Electronic Supplementary Information

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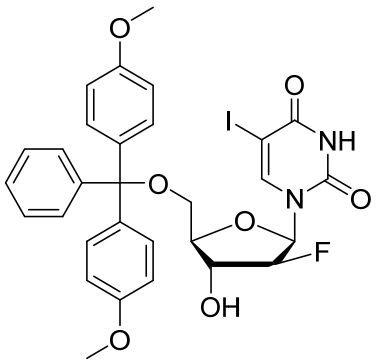
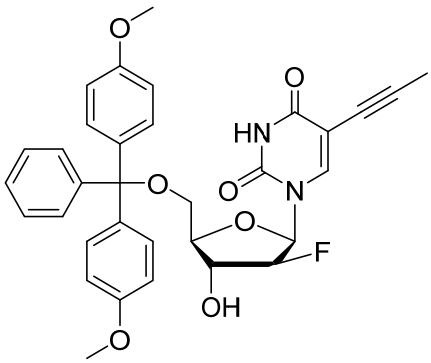
Synthesis and characterization of nucleosides

General

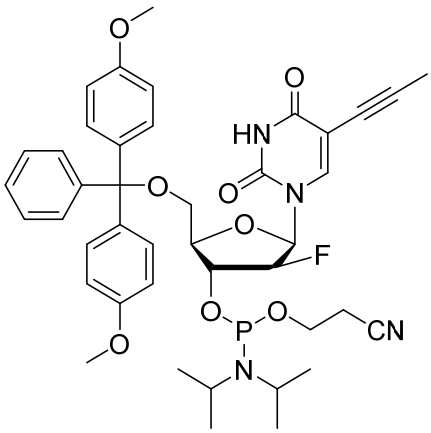
Unless otherwise stated, all chemicals and solvents were purchased from MilliporeSigma (St. Louis, MO) or Thermo Fisher Scientific (Waltham, MA). Nucleoside starting material **2** was synthesized according to previously published procedures.[30,38] All anhydrous solvents dichloromethane (DCM), tetrahydrofuran (THF), dimethylformamide (DMF), acetonitrile (ACN), and pyridine (Py) were used as purchased unless otherwise stated. Flash column chromatography was performed using silica gel 60 (230–400 mesh) purchased from Silicycle (Quebec City, QC). Thin layer chromatography (TLC) was carried out with precoated TLC plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) purchased from EMD Chemicals Inc. (Gibbstown, NJ). NMR spectra were recorded on a Varian 500 MHz NMR spectrometer at room temperature. ^{1}H NMR spectra were recorded at a frequency of 500.0 MHz and chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. ^{19}F NMR spectra were recorded at a frequency of 470.6 MHz and reported in ppm downfield from trichlorofluoromethane. ^{13}C NMR spectra (^{1}H decoupled) were recorded at a frequency of 125.7 MHz and chemical shifts were reported in ppm with tetramethylsilane as a reference. ^{31}P NMR spectra (^{1}H decoupled) were recorded at a frequency of 202.3 MHz and chemical shifts were reported in ppm with H_3PO_4 used as an external standard. Mass spectrometry analysis of nucleoside derivatives was performed on a Thermo LTQ Orbitrap Velos mass spectrometer equipped with a heated electrospray ion source in positive mode. MS spectra (*m/z* 300-2000) were acquired in the Orbitrap at a resolution of 60,000.



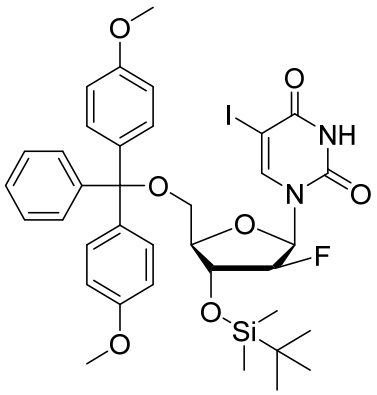
1-[5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl]-5-iodouracil (**3**). To a solution of 1-[2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl]uracil (**2**, 1.60 g, 6.50 mmol) in anhydrous acetonitrile (30 mL) was added cerium(IV) ammonium nitrate (2.51 g, 4.58 mmol) and iodine (1.33 g, 5.24 mmol) with stirring. The mixture was briefly sonicated (2 minutes) then stirred at 80 °C for 90 minutes. Following, the reaction mixture was allowed to cool to room temperature and quenched by the addition of sodium thiosulfate (aqueous, 10 % w/v) until a clear yellow solution was observed (approximately 5 mL). The resulting solution was concentrated *in vacuo* to remove acetonitrile and the remaining solution was poured into a separatory funnel containing distilled water (40 mL). The iodinated analogue was extracted with EtOAc (3 x 100 mL), dried over anhydrous sodium sulfate (2 g) and concentrated. Following, the resulting solid was coevaporated with anhydrous pyridine (2 x 10 mL) and flushed with argon. The resulting oil was dissolved in anhydrous pyridine (25 mL) and 4,4'-dimethoxytritylchloride (2.67 g, 7.87 mmol) was added in three equal portions over a 30-minute period with stirring under an argon atmosphere. The reaction was left at room temperature for 24 hours which revealed consumption of starting material as evident by TLC (5 % MeOH/DCM). The solution was concentrated, poured into 3 % $NaHCO_3$ (75 mL) and extracted with DCM (2 x 50 mL). The combined organic layer was washed with brine (2 x 75 mL), dried over anhydrous sodium sulfate (2 g) and concentrated. Following, the yellow oil was purified by flash column chromatography using DCM/MeOH (100/0 to 98/2, v/v) as eluent to yield the title compound as an off-white foam (pyridine salt, 3.44 g, 5.10 mmol, 78 % over 2 steps). R_f (SiO_2 TLC): 0.29 CH_2Cl_2/MeOH (95/5). λ_{max} (ACN) = 234 and 275 nm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 9.20 (bs, 1H, NH), 8.60-8.59 (m, 2H, Py), 7.97 (d, 1H, H6, *J* = 1.77 Hz), 7.71-7.67 (m, 1H, Py), 7.97 (s, 1H, H6), 7.46-7.21 (m, 11H, Ar), 7.25-7.21 (m, 4H, Ar), 6.20 (dd, 1H, H1', *J* = 3.40 Hz, 19.4 Hz), 5.14 (ddd, 1H, H2', *J* = 1.37 Hz, 3.40 Hz, 51.7 Hz), 4.49-4.44 (m, 1H, H3'), 4.04 (q, 1H, H4'), 3.79 (s, 6H, 2 x OCH_3), 3.44-3.39 (m, 2H, H5' and H5''). ^{19}F NMR (470 MHz, $CDCl_3$, ppm): -197.4 (dt, F2', *J* = 19.4 Hz, 51.7 Hz) ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): δ 160.1, 158.5, 149.9, 149.2, 145.5, 144.4, 136.5, 135.6, 135.6, 130.0, 129.9, 128.0, 126.9, 123.9, 113.3, 95.7, 94.2, 86.5, 84.4, 84.3, 83.8, 77.2, 77.0, 76.7, 75.4, 75.2, 67.9, 67.9, 62.5, 55.2, 25.5. HRMS (ESI-MS) *m/z* calculated for $C_{30}H_{28}FIN_2O_7$ 674.0925: found 674.0920 $[M]^+$.



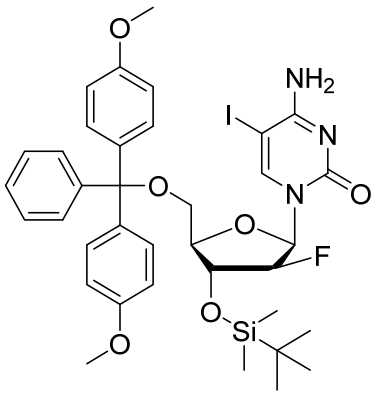
1-[5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl]-5-propynyluracil (**4**). Compound **3** (0.60 g, 0.89 mmol) was added to an oven-dried round-bottom flask equipped with a stir bar and flushed with anhydrous argon. The solid was dissolved in a mixture of anhydrous tetrahydrofuran (4 mL), dimethylformamide (1 mL) and triethylamine (8 mL) while stirring under argon. To the resulting solution was added copper(I) iodide (17 mg, 89 µmol), bis(triphenylphosphine)palladium(II) dichloride (63 mg, 89 µmol) and propyne gas was bubbled through for approximately 5 minutes with vigorous stirring. The flask containing the darkened solution was sealed and left to stir overnight at room temperature. The resulting reaction mixture was concentrated *in vacuo*, filtered over celite, and poured into aqueous EDTA (5% w/v, 50 mL). The compound was extracted with DCM (2 x 50 mL) and the combined organic layers were washed consecutively with aqueous EDTA (5% w/v, 2 x 50 mL), and brine (4 x 50 mL). The organic layer was dried over anhydrous sodium sulfate (2 g) and concentrated *in vacuo*. Following, the yellow oil was purified by flash column chromatography using DCM/MeOH (100/0 to 99/1, v/v) as eluent to yield the title compound as a white foam (342 mg, 0.58 mmol, 65 %). R_f (SiO_2 TLC): 0.22 CH_2Cl_2/MeOH (97/3). λ_{max} (ACN) = 232 and 282 nm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 9.24 (bs, 1H, NH), 7.77 (s, 1H, H6), 7.47-7.19 (m, 9H, Ar), 6.86-6.84 (m, 4H, Ar), 6.22-6.18 (m, 1H, H1'), 5.10-4.99 (m, 1H, H2'), 4.38-4.34 (m, 1H, H3'), 4.08-4.07 (m, 1H, H4'), 3.78 (s, 6H, 2 x OCH_3), 3.43-3.34 (m, 2H, H5' and H5''), 3.16 (bs 1H, OH), 1.89 (s, 3H, CH_3-alkyne). ^{19}F NMR (470 MHz, $CDCl_3$, ppm): -197.8 (dt, F2', *J* = 19.1 Hz, 51.5 Hz) ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): δ 161.9, 158.5, 149.2, 144.5, 142.8, 135.7, 135.6, 129.9, 129.9, 128.0, 127.9, 126.9, 113.2, 100.5, 95.5, 93.9, 91.0, 86.5, 84.4, 84.5, 83.6, 75.6, 75.4, 70.0, 62.8, 55.2, 4.4. HRMS (ESI-MS) *m/z* calculated for $C_{33}H_{32}FN_2O_7H^+$ 587.2194: found 587.2186 $[M + H]^+$.



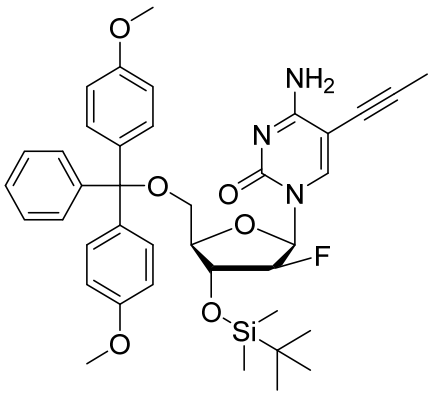
1-[5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl]-5-propynyluracil (**5**). To a solution of compound **4** (0.30 g, 0.51 mmol) in anhydrous tetrahydrofuran (10 mL) and anhydrous diisopropylethylamine (183 µL, 1.02 mmol) was added 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (182 µL, 0.82 mmol). The solution was stirred under argon at room temperature until no more starting material was observed as evident by TLC (1 hour). The reaction mixture was concentrated, poured into 3 % $NaHCO_3$ (50 mL) and extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous sodium sulfate (2 g), and concentrated *in vacuo*. The resulting yellow oil was purified by flash column chromatography on triethylamine treated silica using EtOAc/Hex (8/2, v/v) as eluent to yield the title compound as a white foam (150 mg, 0.58 mmol, 37 %). R_f (SiO_2 TLC): 0.69 and 0.75 CH_2Cl_2/MeOH (95/5). λ_{max} (ACN) = 232 and 282 nm. ^{1}H NMR (500 MHz, d_6-acetone, ppm): 10.38 (bs, 1H, NH), 7.83-7.81 (m, 1H, H6), 7.55-7.23 (m, 9H, Ar), 6.94-6.90 (m, 4H, Ar), 6.27-6.22 (m, 1H, H1'), 5.32-4.17 (m, 1H, H2'), 4.71-4.56 (m, 1H, H3'), 4.28-4.23 (m, 1H, H4'), 3.95-3.59 (m, 10H, 2 x CHNP, CH_2OP, 2 x OCH_3), 3.47-3.40 (m, 2H, H5' and H5''), 2.77-2.61 (m, 2H, CH_2CN), 1.88-1.85 (m, 3H, CH_3-alkyne), 1.21-1.83 (m, 9H, *i*-Pr-CH_3), 1.10-1.08 (m, 3H, *i*-Pr-CH_3). ^{19}F NMR (470 MHz, d_6-acetone, ppm): -197.9 - -198.2 (m, F2'). ^{31}P NMR (202.3 MHz, d_6-acetone, ppm): δ 150.7, 150.4. HRMS (ESI-MS) *m/z* calculated for $C_{42}H_{48}FN_4O_8\,H^+$ 787.3272: found 787.3268 $[M + H]^+$.



