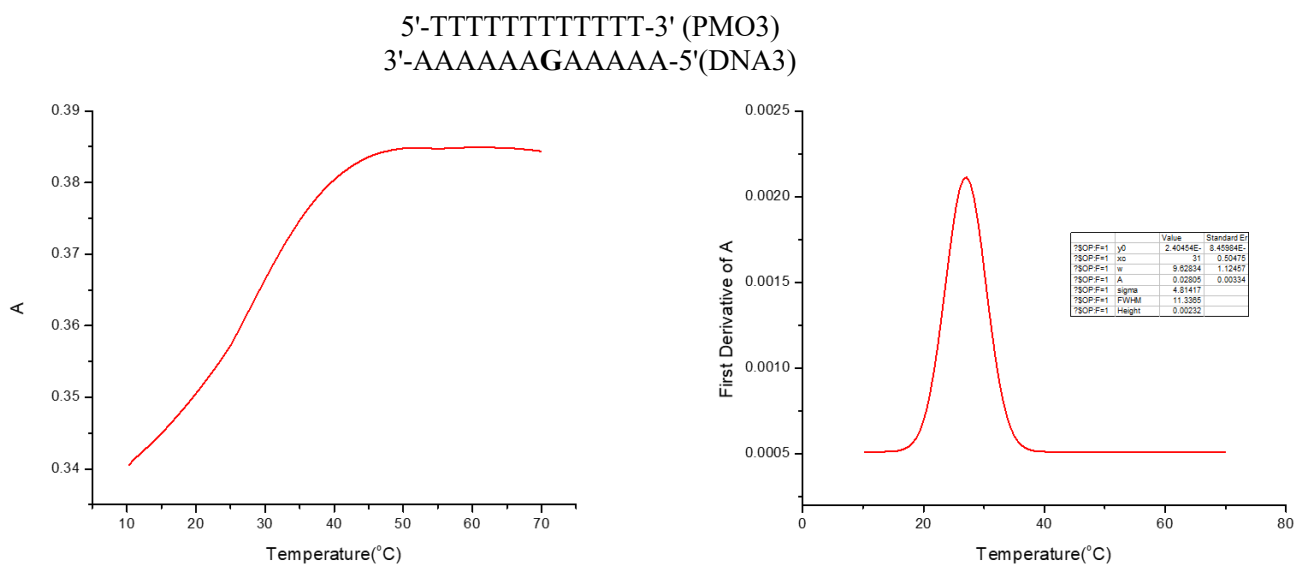


Figure S54: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO3-DNA3 duplex

5'-TTTTTTTTTTTTT-3' (PMO3)
3'-AAAAAACAAAAA-5'(DNA4)

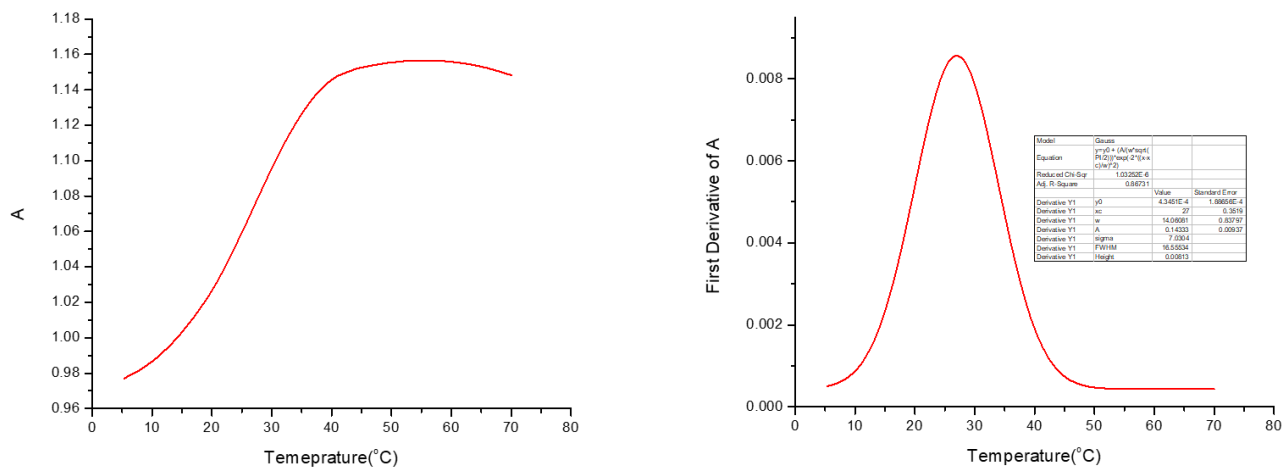


Figure S55: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO3-DNA4 duplex

5'-TTTTTT^cTTTTTT-3' (PMO4)
3'-AAAAAA^TAAAAA-5'(DNA2)

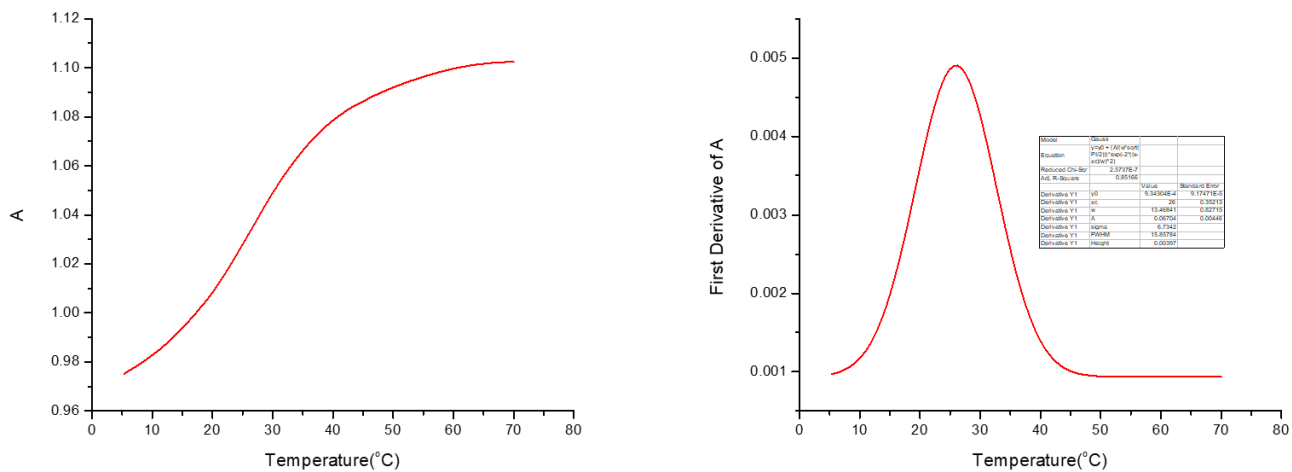


Figure S56: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO4-DNA2 duplex

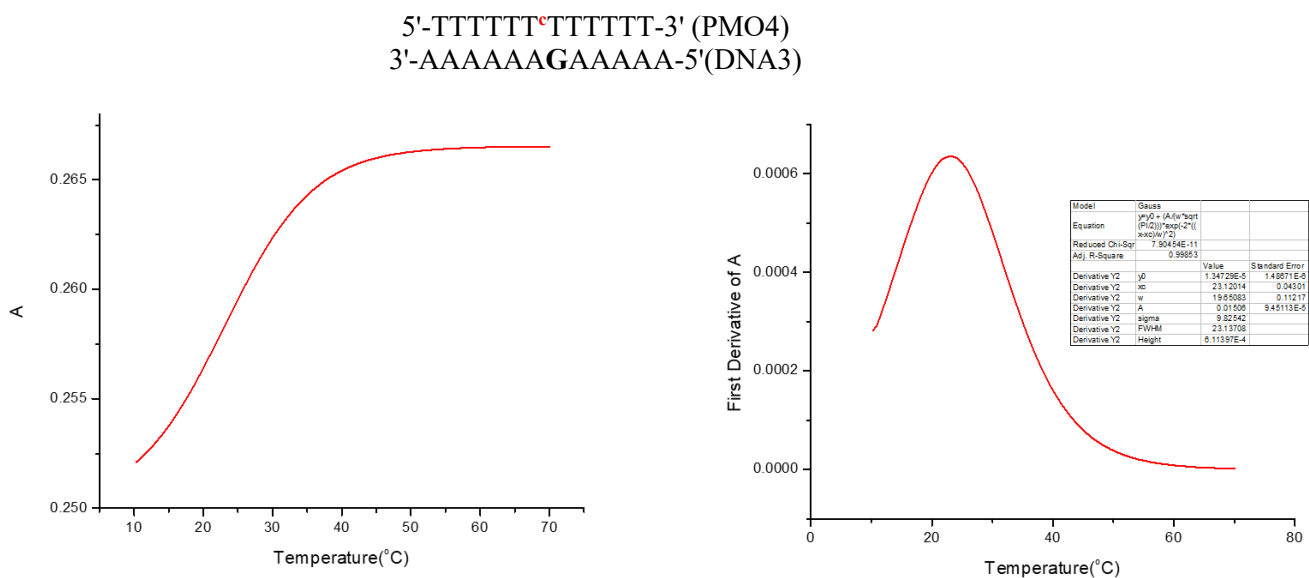


Figure S57: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO4-DNA3 duplex

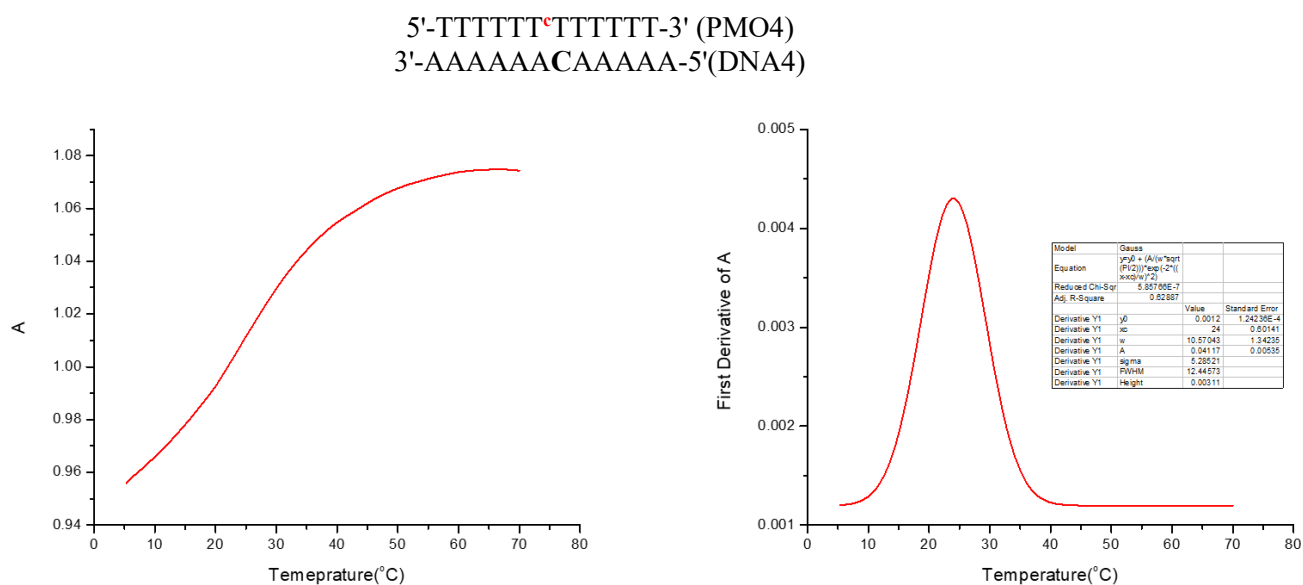


Figure S58: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO4-DNA4 duplex

5'-TTTTTT^uTTTTTT-3' (PMO5)
3'-AAAAAATAAAAA-5'(DNA2)

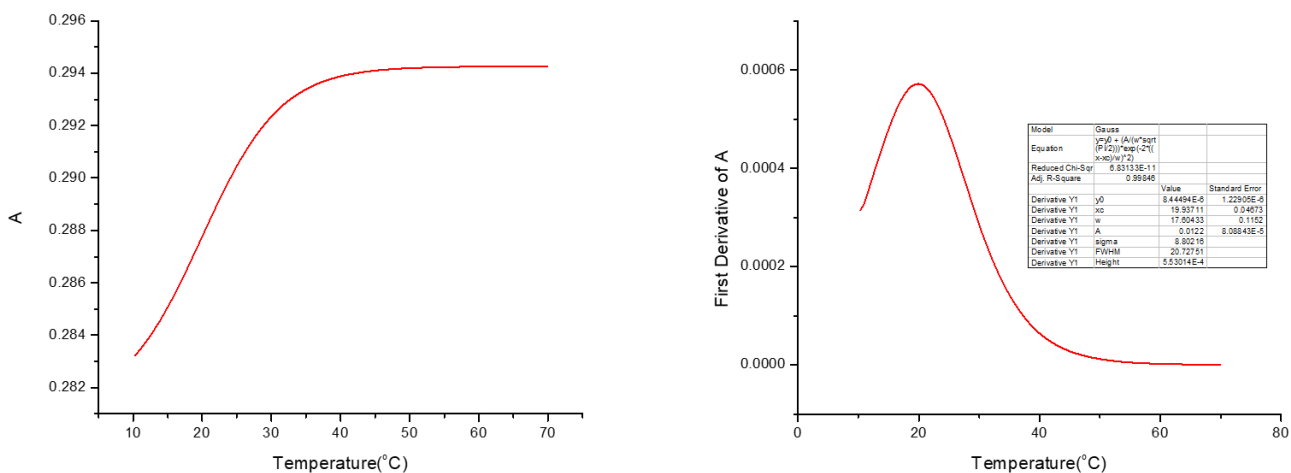


Figure S59: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO5-DNA2 duplex

5'-TTTTTT^uTTTTTT-3' (PMO5)
3'-AAAAAAGAAAAA-5'(DNA3)

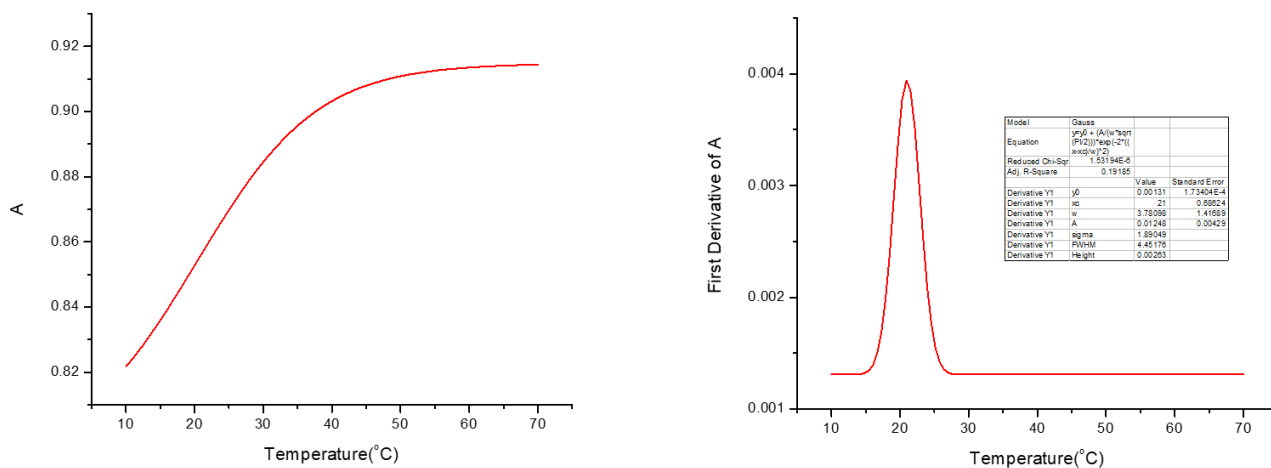


Figure S60: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO5-DNA3 duplex

5'-TTTTTT^uTTTTTT-3' (PMO5)
3'-AAAAAACAAAAA-5'(DNA4)

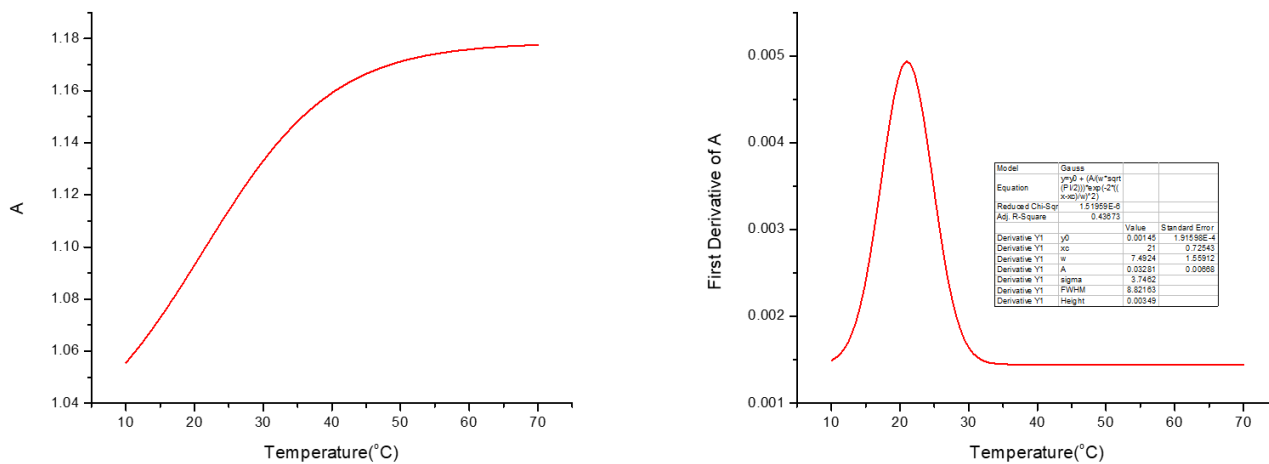


Figure S61: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO5-DNA4 duplex

5'-TTTTTT^mTTTTTT-3' (PMO6)
3'-AAAAAATAAAAA-5'(DNA2)