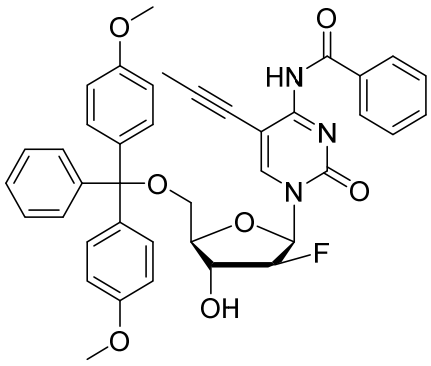
1-[5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-3'-O-(*tert*-butyldimethylsilyl)-ß-D-arabinofuranosyl]-5-iodouracil (**6**). To a solution of compound **3** (2.50 g, 3.32 mmol) in anhydrous DCM (25 mL) was added imidazole (0.90 g, 13.3 mmol) and *tert*-butyldimethylsilyl chloride (1.00 g, 6.67 mmol) with stirring under an argon atmosphere. The cloudy solution was stirred for 16 hours at room temperature which revealed consumption of the starting material as evident by TLC. The mixture was poured into 3 % $NaHCO_3$ (75 mL) and extracted with DCM (2 x 50 mL). The organic layer was further washed with brine (75 mL), dried over anhydrous sodium sulfate (2 g), filtered, and concentrated *in vacuo*. Following, the yellow oil was purified by flash column chromatography using Hex/EtOAc (9/1 to 7/3, v/v) as eluent to yield the title compound as a white foam (2.16 g, 2.74 mmol, 83 %). R_f (SiO_2 TLC): 0.58 CH_2Cl_2/MeOH (95/5). λ_{max} (ACN) = 198 and 275 nm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.93 (bs, 1H, NH), 8.01 (s, 1H, H6), 7.47-7.21 (m, 9H, Ar), 6.86-6.85 (m, 4H, Ar), 6.20-6.15 (m, 1H, H1'), 4.97-4.87 (m, 1H, H2'), 4.46-4.41 (m, 1H, H3'), 3.96-3.95 (m, 1H, H4'), 3.79 (s, 6H, 2 x OCH_3), 3.42-3.28 (m, 2H, H5' and H5''), 0.83 (s, 9H, Si-C(CH_3)$_3$), 0.06 (s, 3H, Si-CH_3), 0.01 (s, 3H, Si-CH_3). ^{19}F NMR (470 MHz, $CDCl_3$, ppm): -196.5 (dt, F2', *J* = 19.2 Hz, 52.1 Hz). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): δ 159.7, 158.6, 149.6, 145.4, 144.3, 135.5, 135.5, 129.9, 129.9, 128.0, 128.0, 126.9, 113.3, 96.3, 94.7, 86.5, 84.3, 84.2, 75.9, 75.7, 67.6, 61.9, 55.2, 25.5, 17.8, -4.8, -5.0. HRMS (ESI-MS) *m/z* calculated for $C_{36}H_{42}FIN_2O_7Si$ 788.1790: found 788.1787 $[M + H]^+$.



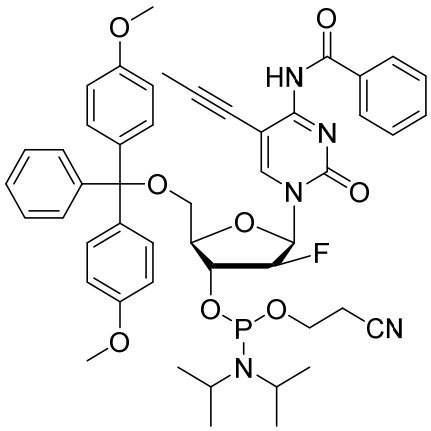
1-[5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-3'-O-(*tert*-butyldimethylsilyl)-ß-D-arabinofuranosyl]-5-iodocytosine (**7**). Compound **6** (2.16 g, 2.74 mmol) was dissolved in anhydrous acetonitrile (20 mL), DCM (7 mL) and triethylamine (5.70 mL, 41.1 mmol). The solution was flushed with argon and placed on an ice-water bath before the addition of 1,2,4-triazole (1.92 g, 27.4 mmol) and phosphorus(V) oxychloride (0.63 mL, 6.84 mmol) dropwise. The reaction was stirred at room temperature for 2 hours which revealed complete consumption of the starting material as evident by TLC. The reaction mixture was concentrated *in vacuo* diluted with DCM (50 mL) and poured into 3 % $NaHCO_3$ (75 mL). The organic layer was collected, and the aqueous layer was extracted with DCM (2 x 40 mL). The organic washes were combined, concentrated *in vacuo,* and dissolved in dioxane (22 mL). Fresh aqueous NH_4OH (28%, 8.0 mL) was added, and the reaction was stirred for 30 minutes at room temperature. Following, the reaction mixture was concentrated *in vacuo*, poured into distilled H_2O (50 mL) and extracted with DCM (3 x 50 mL). The combined organic layers were further washed with brine (75 mL), dried over anhydrous sodium sulfate (2 g) and concentrated *in vacuo*. The yellow oil was purified by flash column chromatography using Hex/EtOAc (1/1 to 3/7, v/v) as eluent to yield the title compound as a white foam (2.04 g, 2.59 mmol, 95 %). R_f (SiO_2 TLC): 0.24 CH_2Cl_2/MeOH (97/3). λ_{max} (ACN) = 204 and 274 nm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.16 (bs, 1H, NH), 7.97 (s, 1H, H6), 7.47-7.21 (m, 9H, Ar), 6.86-6.84 (m, 4H, Ar), 6.24-6.20 (m, 1H, H1'), 5.57 (bs, 1H, NH), 5.07-4.94 (m, 1H, H2'), 4.42-4.37 (m, 1H, H3'), 3.98-3.96 (m, 1H, H4'), 3.79 (s, 6H, 2 x OCH_3), 3.41-3.26 (m, 2H, H5' and H5''), 0.83 (s, 9H, Si-C(CH_3)$_3$), 0.04 (s, 3H, Si-CH_3), 0.01 (s, 3H, Si-CH_3). ^{19}F NMR (470 MHz, $CDCl_3$, ppm): -196.9 (dt, F2', J = 19.3 Hz, 51.8 Hz). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): δ 163.8, 158.5, 154.3, 147.9, 144.4, 135.7, 129.9, 129.9, 128.0, 127.9, 126.9, 113.2, 95.9, 94.4, 86.4, 85.4, 85.2, 84.3, 76.3, 76.0, 62.1, 55.8, 55.2, 25.6, 17.8, -4.8, -5.0. HRMS (ESI-MS) *m/z* calculated for $C_{36}H_{43}FIN_3O_6Si\,H^+$ 788.2028: found 708.2022 $[M + H]^+$.



1-[5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-3'-O-(*tert*-butyldimethylsilyl)-ß-D-arabinofuranosyl]-5-propynylcytosine (**8**). Compound **7** (2.04 g, 2.59 mmol) was added to an oven-dried round-bottom flask equipped with a stir bar and flushed with anhydrous argon. The solid was dissolved in a mixture of anhydrous tetrahydrofuran (7 mL), dimethylformamide (1.5 mL) and triethylamine (14 mL) while stirring under argon. To the resulting solution was added copper(I) iodide (49 mg, 0.25 mmol), bis(triphenylphosphine)palladium(II) dichloride (182 mg, 0.25 mmol) and propyne gas was bubbled through for approximately 5 minutes with vigorous stirring. The flask containing the darkened solution was sealed and left to stir overnight at room temperature. The resulting reaction mixture was concentrated *in vacuo*, filtered over celite, and poured into aqueous EDTA (5% w/v, 75 mL). The compound was extracted with DCM (2 x 60 mL) and the combined organic layers were washed consecutively with aqueous EDTA (5% w/v, 2 x 75 mL), and brine (4 x 65 mL). The organic layer was dried over anhydrous sodium sulfate (2 g) and concentrated *in vacuo*. The yellow oil was purified by flash column chromatography using CH_2Cl_2/MeOH (100/0 to 97.5/2.5, v/v) as eluent to yield the title compound as a white foam (1.74 g, 2.48 mmol, 96 %). R_f (SiO_2 TLC): 0.19 CH_2Cl_2/MeOH (97/3). λ_{max} (ACN) = 238 and 300 nm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.01 (bs, 1H, NH), 7.81 (s, 1H, H6), 7.48-7.20 (m, 9H, Ar), 6.85-6.82 (m, 4H, Ar), 6.26-6.21 (m, 1H, H1'), 5.74 (bs, 1H, NH), 5.04-4.93 (m, 1H, H2'), 4.34-4.30 (m, 1H, H3'), 3.99-3.93 (m, 1H, H4'), 3.79 (s, 6H, 2 x OCH_3), 3.35-3.27 (m, 2H, H5' and H5''), 1.95 (s, 3H, CH_3-alkyne), 0.83 (s, 9H, Si-C(CH_3)$_3$), 0.04 (s, 3H, Si-CH_3), -0.01 (s, 3H, Si-CH_3). ^{19}F NMR (470 MHz, $CDCl_3$, ppm): -197.6 (dt, F2', *J* = 19.1 Hz, 51.8 Hz). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): δ 165.1, 158.5, 154.0, 144.5, 144.0, 135.7, 135.7, 129.9, 129.9, 128.0, 127.8, 126.8, 113.1, 95.7, 94.2, 91.8, 91.7, 86.4, 85.4, 85.30, 84.19, 76.31, 76.1, 70.48, 62.5, 55.2, 25.6, 17.8, 4.3, -4.8, -5.0. HRMS (ESI-MS) *m/z* calculated for $C_{39}H_{46}FN_3O_7Si\,H^+$ 700.3218: found 700.3213 $[M + H]^+$.



1-[5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl]-N^4-benzoyl-5-propynylcytosine (**9**). Compound **8** (1.71 g, 2.45 mmol) was dissolved in anhydrous dimethylformamide (8 mL) and flushed with argon. Following, benzoic anhydride (0.66 g, 2.93 mmol) was added, and solution was stirred at room temperature for 36 hours. The reaction mixture was poured into saturated $NaHCO_3$ (50 mL) and extracted with DCM (2 x 50 mL). The organic extractions were combined and washed consecutively with saturated $NaHCO_3$ (2 x 50 mL) and brine (4 x 75 mL). The organic layer was dried over anhydrous sodium sulfate (2 g) and concentrated *in vacuo*. The yellow oil was adhered to a short silica column and rinsed with DCM (200 mL) followed by DCM/MeOH (98/2) to elute the benzoylated intermediate. The fractions containing the product were concentrated *in vacuo* and dissolved in tetrahydrofuran (50 mL). To the solution was added tetrabutylammonium fluoride (1 M solution in THF, 2.66 mL, 2.66 mmol) with stirring under argon. The reaction mixture was stirred for 1 hour at room temperature and concentrated *in vacuo*. The yellow syrup was poured into $NaHCO_3$ (75 mL), extracted with DCM (2 x 50 mL) and washed with brine (75 mL). The organic layer was dried over anhydrous sodium sulfate (2 g), concentrated *in vacuo* and purified by flash column chromatography using DCM/MeOH (99/1 to 98/2, v/v) as eluent to yield the title compound as a white foam (1.17 g, 1.70 mmol, 69 %). R_f (SiO_2 TLC): 0.33 CH_2Cl_2/MeOH (95/5). λ_{max} (ACN) = 238 and 341 nm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.05 (bs, 1H, NH), 8.03 (s, 1H, H6), 7.57-7.21 (m, 14H, Ar), 6.86-6.80 (m, 4H, Ar), 6.31-6.27 (m, 1H, H1'), 5.30-5.15 (m, 1H, H2'), 4.42-4.39 (m, 1H, H3'), 4.25-4.20 (m, 1H, H4'), 3.78 (s, 6H, 2 x OCH_3), 3.44-3.34 (m, 2H, H5' and H5''), 1.99 (s, 3H, CH_3-alkyne). ^{19}F NMR (470 MHz, $CDCl_3$, ppm): -197.2 - -197.4 (m, F2'). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): δ 158.5, 144.6, 135.8, 135.7, 132.8, 129.9, 129.9, 128.4, 128.0, 127.9, 126.8, 113.2, 113.2, 86.3, 75.6, 75.4, 63.0, 55.1, 4.4. HRMS (ESI-MS) *m/z* calculated for $C_{40}H_{36}FN_3O_7H^+$ 690.2616: found 690.2613 $[M + H]^+$.



1-[5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl]-N^4-benzoyl-5-propynylcytosine (**10**). To a solution of compound **9** (300 mg, 0.43 mmol) in anhydrous tetrahydrofuran (6 mL) was added anhydrous diisopropylethylamine (186 µL, 1.04 mmol). The reaction vessel was purged with argon with vigorous stirring for approximately 5 minutes followed by the drop-wise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (155 µL, 0.70 mmol). The reaction mixture was stirred for 1 hour at room temperature which revealed complete consumption of the starting material as evident by TLC. The solution was concentrated *in vacuo* and the resulting yellow oil was poured into poured into $NaHCO_3$ (50 mL), extracted with EtOAc (2 x 40 mL) and washed with brine (75 mL). The organic layer was dried over anhydrous sodium sulfate (2 g), filtered, and concentrated *in vacuo*. The yellow oil was purified by flash column chromatography with TEA treated silica, using EtOAc/Hex, 3/7, v/v) as eluent to yield the title compound as a white foam (277 mg, 0.31 mmol, 72 %). R_f (SiO_2 TLC): 0.65, 0.70 EtOAc/Hex (8/2). λ_{max} (ACN) = 238 and 342 nm. ^{1}H NMR (500 MHz, acetone-d_6, ppm): 8.30 (bs, 1H, NH), 8.07-7.92 (m, 1H, H6), 7.60-6.81 (m, 18H, Ar), 6.30-6.27 (m, 1H, H1'), 5.39-5.25 (m, 1H, H2'), 4.75-4.60 (m, 1H, H3'), 4.34-4.31 (m, 1H, H4'), 3.92-3.64 (m, 10H, 2 x CHNP, CH_2OP, 2 x OCH_3), 3.48-3.45 (m, 2H, H5' and H5''), 2.77-2.63 (m, 2H, CH_2CN), 1.94 (s, 3H, CH_3-alkyne), 1.19-1.09 (m, 12H, *i*-Pr-CH_3). ^{19}F NMR (470 MHz, acetone-d_6, ppm): -197.6 - -197.9 (m, F2'). ^{31}P NMR (202.3 MHz, d_6-acetone, ppm): δ 150.8, 150.4. HRMS (ESI-MS) m/z calculated for $C_{49}H_{53}FN_5O_8$ H^+ 890.3694: found 890.3691 $[M + H]^+$.