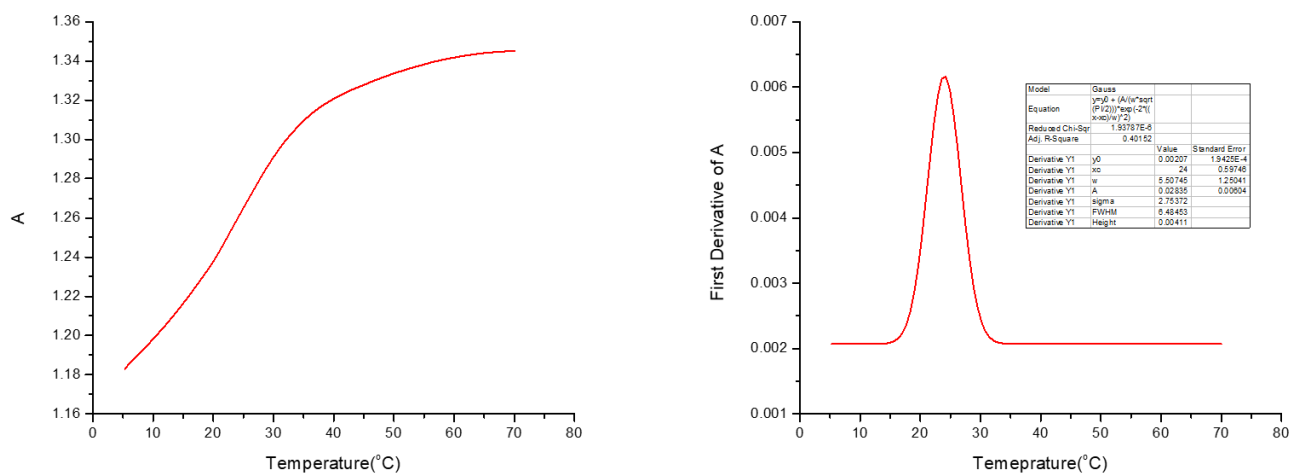


Figure S62: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO6-DNA2 duplex

5'-TTTTTT^mTTTTTT-3' (PMO6)
3'-AAAAAAGAAAAA-5'(DNA3)

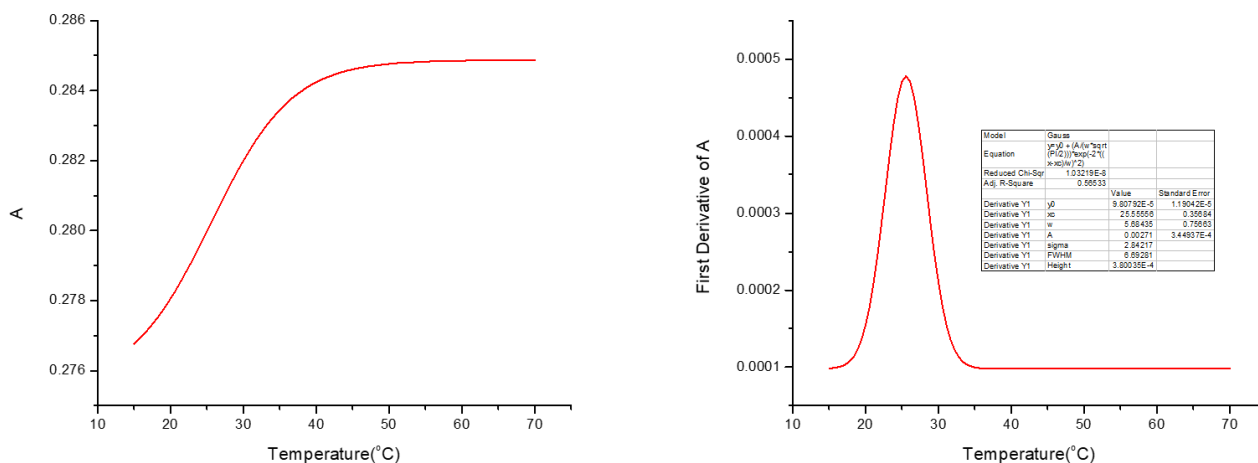


Figure S63: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO6-DNA3 duplex

5'-TTTTTT^mTTTTTT-3' (PMO6)
3'-AAAAAACAAAAA-5'(DNA4)

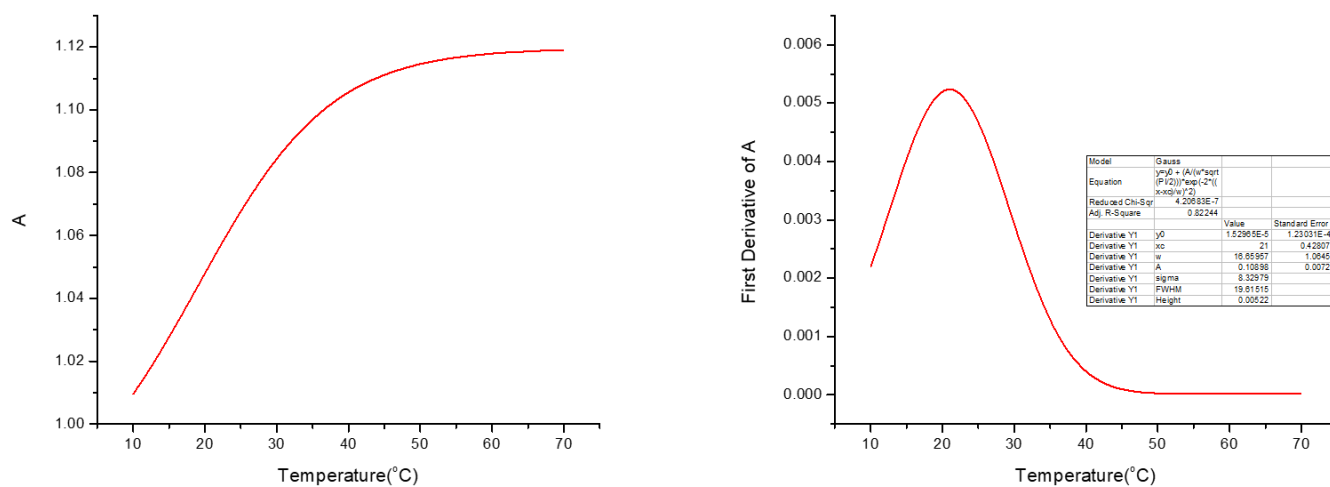


Figure S64: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO6-DNA4 duplex

5'-TTTTTT^gTTTTTT-3' (PMO7)
3'-AAAAAATAAAAA-5' (DNA2)

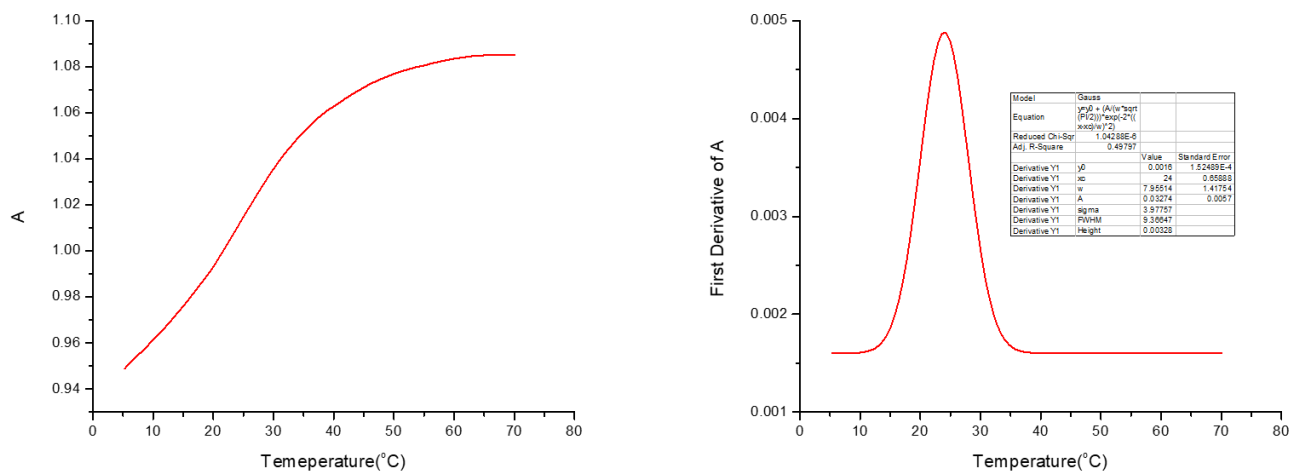


Figure S65: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO7-DNA2 duplex

5'-TTTTTT^gTTTTTT-3' (PMO7)
3'-AAAAAAGAAAAA-5' (DNA3)