Fig. S1: 500 MHz ^{1}H NMR spectrum of compound **3** in $CDCl_3$

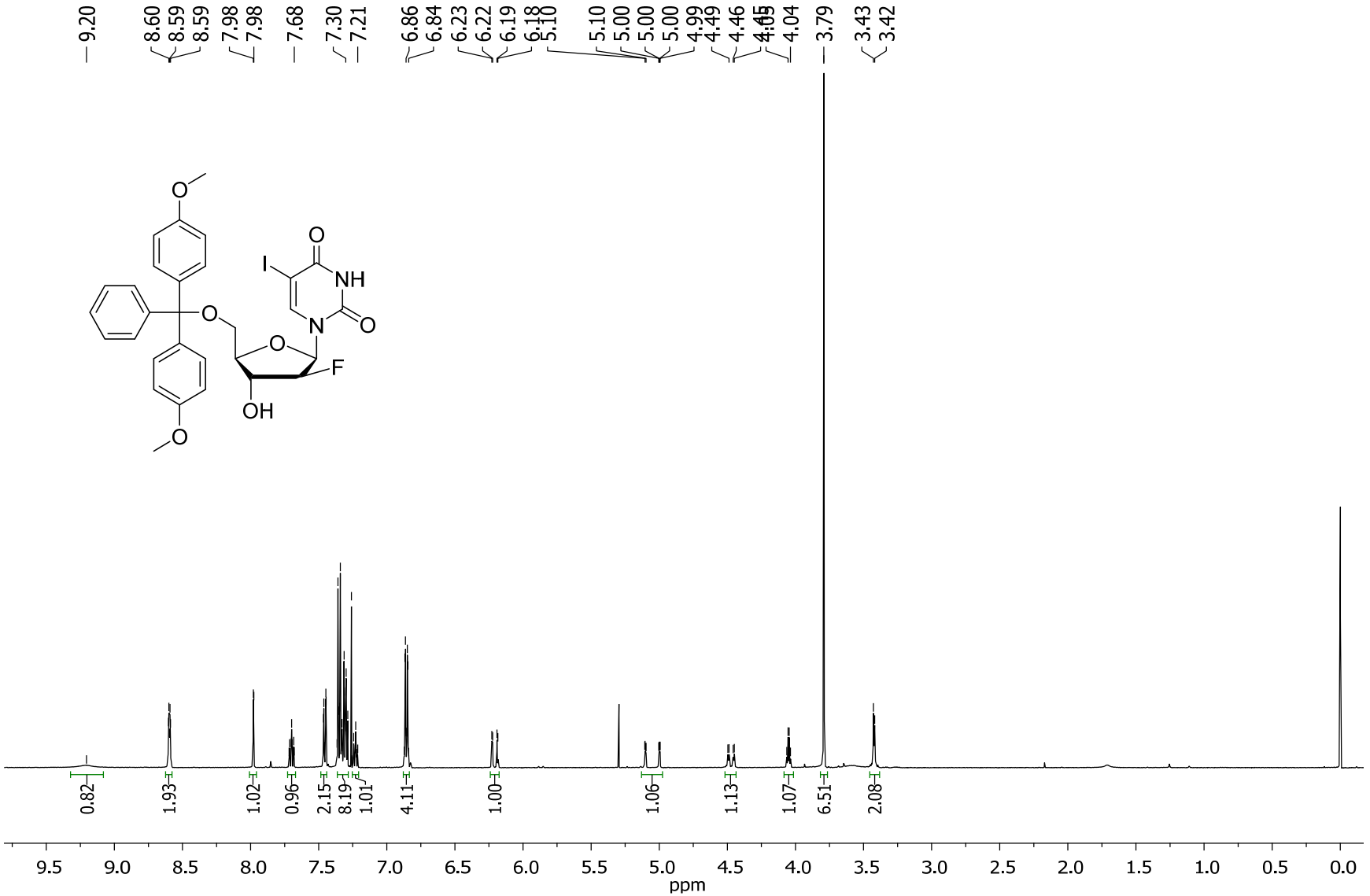


Fig. S2: 470.6 MHz ^{19}F NMR spectrum of compound **3** in $CDCl_3$

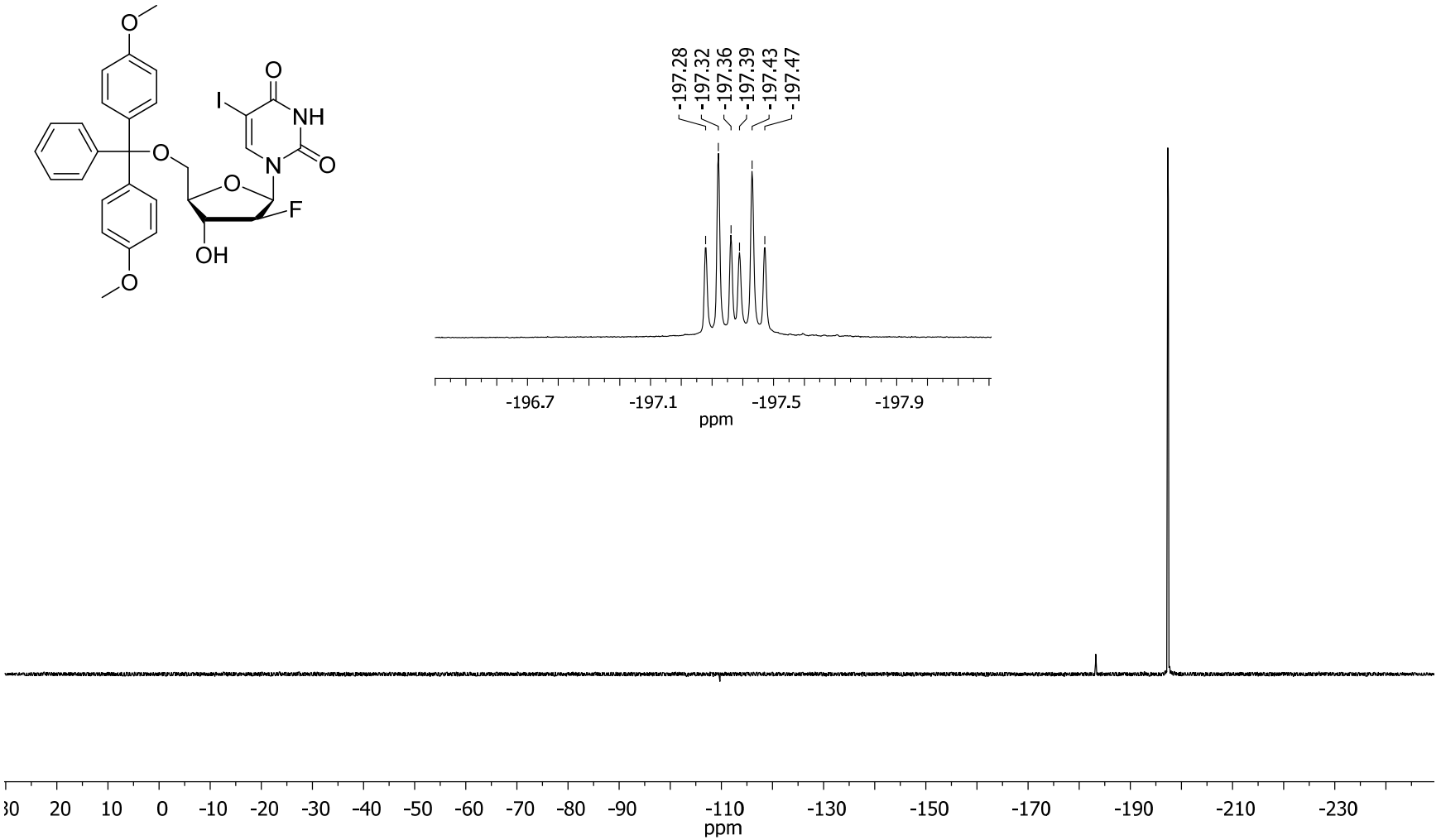


Fig. S3: 125.7 MHz ^{13}C NMR spectrum of compound **3** in $CDCl_3$

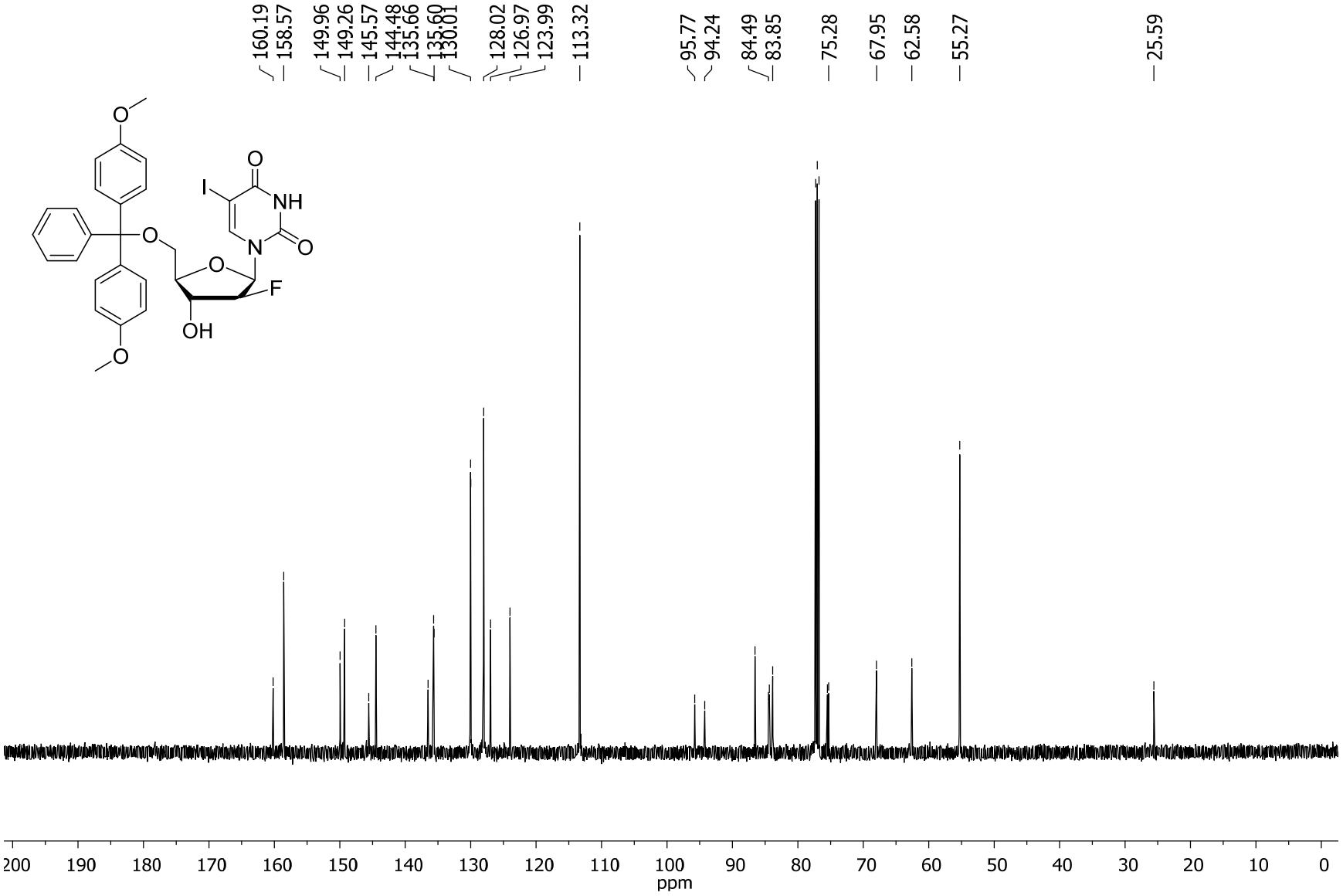


Fig. S4: 500 MHz ^{1}H NMR spectrum of compound **4** in $CDCl_3$

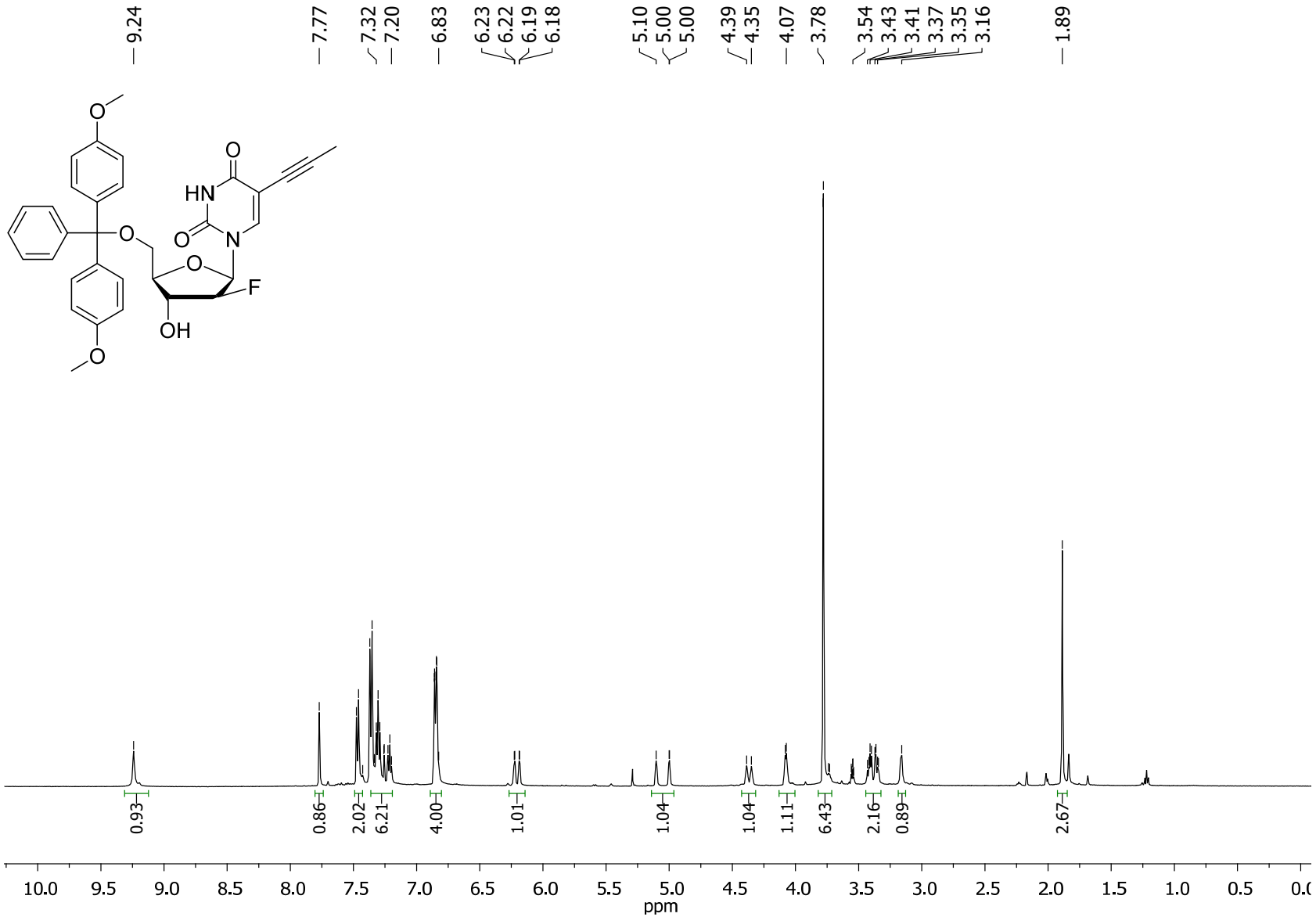