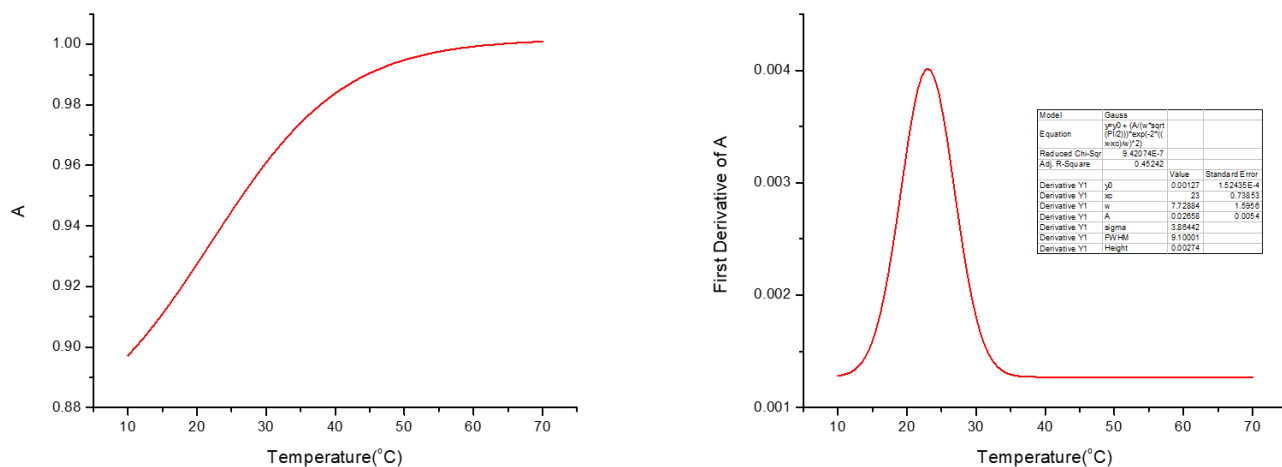


Figure S66: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO7-DNA3 duplex

5'-TTTTTTT^gTTTTTTT-3' (PMO7)
3'-AAAAAACAAAAA-5'(DNA4)

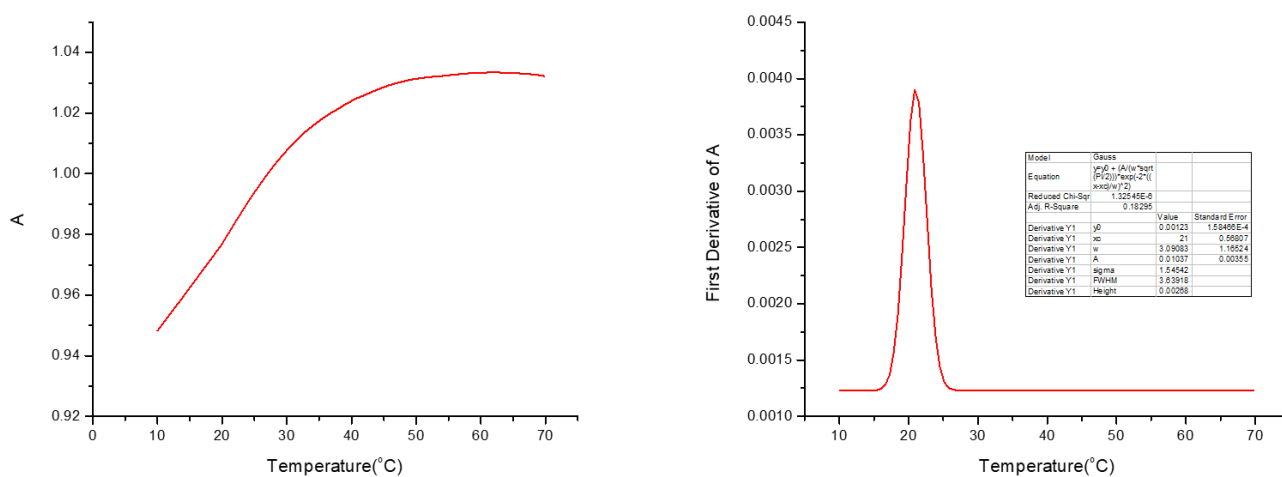


Figure S67: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO7-DNA4 duplex

5'-TGGAGTTCCTGT-3' (PMO8)
3'-ACCTCATGGACA-5'(DNA6)

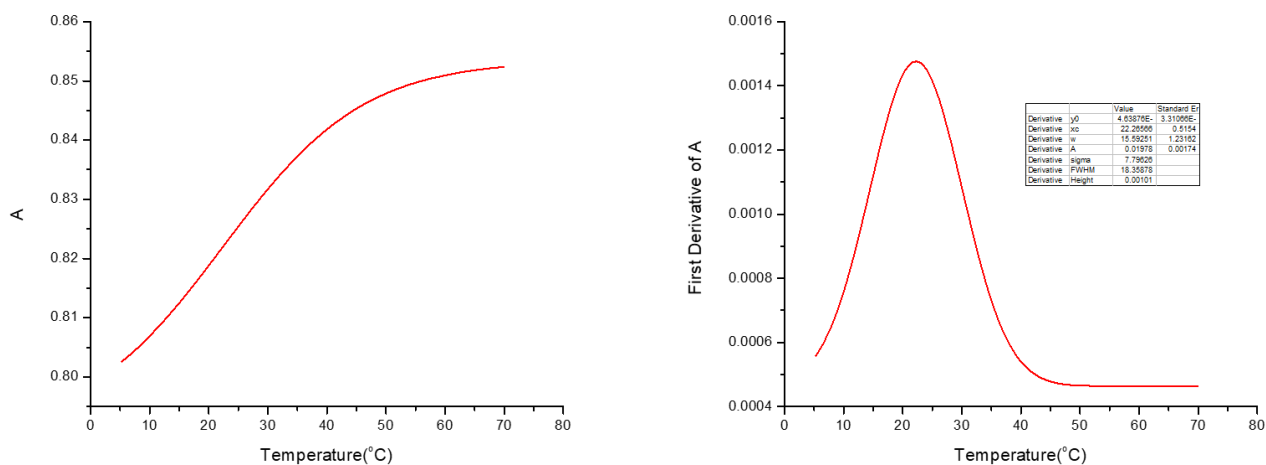


Figure S68: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO8-DNA6 duplex

5'-TGGAGTTCCTGT-3' (PMO8)
3'-ACCTCAGGGACA-5'(DNA7)

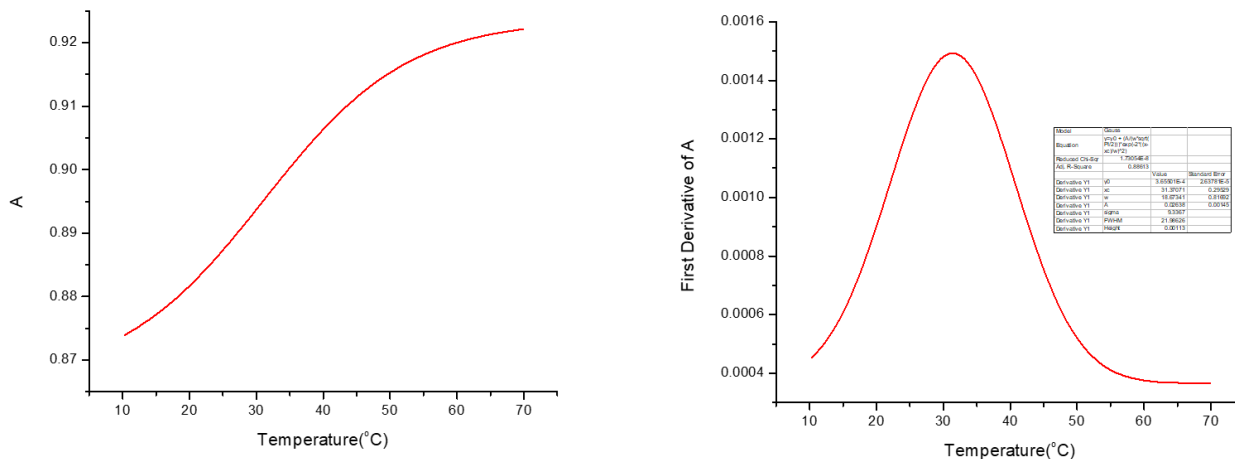


Figure S69: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO8-DNA7 duplex

5'-TGGAGTTCCTGT-3' (PMO8)
3'-ACCTCACGGACA-5'(DNA8)

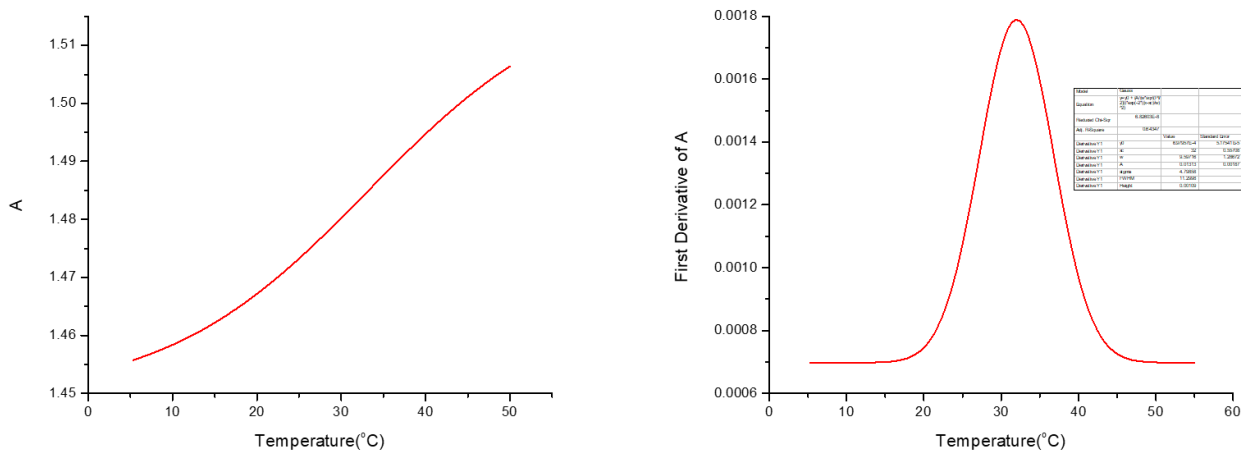


Figure S70: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO8-DNA8 duplex

5'-TGGAGT^oTCCTGT-3' (PMO9)
3'-ACCTCATGGACA-5'(DNA6)

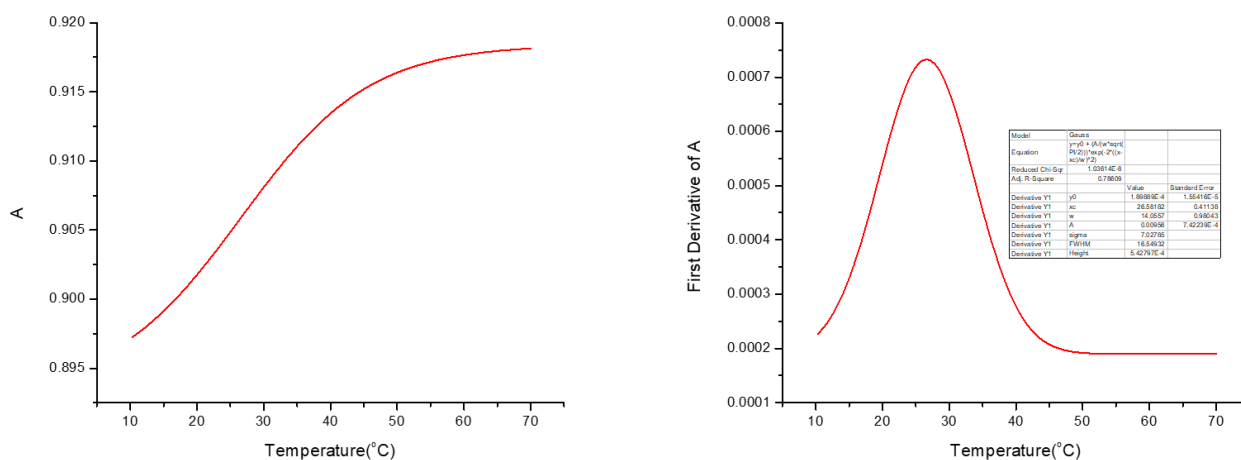


Figure S71: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO9-DNA6 duplex

5'-TGGAGT^oTCCTGT-3' (PMO9)
3'-ACCTCAGGGACA-5'(DNA7)

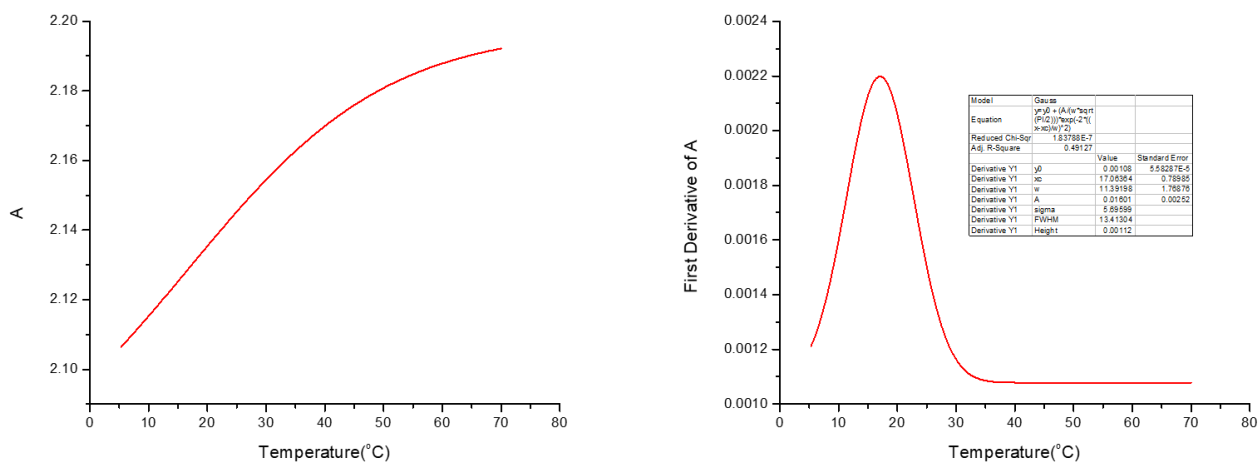


Figure S72: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO9-DNA7 duplex

5'-TGGAGT^cTCCTGT-3' (PMO9)
3'-ACCTCACGGACA-5'(DNA8)

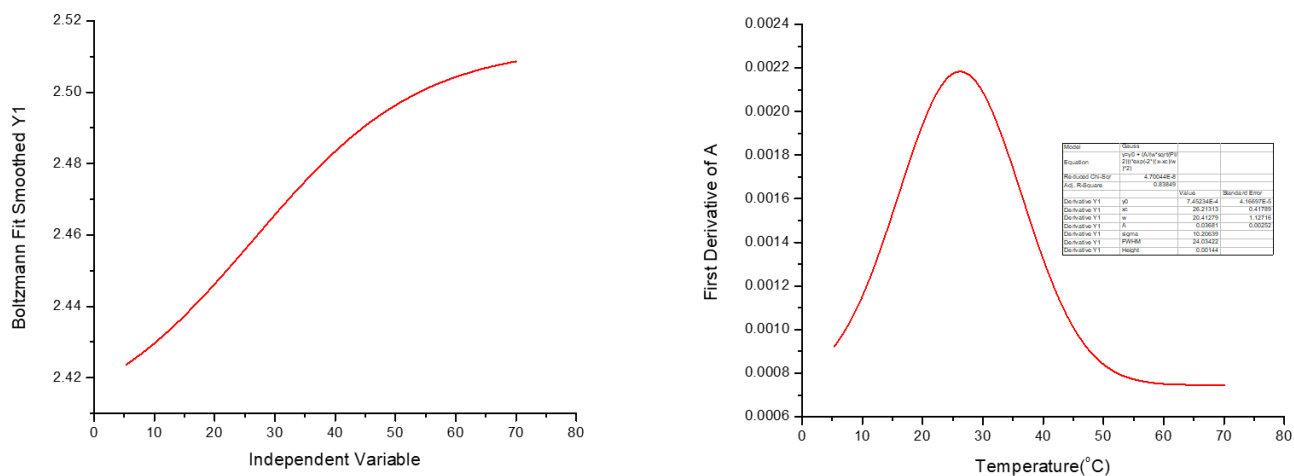


Figure S73: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO9-DNA8 duplex

5'-TGGAGT^uTCCTGT-3' (PMO10)
3'-ACCTCATGGACA-5'(DNA6)

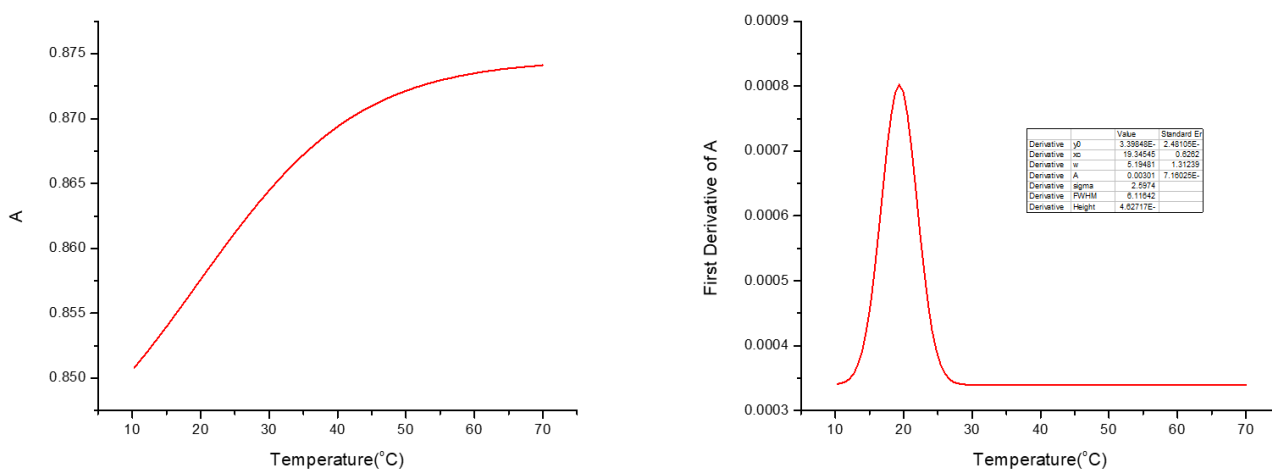


Figure S74: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO10-DNA6 duplex

5'-TGGAGT^mTCCTGT-3' (PMO10)
3'-ACCTCAGGGACA-5'(DNA7)