Fig. S5: 470.6 MHz ^{19}F NMR spectrum of compound **4** in $CDCl_3$

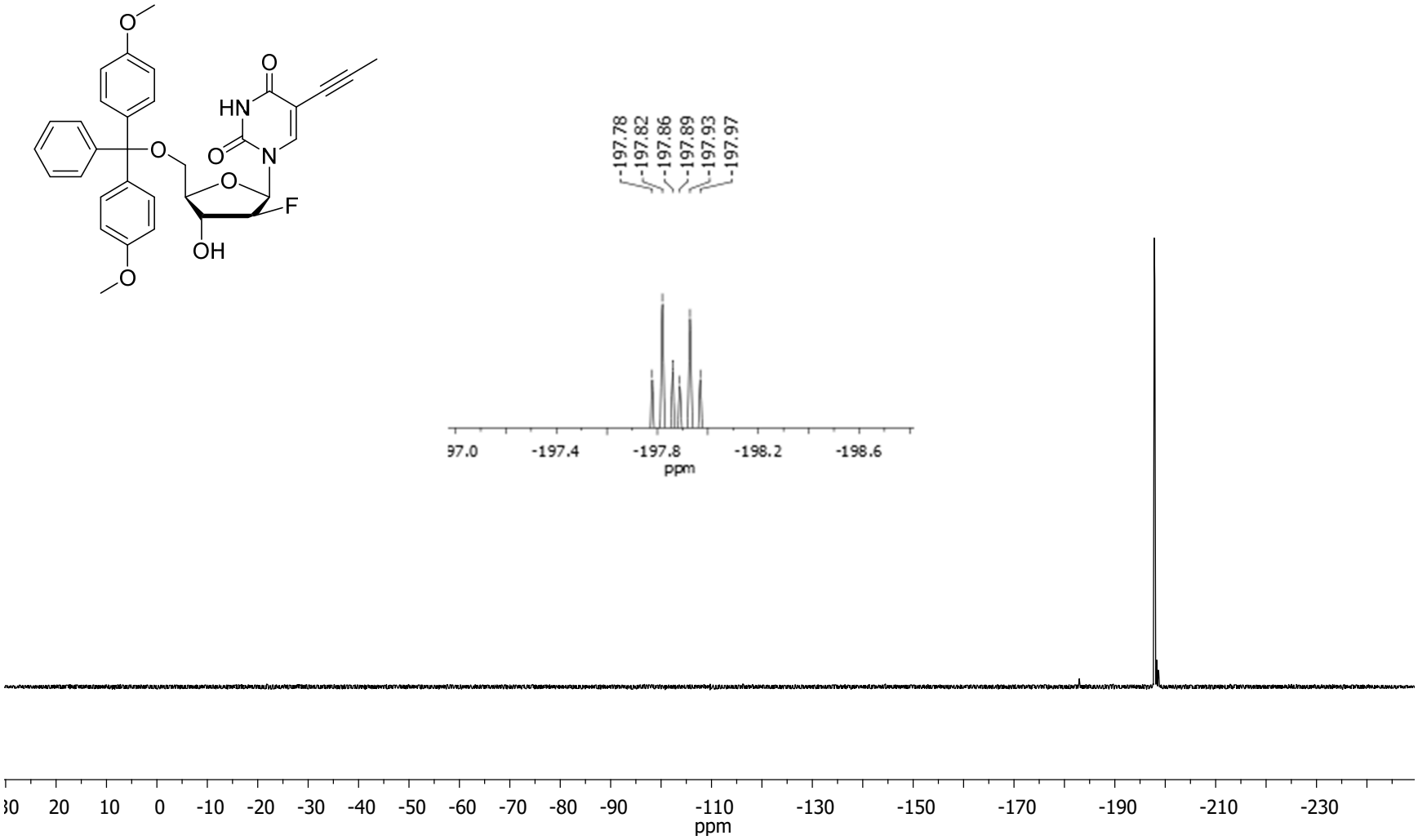


Fig. S6: 125.7 MHz ^{13}C NMR spectrum of compound **4** in $CDCl_3$

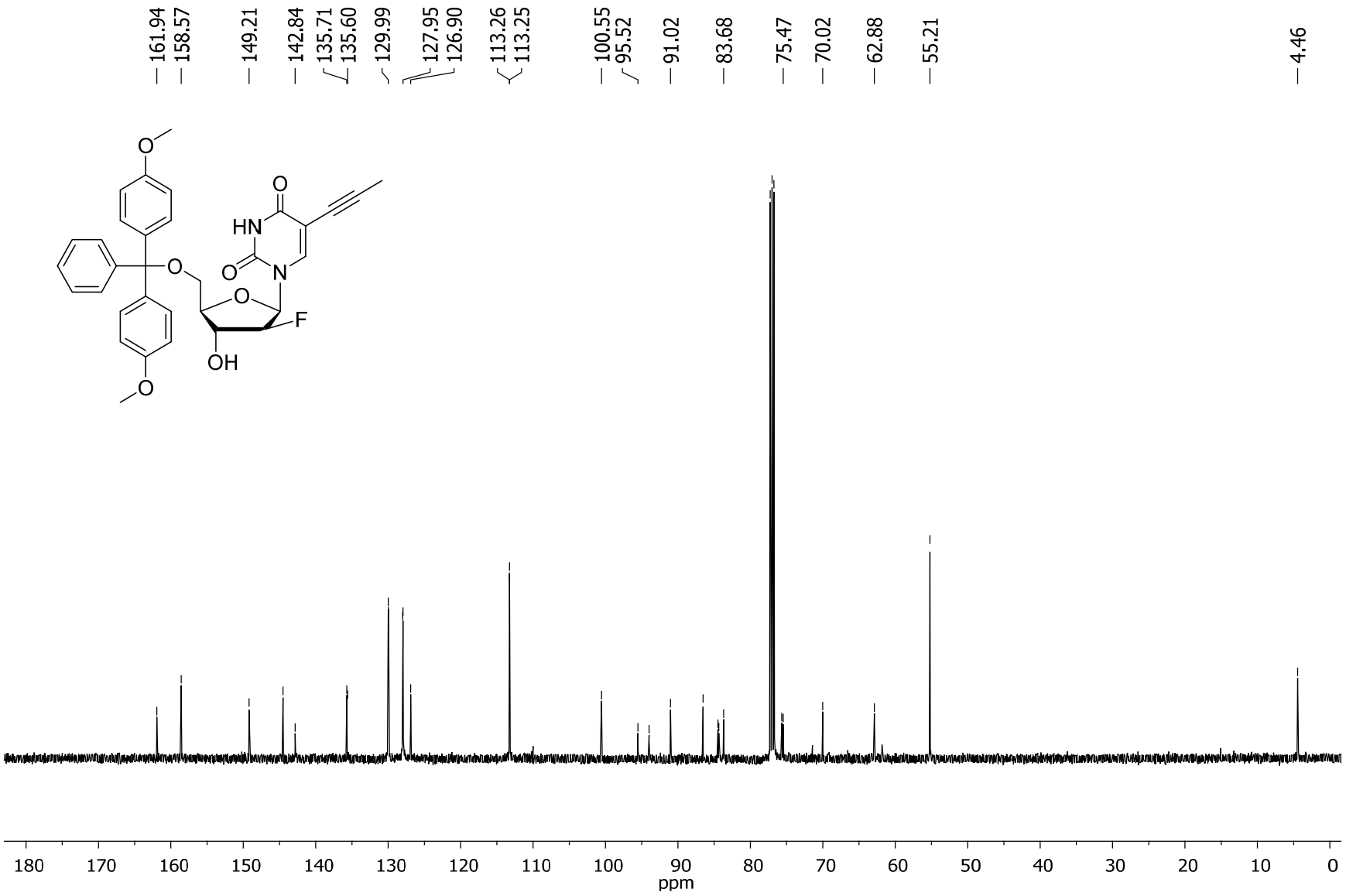


Fig. S7: 500 MHz ^{1}H NMR spectrum of compound **5** in acetone-d_6

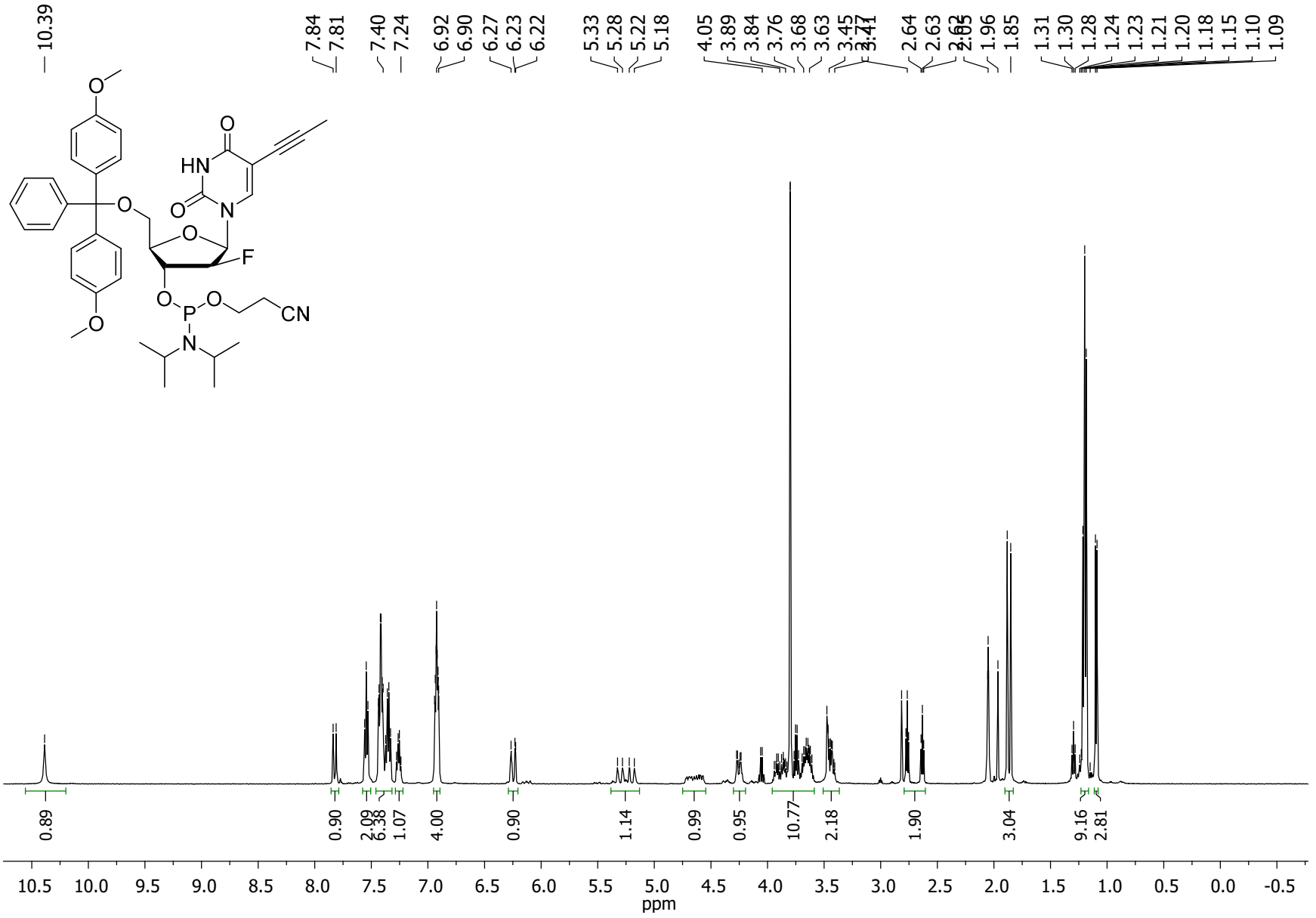


Fig. S8: 470.6 MHz ^{19}F NMR spectrum of compound **5** in acetone-d_6