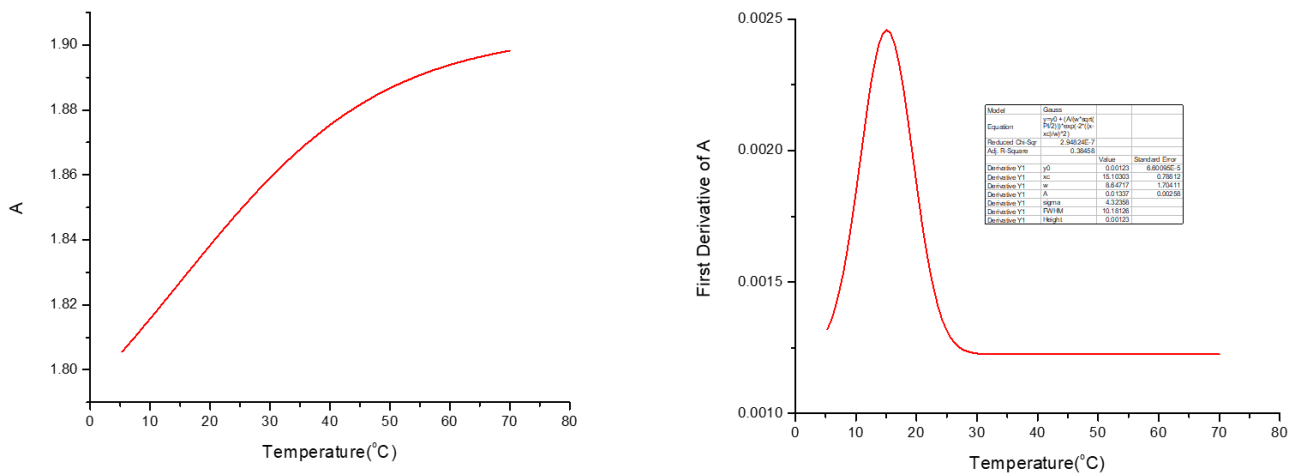


Figure S75: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO10-DNA7 duplex

5'-TGGAGT^mTCCTGT-3' (PMO10)
3'-ACCTCACGGACA-5'(DNA8)

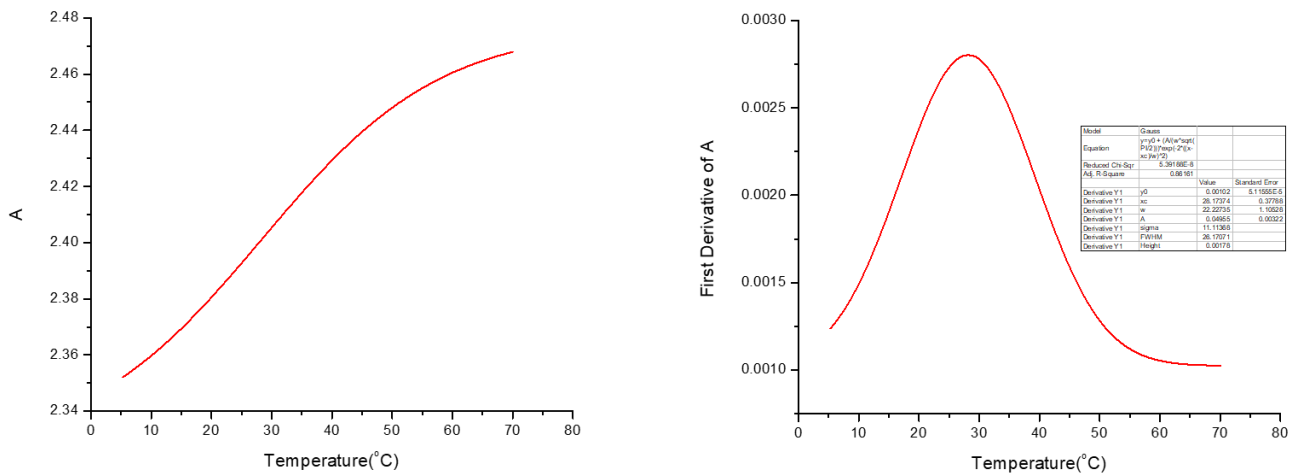


Figure S76: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO10-DNA8 duplex

5'-TGGAGT^mTCCTGT-3' (PMO11)
3'-ACCTCATGGACA-5'(DNA6)

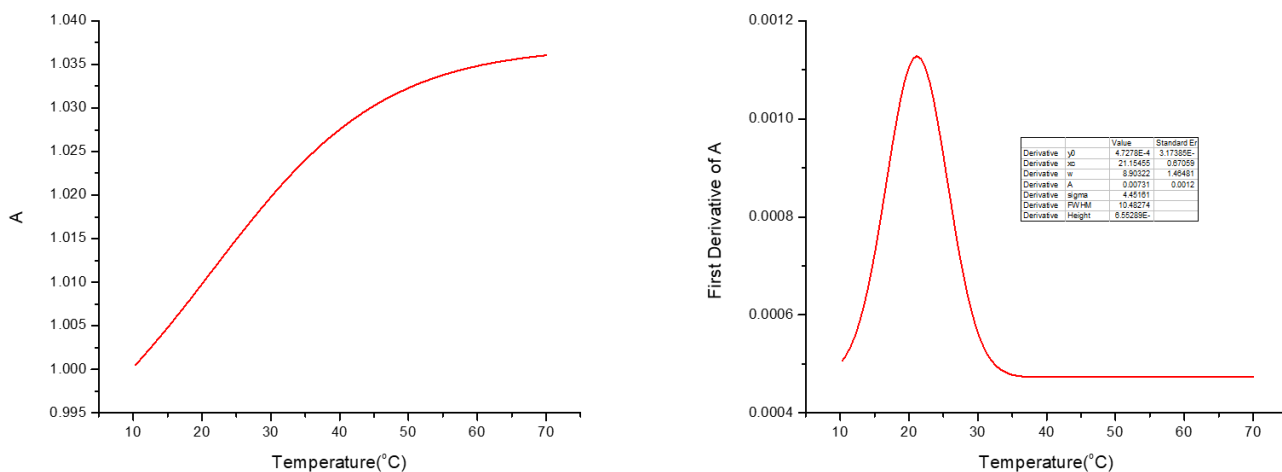


Figure S77: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO11-DNA6 duplex

5'-TGGAGT^mTCCTGT-3' (PMO11)
3'-ACCTCAGGGACA-5'(DNA7)

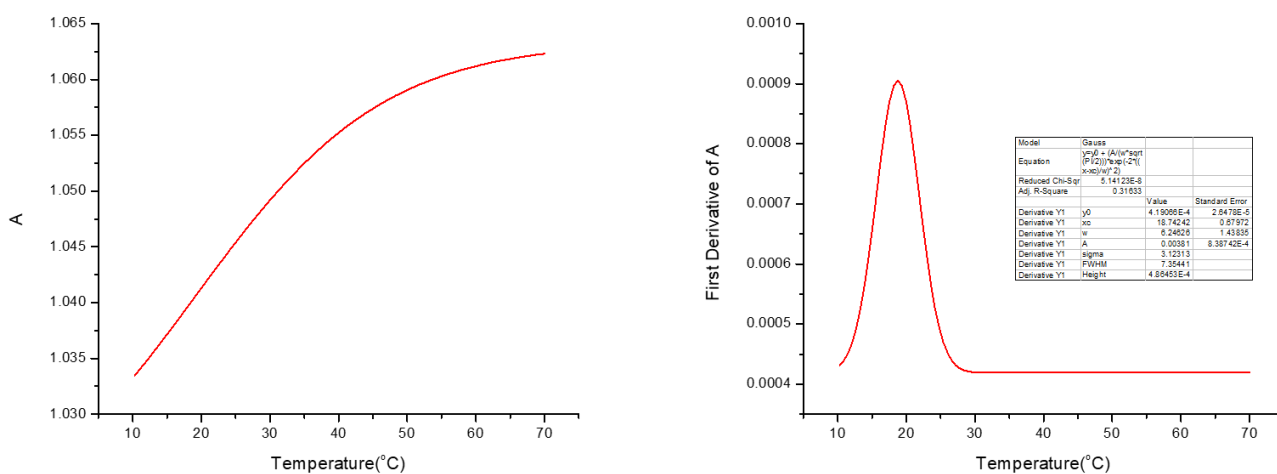


Figure S78: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO11-DNA7 duplex

5'-TGGAGT^mTCCTGT-3' (PMO11)
3'-ACCTCACGGACA-5'(DNA8)