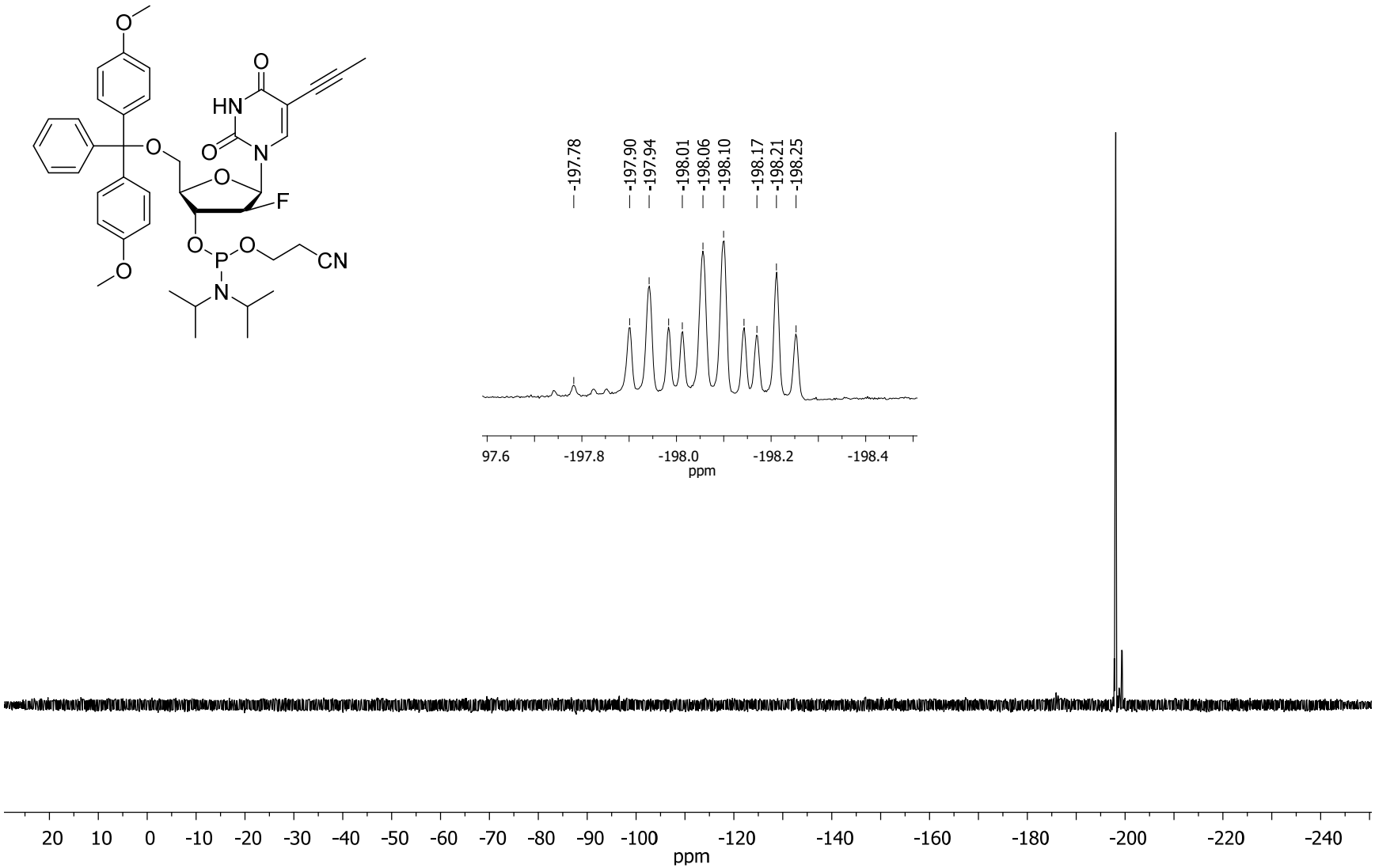


Fig. S9: 202.3 MHz ^{31}P NMR spectrum of compound **5** in acetone-d_6

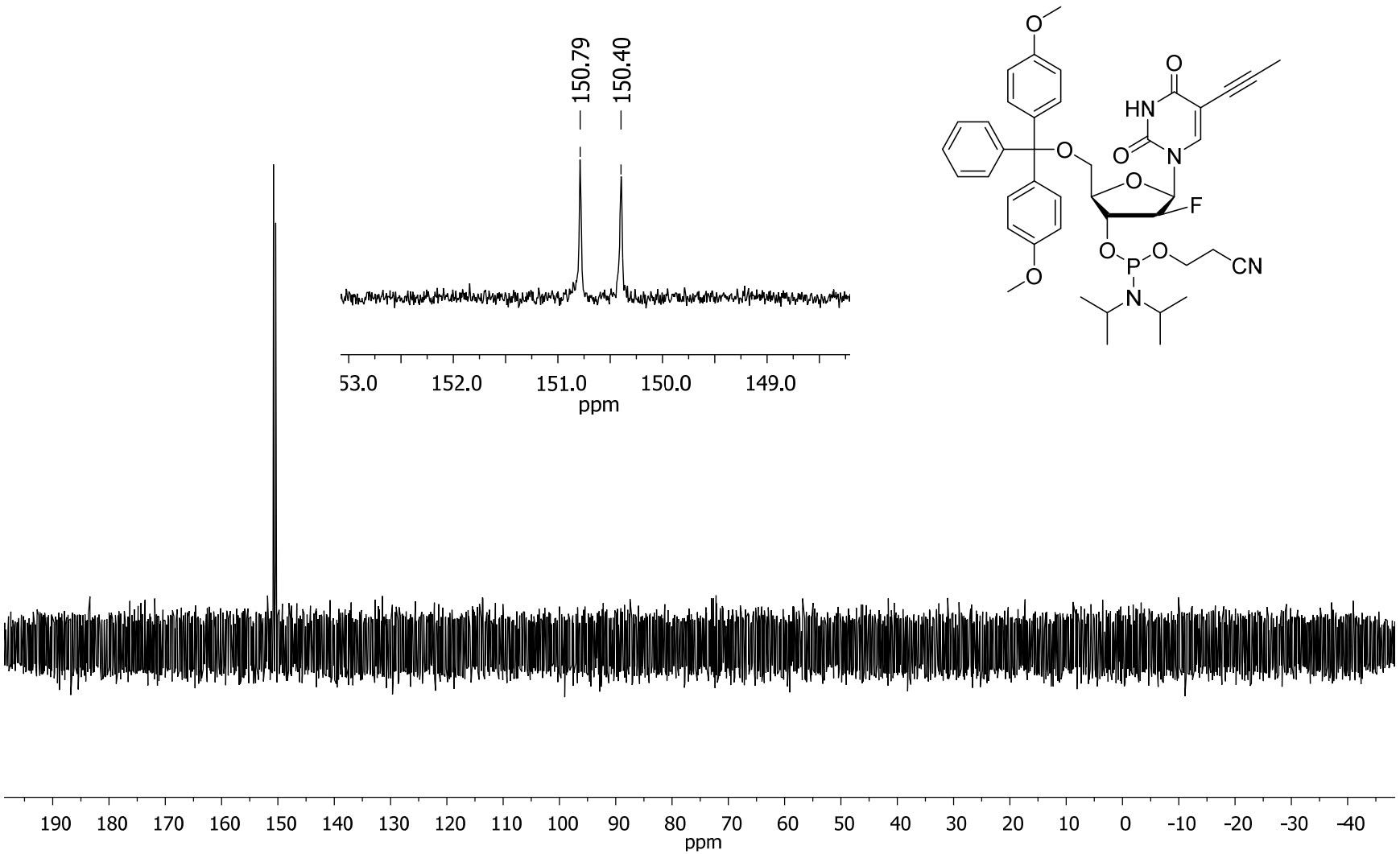


Fig. S10: 500 MHz ^{1}H NMR spectrum of compound **6** in $CDCl_3$

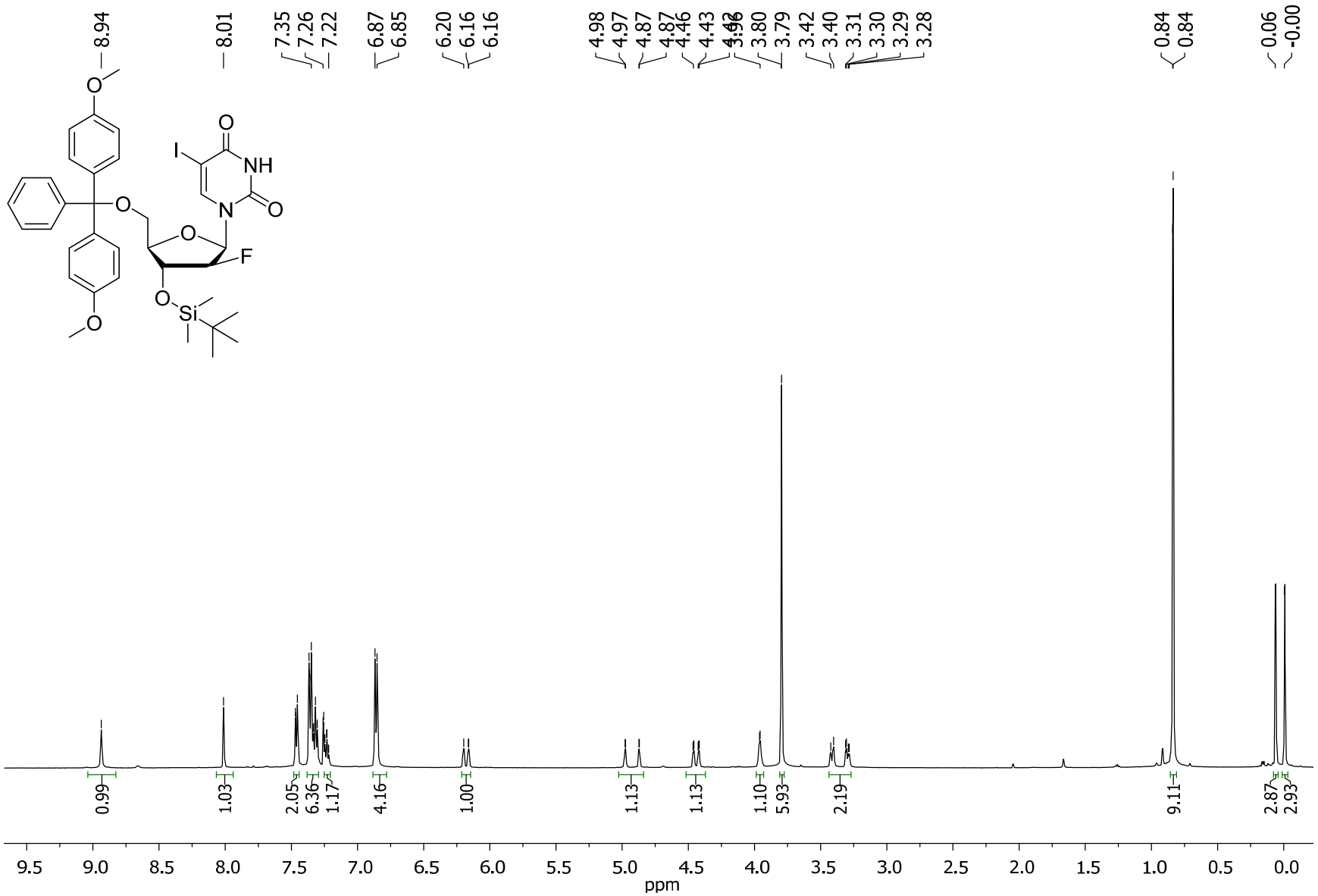


Fig. S11: 470.6 MHz ^{19}F NMR spectrum of compound **6** in $CDCl_3$

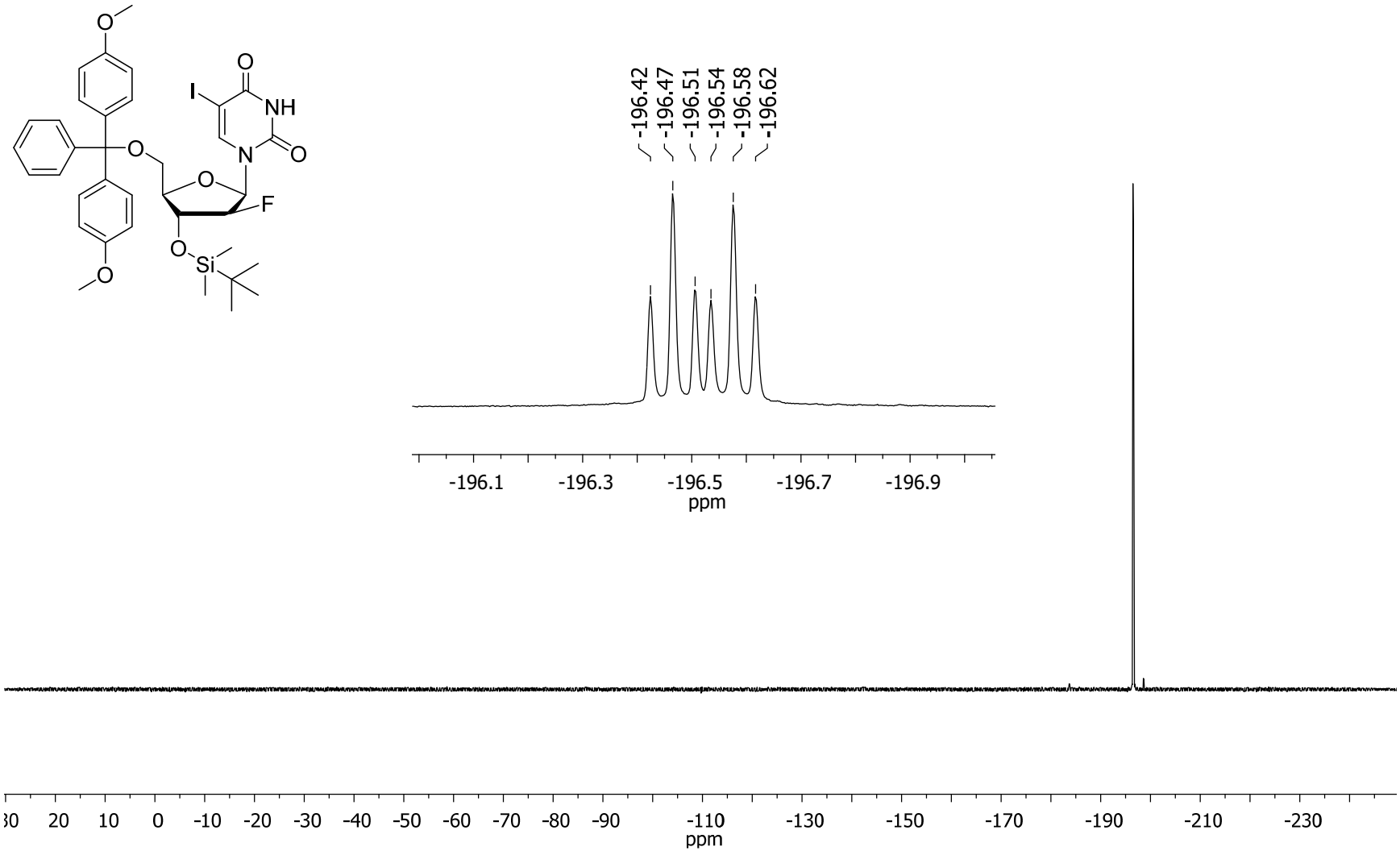


Fig. S12: 125.7 MHz ^{13}C NMR spectrum of compound **6** in $CDCl_3$

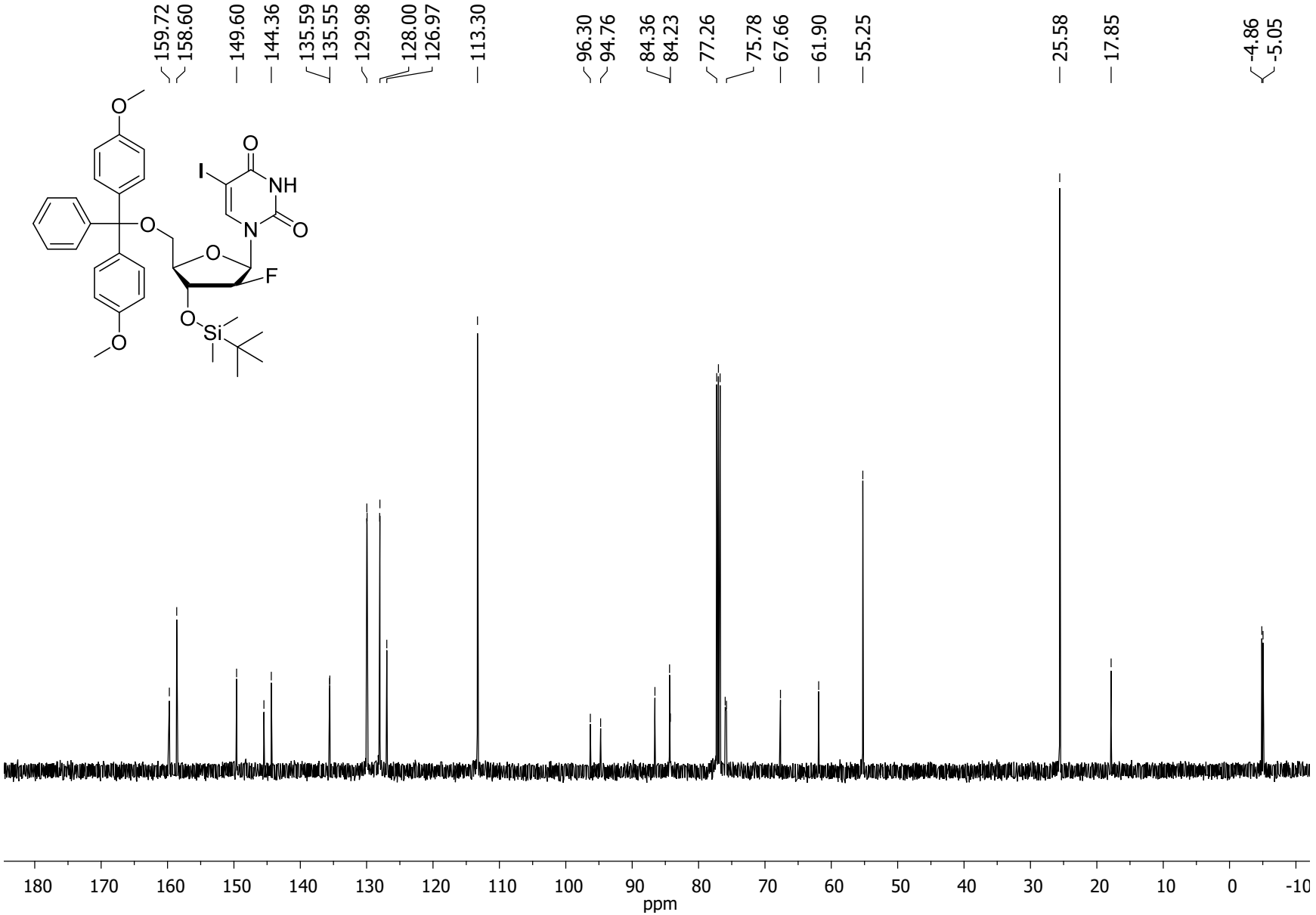


Fig. S13: 500 MHz ^{1}H NMR spectrum of compound **7** in $CDCl_3$

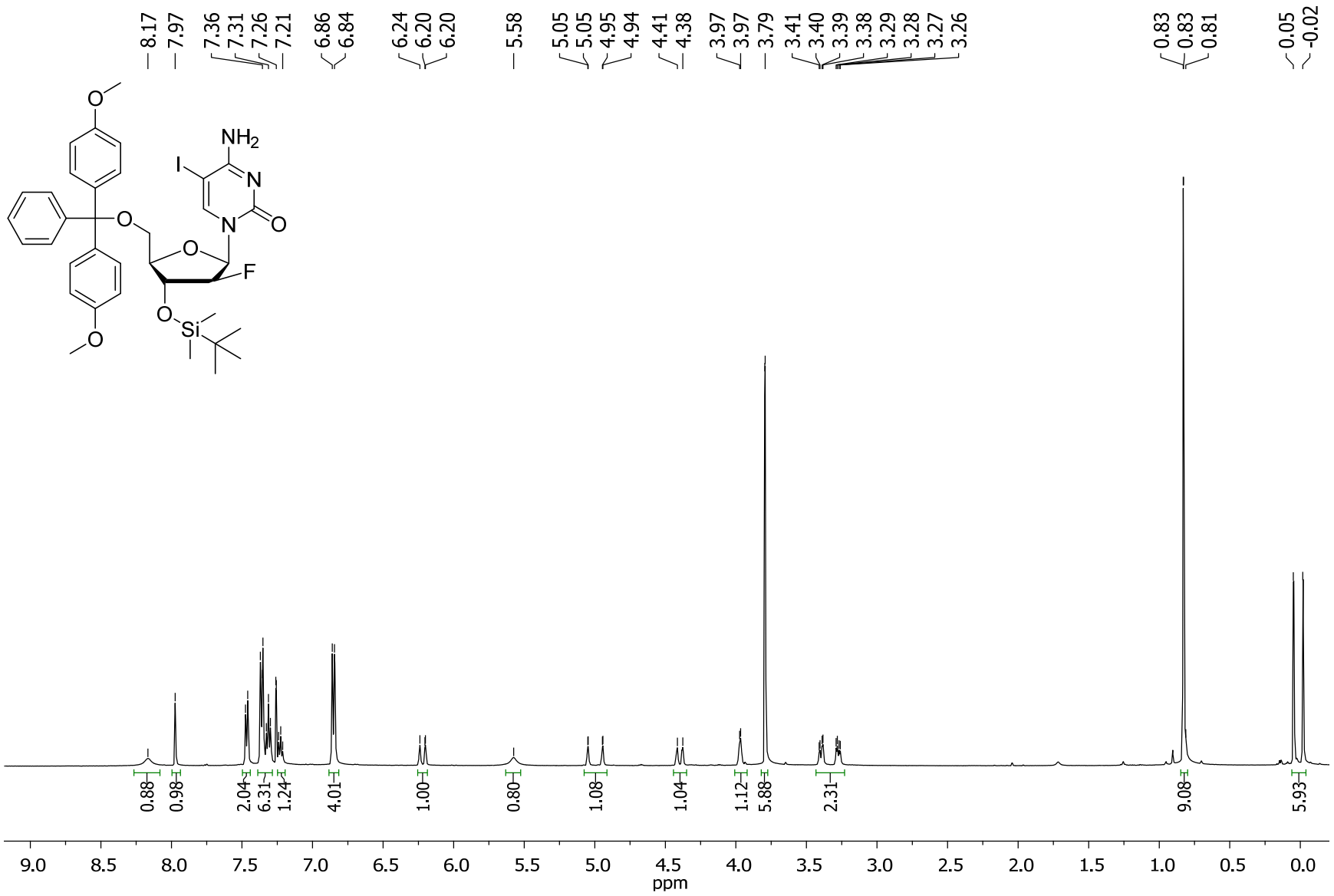


Fig. S14: 470.6 MHz ^{19}F NMR spectrum of compound **7** in $CDCl_3$

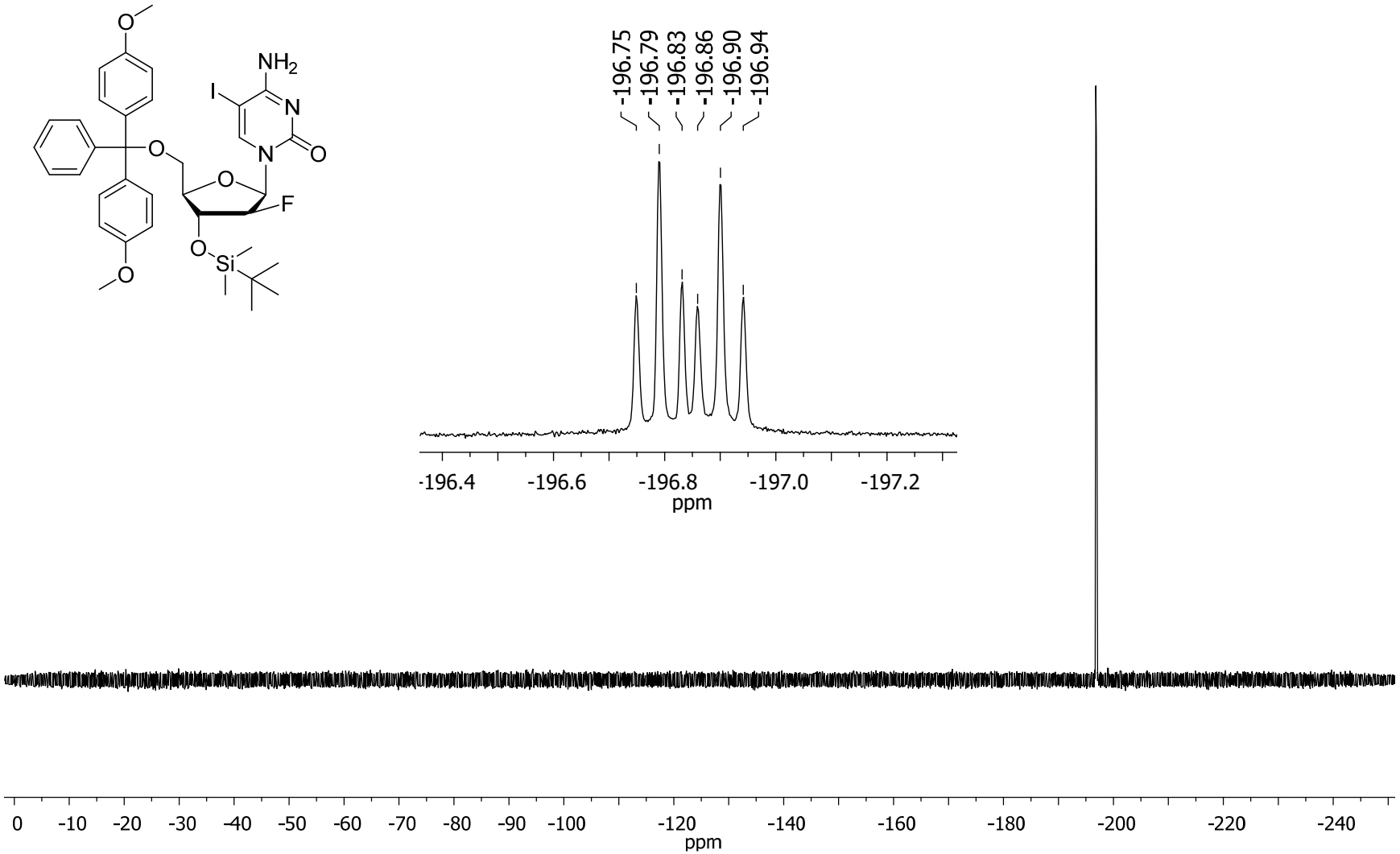


Fig. S15: 125.7 MHz ^{13}C NMR spectrum of compound **7** in $CDCl_3$

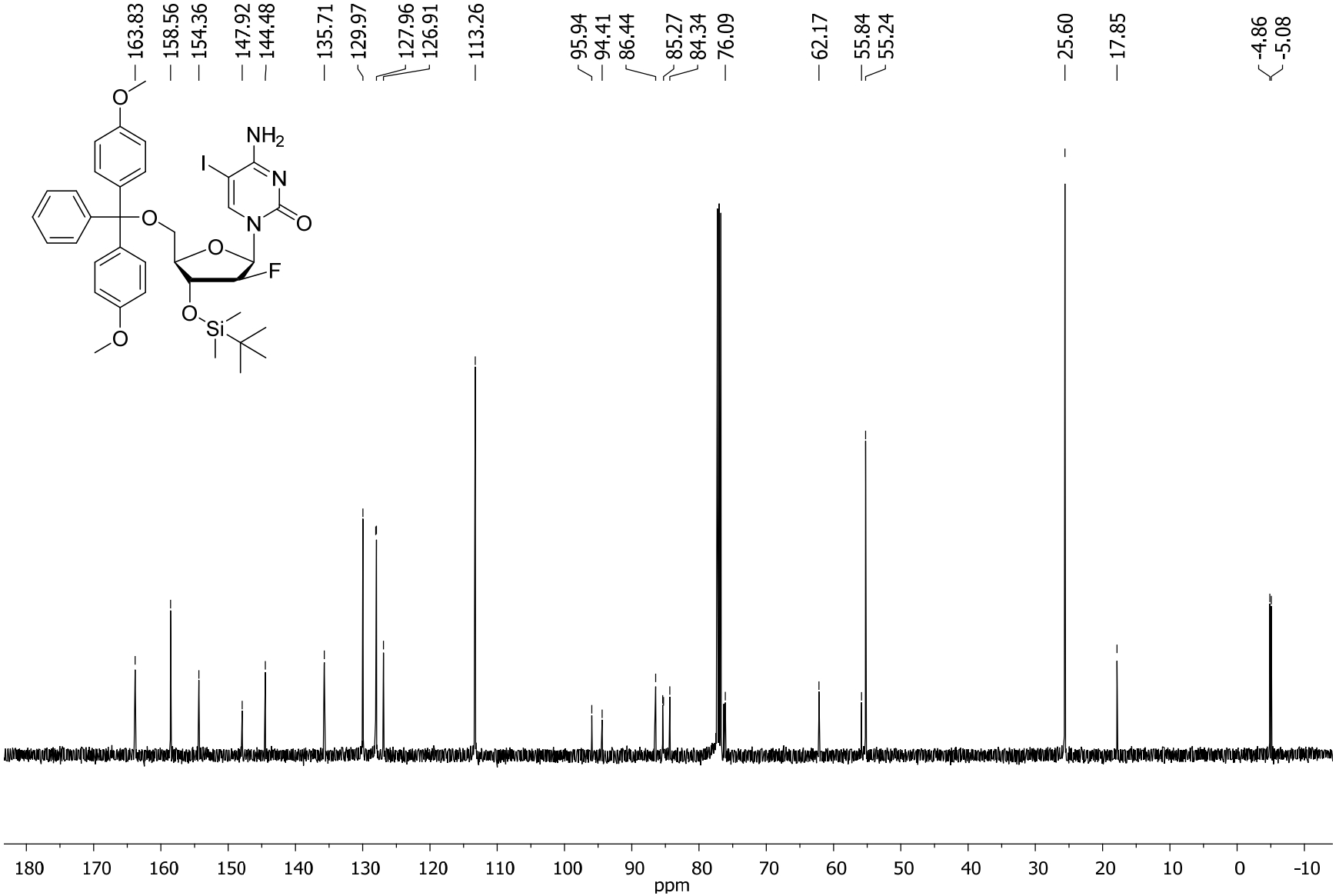