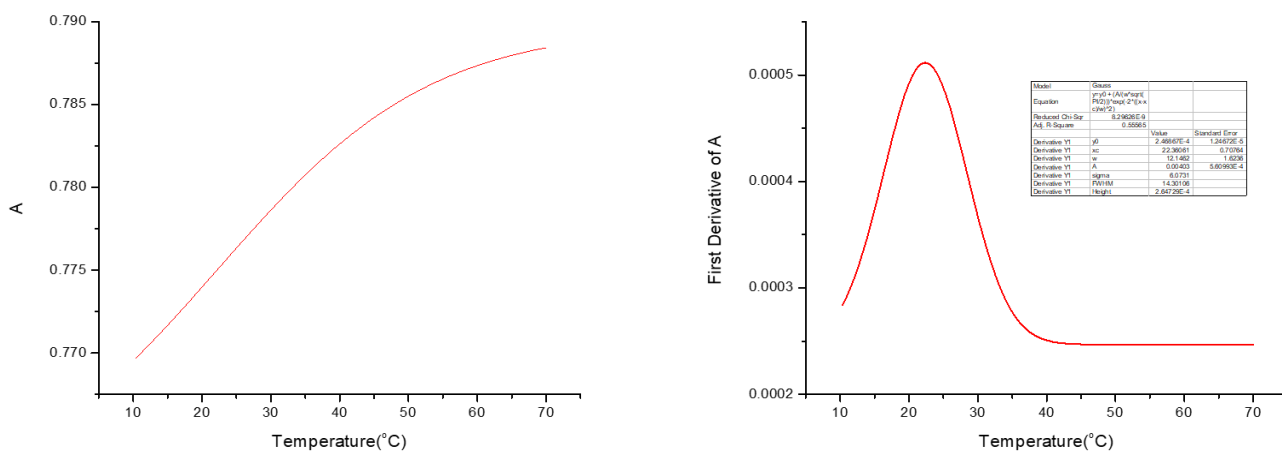


Figure S79: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO11-DNA8 duplex

5'-TGGAGT^gTCCTGT-3' (PMO12)
3'-ACCTCATGGACA-5'(DNA6)

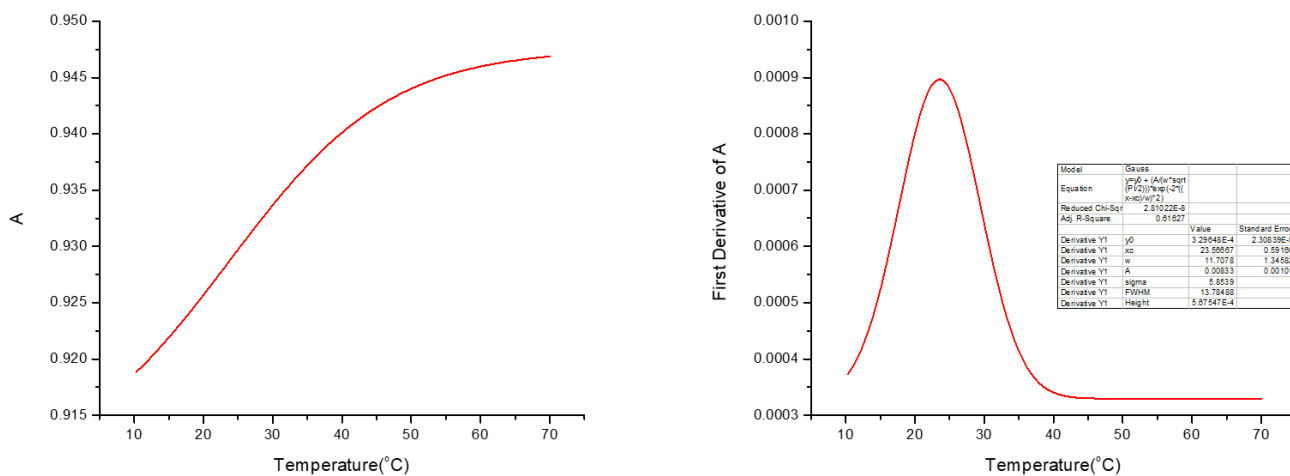


Figure S80: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO12-DNA6 duplex

5'-TGGAGT^gTCCTGT-3' (PMO12)
3'-ACCTCAGGGACA-5'(DNA7)

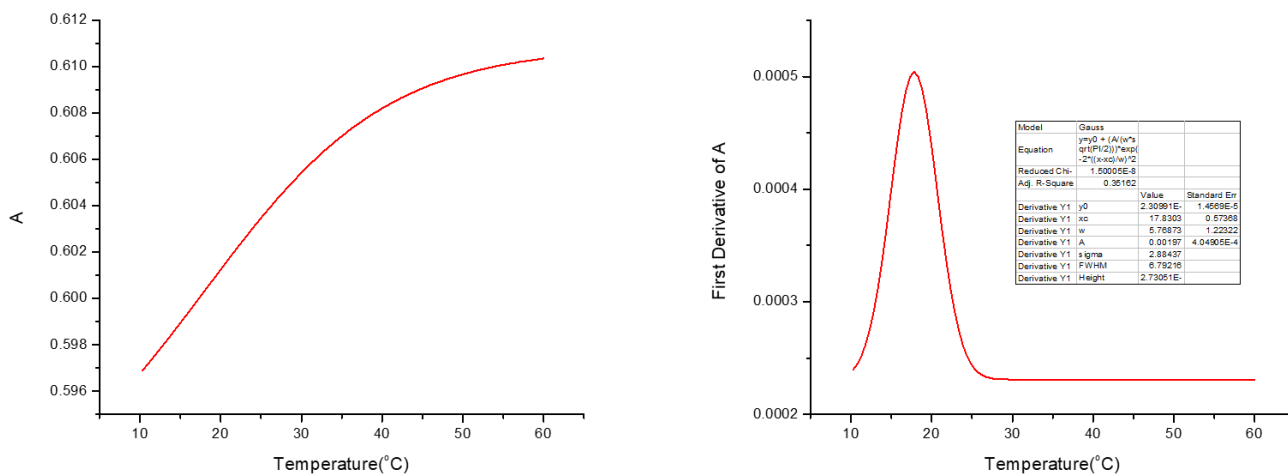


Figure S81: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO12-DNA7 duplex

5'-TGGAGT^gTCCTGT-3' (PMO12)
3'-ACCTCACGGACA-5'(DNA8)

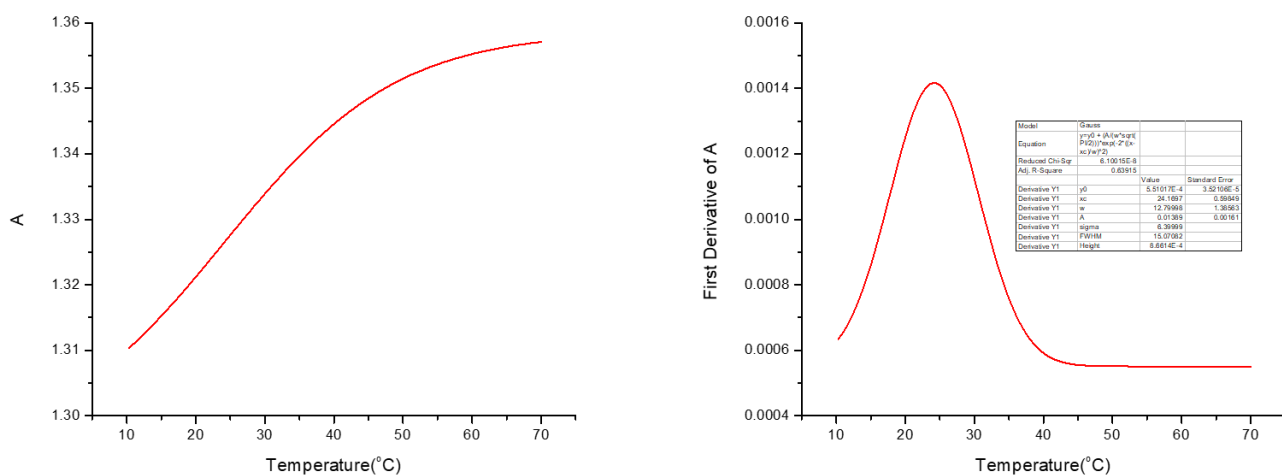


Figure S82: Thermal melting plot of Absorption vs temperature and first order derivative of A vs Temperature of PMO12-DNA8 duplex

15. Table for thermal melting data of modified PMOs with single mismatched DNA

Table S2: Thermal melting data of modified PMOs with single mismatch DNA^a

Sl. No	PMO sequence with target DNA	Tm (ΔTm) in °C	Sl. No	PMO sequence with target DNA	Tm (ΔTm) in °C
1	5'-TTTTTTTTTTTT-3' (PMO3) 3'-AAAAAAAAAAAAA-5'(DNA1)	41	21	5'-TGGAGTTCCTGT-3' (PMO8) 3'-ACCTCAAGGACA-5'(DNA5)	45
2	5'-TTTTTTTTTTTT-3' (PMO3) 3'-AAAAAATAAAAA-5'(DNA2)	30 (-11)	22	5'-TGGAGTTCCTGT-3' (PMO8) 3'-ACCTCATGGACA-5'(DNA6)	22 (-23)
3	5'-TTTTTTTTTTTT-3' (PMO3) 3'-AAAAAAGAAAAA-5'(DNA3)	31 (-10)	23	5'-TGGAGTTCCTGT-3' (PMO8) 3'-ACCTCAGGGACA-5'(DNA7)	31 (-14)
4	5'-TTTTTTTTTTTT-3' (PMO3) 3'-AAAAAACAAAAA-5'(DNA4)	27 (-14)	24	5'-TGGAGTTCCTGT-3' (PMO8) 3'-ACCTCACGGACA-5'(DNA8)	32 (-13)
5	5'-TTTTTT ^c TTTTTT-3' (PMO4) 3'-AAAAAAAAAAAAA-5'(DNA1)	30	25	5'-TGGAGT ^c TCCTGT-3' (PMO9) 3'-ACCTCAAGGACA-5'(DNA5)	28
6	5'-TTTTTT ^c TTTTTT-3' (PMO4) 3'-AAAAAATAAAAA-5'(DNA2)	26 (-4)	26	5'-TGGAGT ^c TCCTGT-3' (PMO9) 3'-ACCTCATGGACA-5'(DNA6)	27 (-1)
7	5'-TTTTTT ^c TTTTTT-3' (PMO4) 3'-AAAAAAGAAAAA-5'(DNA3)	23 (-7)	27	5'-TGGAGT ^c TCCTGT-3' (PMO9) 3'-ACCTCAGGGACA-5'(DNA7)	17 (-11)
8	5'-TTTTTT ^c TTTTTT-3' (PMO4) 3'-AAAAAACAAAAA-5'(DNA4)	24 (-6)	28	5'-TGGAGT ^c TCCTGT-3' (PMO9) 3'-ACCTCACGGACA-5'(DNA8)	26 (-2)
9	5'-TTTTTT ^u TTTTTT-3' (PMO5) 3'-AAAAAAAAAAAAA-5'(DNA1)	31	29	5'-TGGAGT ^u TCCTGT-3' (PMO10) 3'-ACCTCAAGGACA-5'(DNA5)	26
10	5'-TTTTTT ^u TTTTTT-3' (PMO5) 3'-AAAAAATAAAAA-5'(DNA2)	20 (-11)	30	5'-TGGAGT ^u TCCTGT-3' (PMO10) 3'-ACCTCATGGACA-5'(DNA6)	19 (-7)
11	5'-TTTTTT ^u TTTTTT-3' (PMO5) 3'-AAAAAAGAAAAA-5'(DNA3)	21 (-10)	31	5'-TGGAGT ^u TCCTGT-3' (PMO10) 3'-ACCTCAGGGACA-5'(DNA7)	15 (-11)
12	5'-TTTTTT ^u TTTTTT-3' (PMO5) 3'-AAAAAACAAAAA-5'(DNA4)	21 (-10)	32	5'-TGGAGT ^u TCCTGT-3' (PMO10) 3'-ACCTCACGGACA-5'(DNA8)	28 (+2)
13	5'-TTTTTT ^m TTTTTT-3' (PMO6) 3'-AAAAAAAAAAAAA-5'(DNA1)	28	33	5'-TGGAGT ^m TCCTGT-3' (PMO11) 3'-ACCTCAAGGACA-5'(DNA5)	23
14	5'-TTTTTT ^m TTTTTT-3' (PMO6) 3'-AAAAAATAAAAA-5'(DNA2)	24 (-4)	34	5'-TGGAGT ^m TCCTGT-3' (PMO11) 3'-ACCTCATGGACA-5'(DNA6)	21 (-2)
15	5'-TTTTTT ^m TTTTTT-3' (PMO6) 3'-AAAAAAGAAAAA-5'(DNA3)	26 (-2)	35	5'-TGGAGT ^m TCCTGT-3' (PMO11) 3'-ACCTCAGGGACA-5'(DNA7)	19 (-4)
16	5'-TTTTTT ^m TTTTTT-3' (PMO6) 3'-AAAAAACAAAAA-5'(DNA4)	21 (-7)	36	5'-TGGAGT ^m TCCTGT-3' (PMO11) 3'-ACCTCACGGACA-5'(DNA8)	22 (-1)
17	5'-TTTTTT ^g TTTTTT-3' (PMO7) 3'-AAAAAAAAAAAAA-5'(DNA1)	33	37	5'-TGGAGT ^g TCCTGT-3' (PMO12) 3'-ACCTCAAGGACA-5'(DNA5)	35
18	5'-TTTTTT ^g TTTTTT-3' (PMO7) 3'-AAAAAATAAAAA-5'(DNA2)	24 (-9)	38	5'-TGGAGT ^g TCCTGT-3' (PMO12) 3'-ACCTCATGGACA-5'(DNA6)	24 (-11)
19	5'-TTTTTT ^g TTTTTT-3' (PMO7) 3'-AAAAAAGAAAAA-5'(DNA3)	23 (-10)	39	5'-TGGAGT ^g TCCTGT-3' (PMO12) 3'-ACCTCAGGGACA-5'(DNA7)	18 (-17)

20	5'-TTTTTT T TTTTT-3' (PMO7) 3'-AAAAAACAAAAA-5'(DNA4)	21 (-12)	40	5'-TGGAGT T TCCTGT-3' (PMO12) 3'-ACCTCACGGACA-5'(DNA8)	24 (-11)
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^aThermal denaturation study was performed in 40mM phosphate buffer containing 100mM NaCl. For the Entries 21-40, the oligo concentration was 3 μ M. The bold letters indicate the mismatched nucleobase.

16. Circular dichroism spectra of duplexes

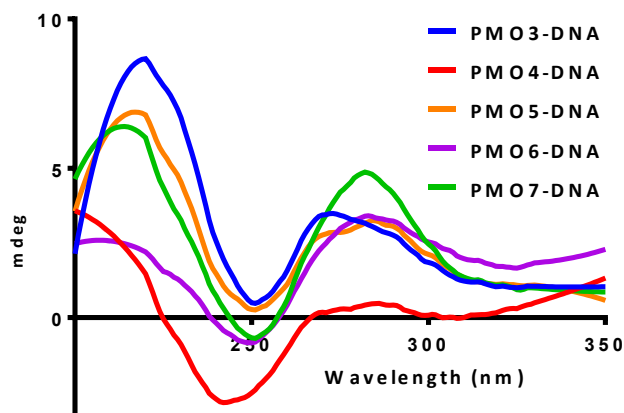


Figure S83: CD-spectra of PMO (3-7) with complementary DNA

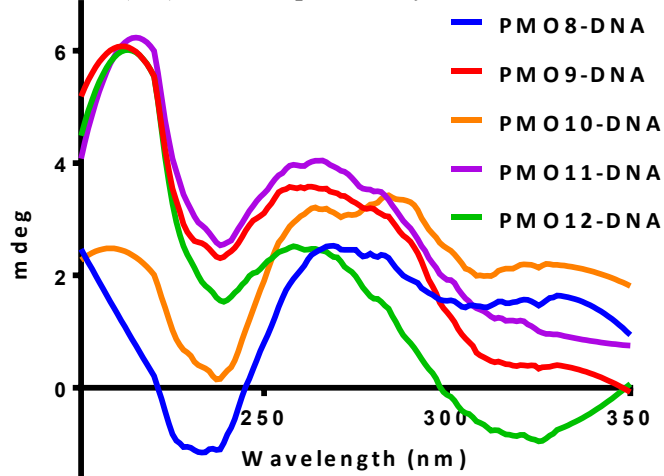


Figure S84: CD-spectra of PMO (8-12) with complementary DNA

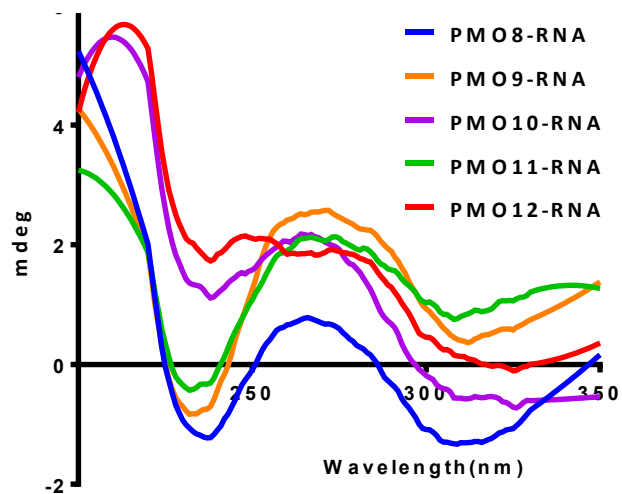


Figure S85: CD-spectra of PMO (8-12) with complementary RNA

17. Reference

1. Ghosh, A.; Banerjee, A.; Gupta, S.; Sinha, S. *J. Am. Chem. Soc.*, 2024, **146(48)**, 32989-33001.