Fig. S16: 500 MHz ^{1}H NMR spectrum of compound **8** in $CDCl_3$

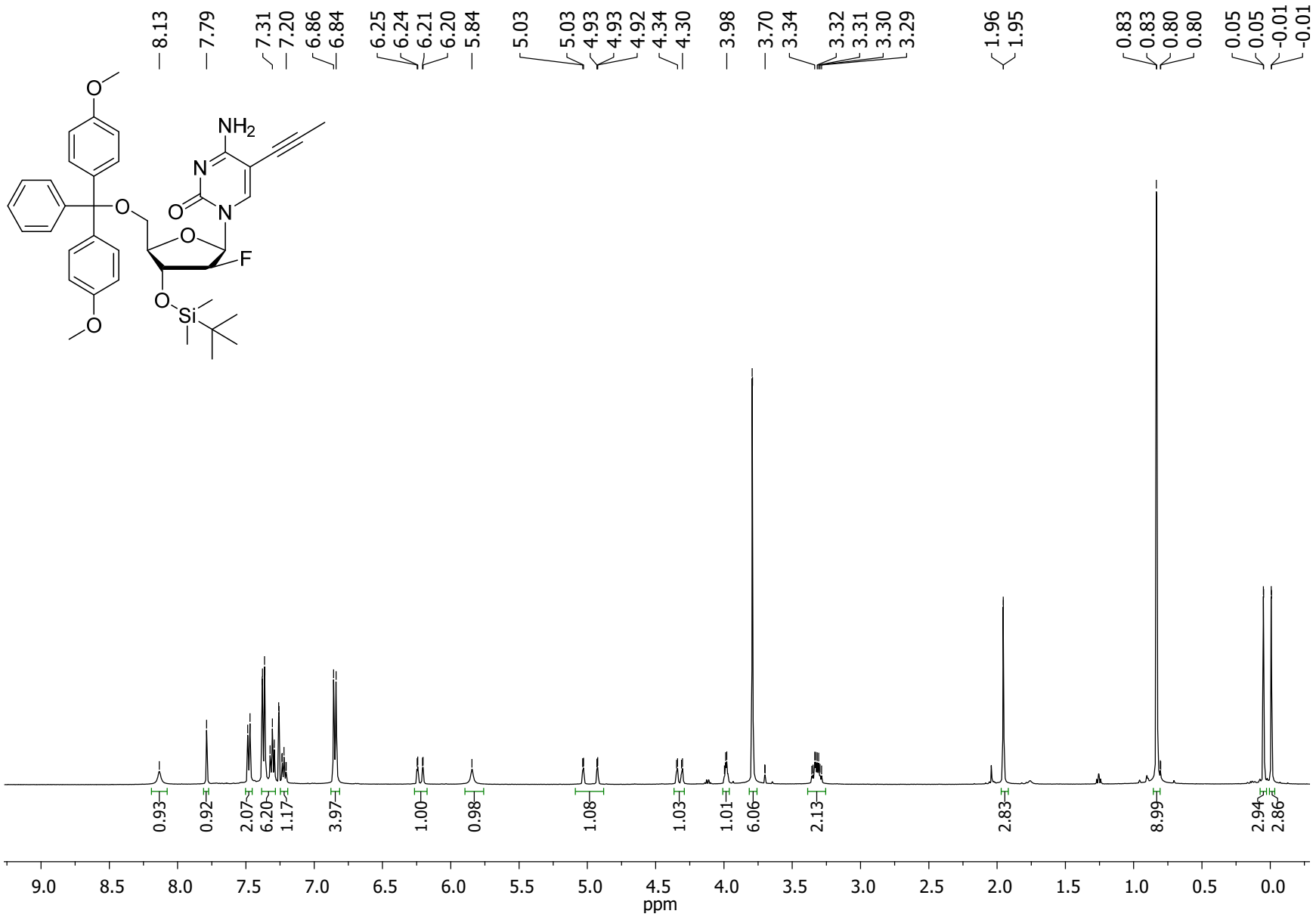


Fig. S17: 470.6 MHz ^{19}F NMR spectrum of compound **8** in $CDCl_3$

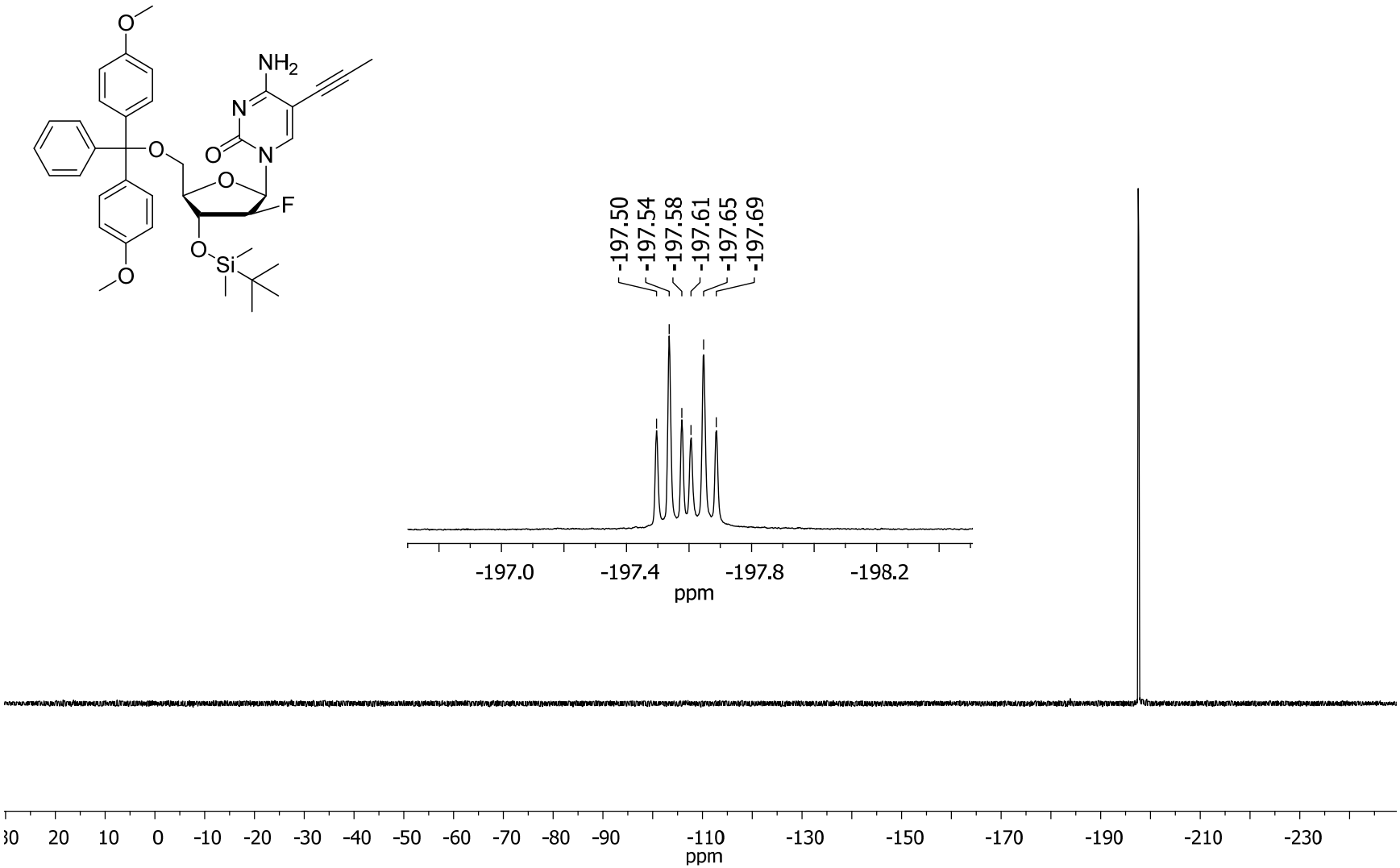


Fig. S18: 125.7 MHz ^{13}C NMR spectrum of compound **8** in $CDCl_3$

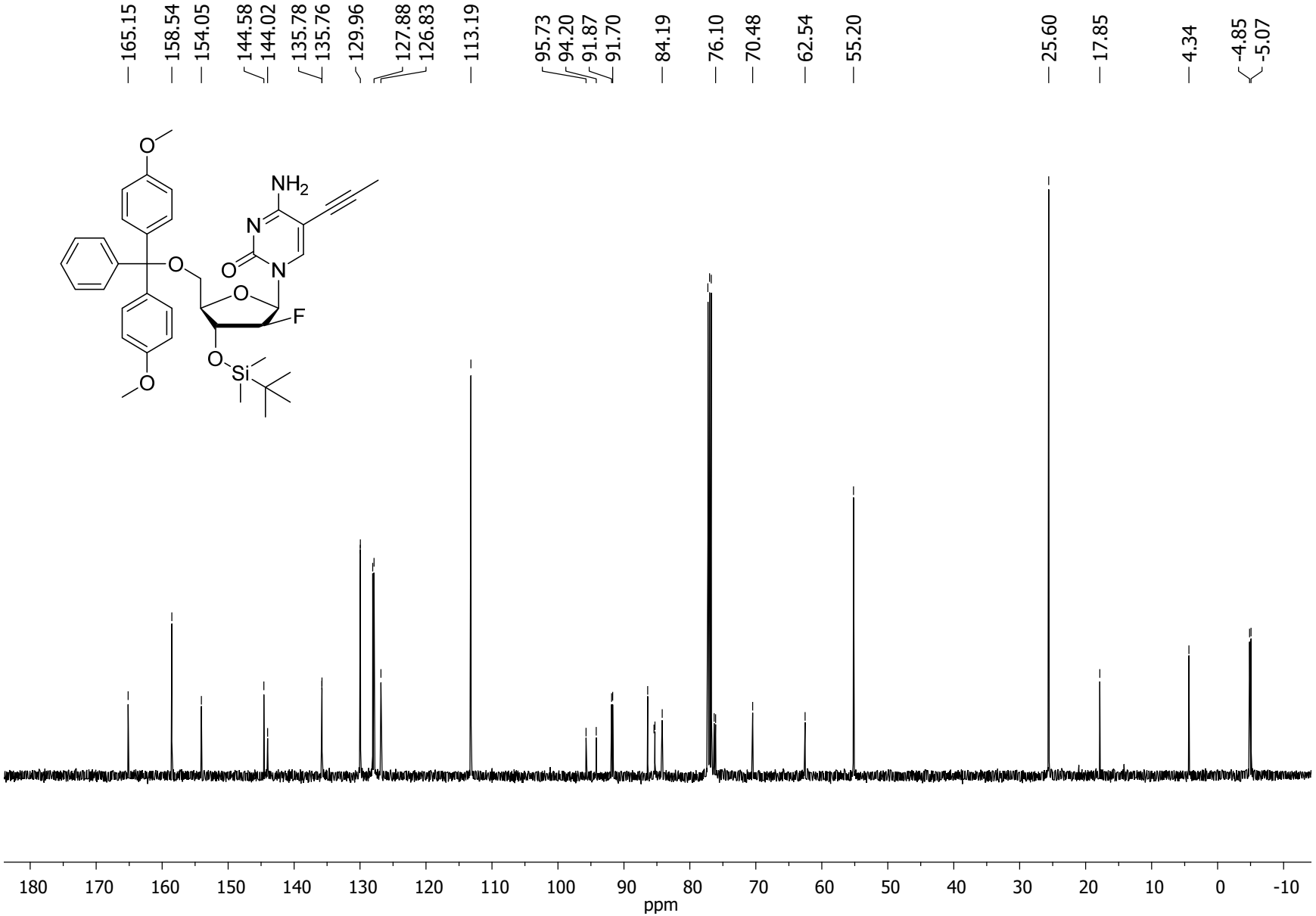


Fig. S19: 500 MHz ^{1}H NMR spectrum of compound **9** in $CDCl_3$

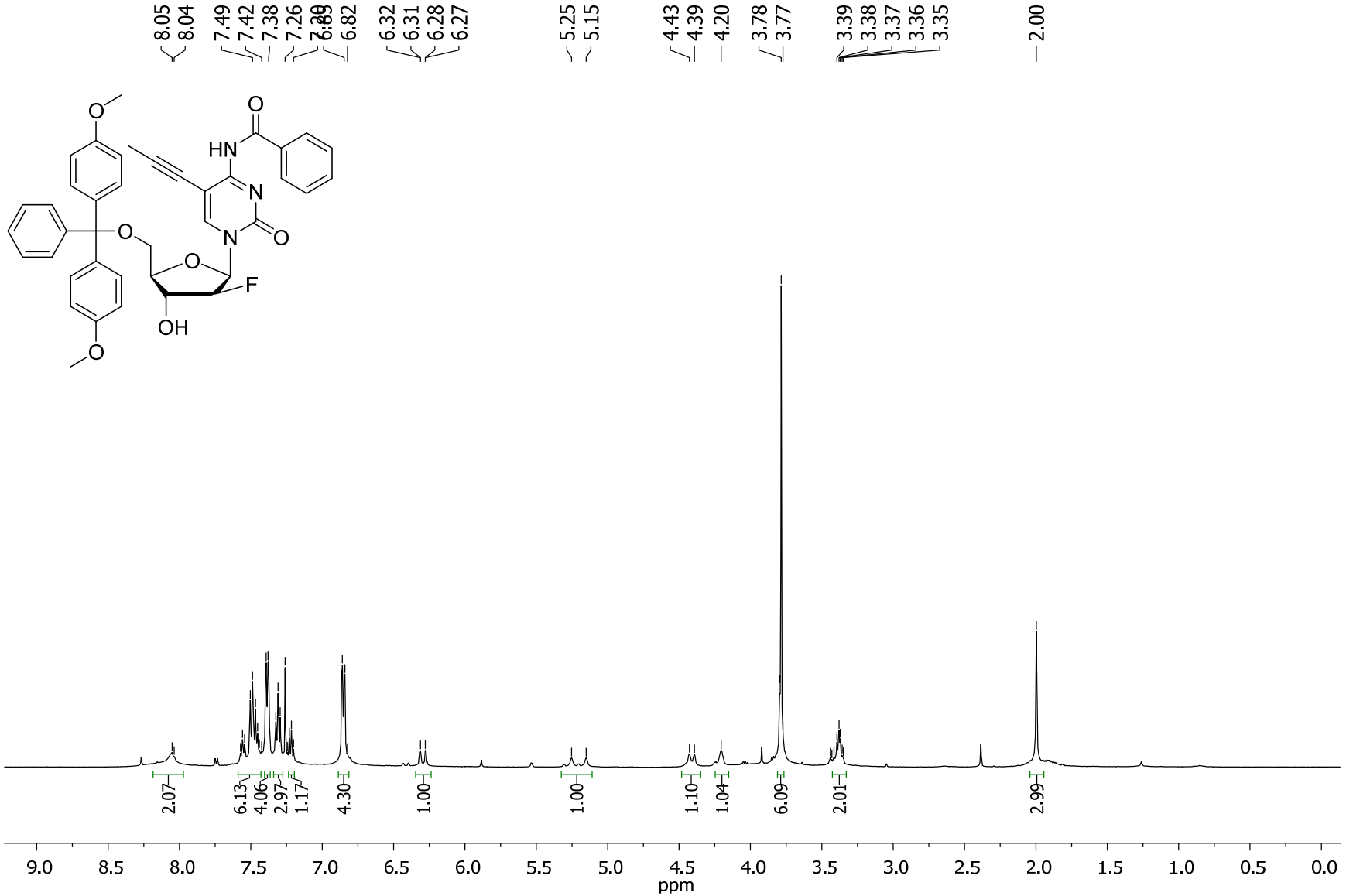


Fig. S20: 470.6 MHz ^{19}F NMR spectrum of compound **9** in $CDCl_3$

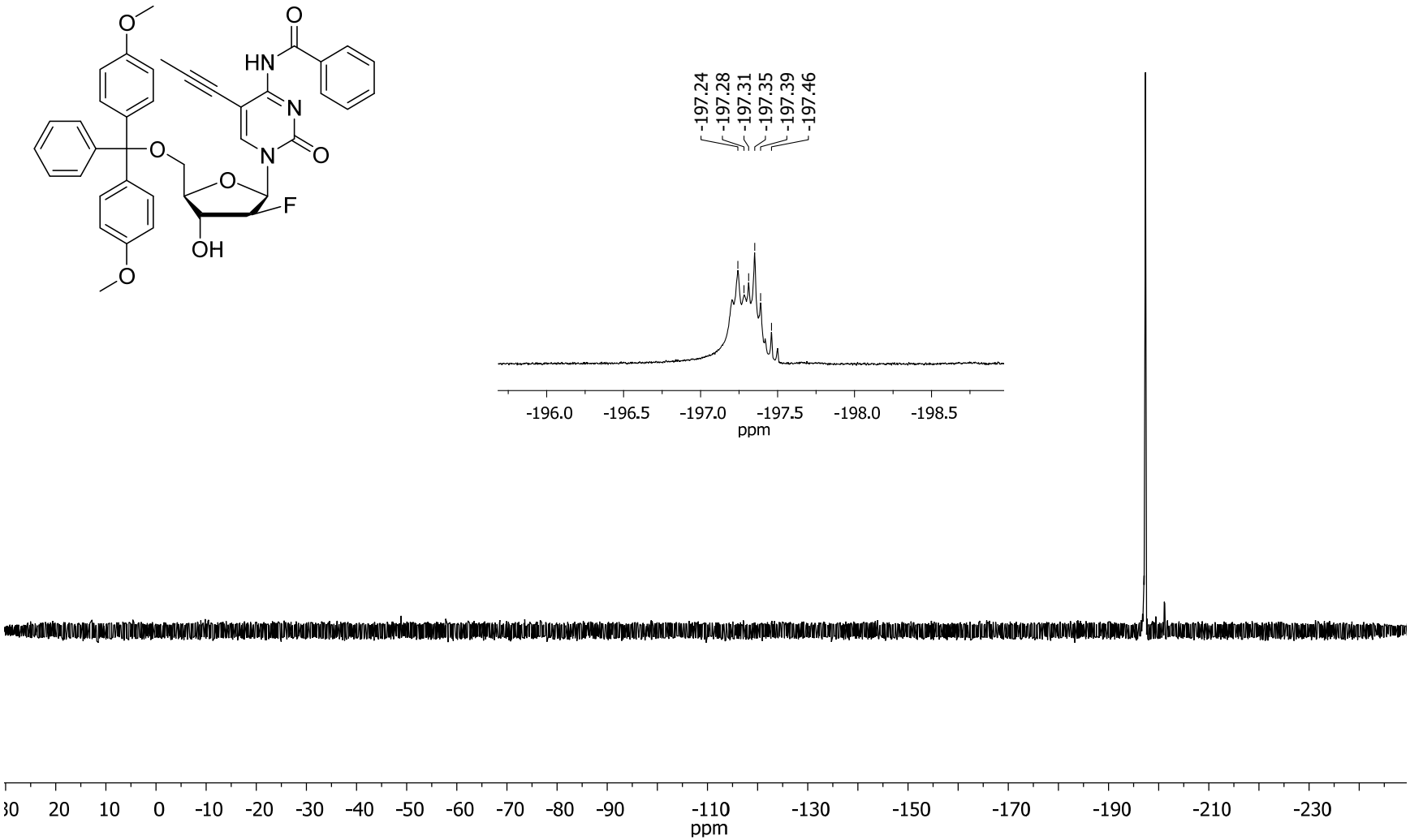


Fig. S21: 125.7 MHz ^{13}C NMR spectrum of compound **9** in $CDCl_3$

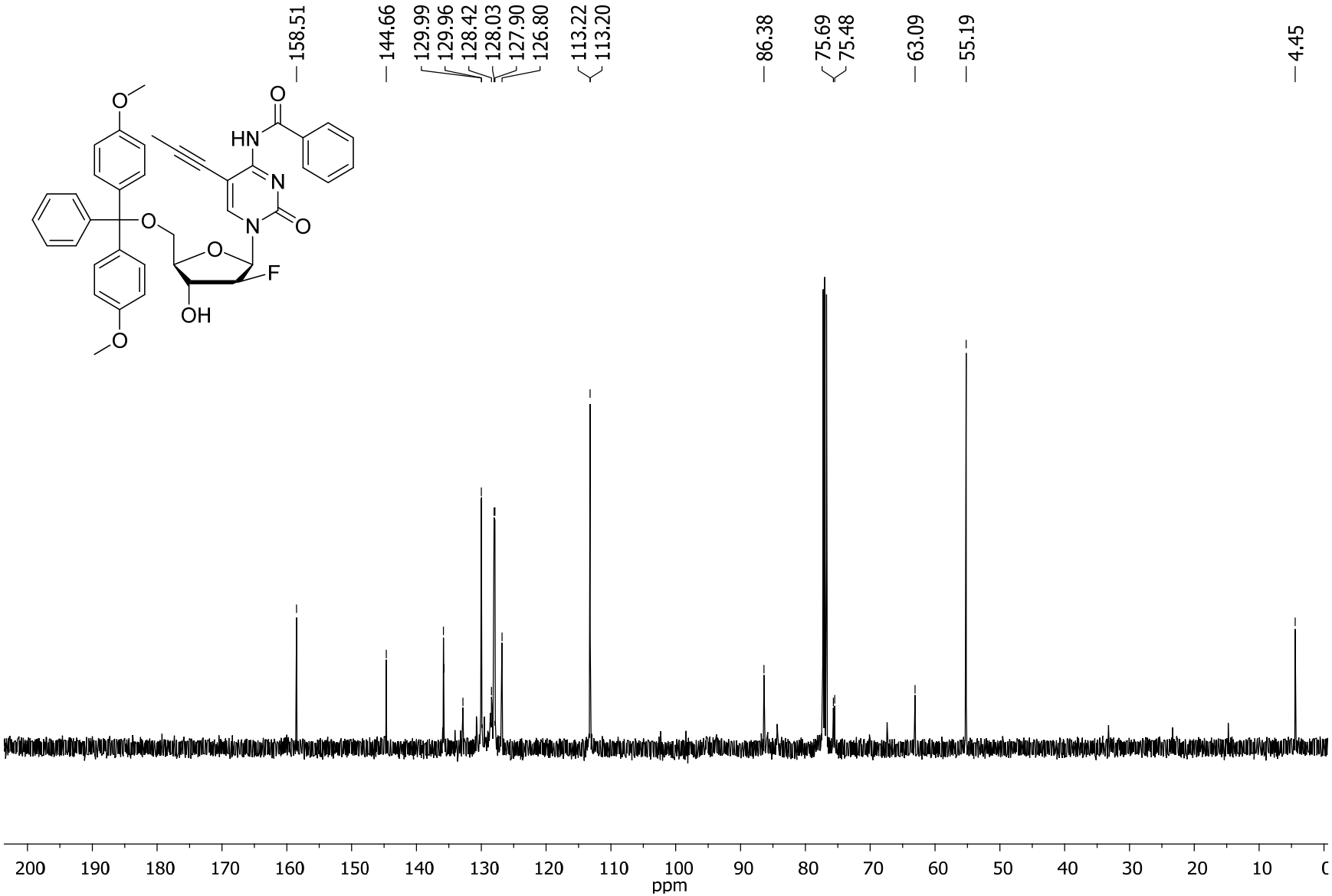


Fig. S22: 500 MHz ^{1}H NMR spectrum of compound **10** in acetone-d_6

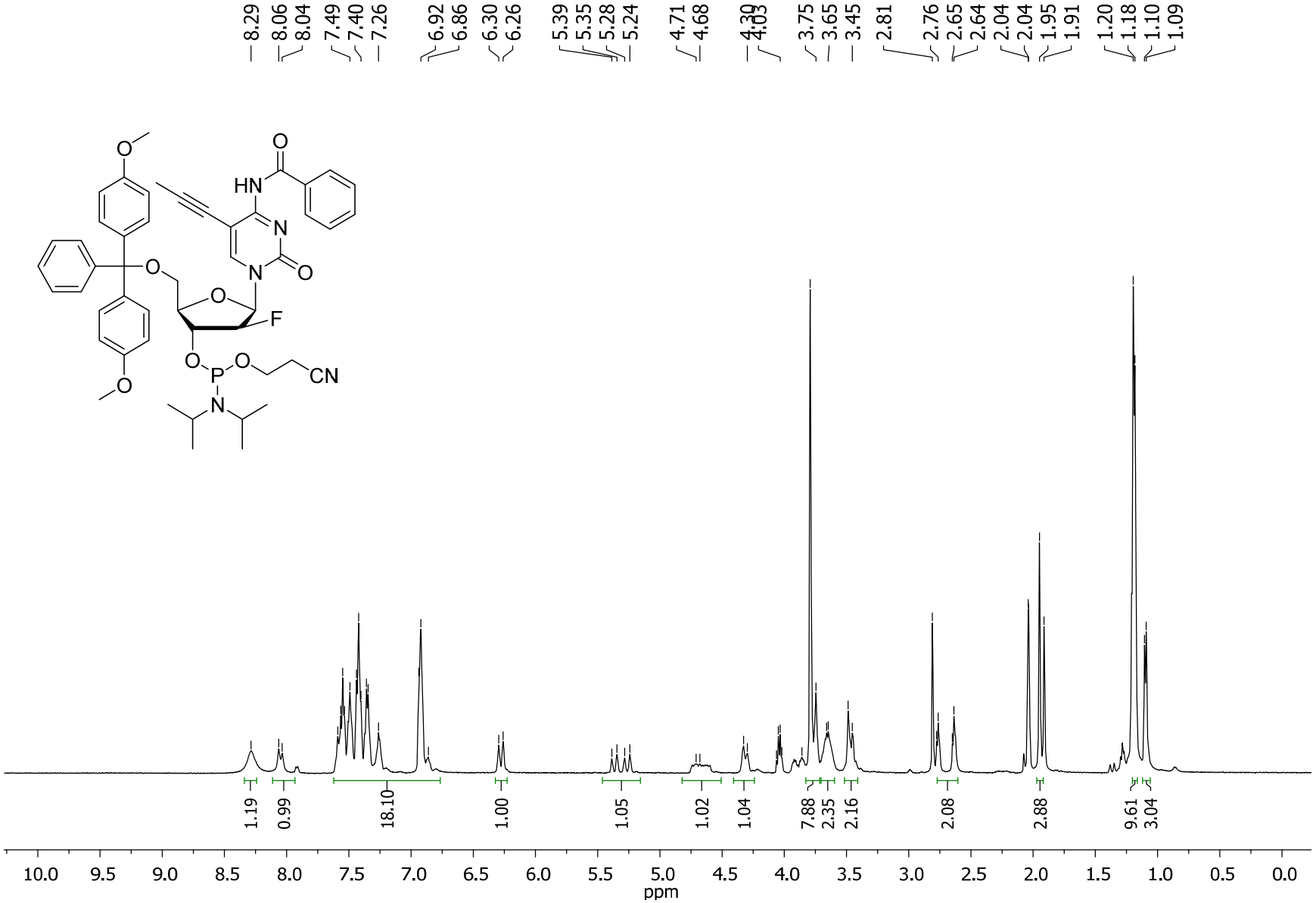


Fig. S23: 470.6 MHz ^{19}F NMR spectrum of compound **10** in acetone-d_6

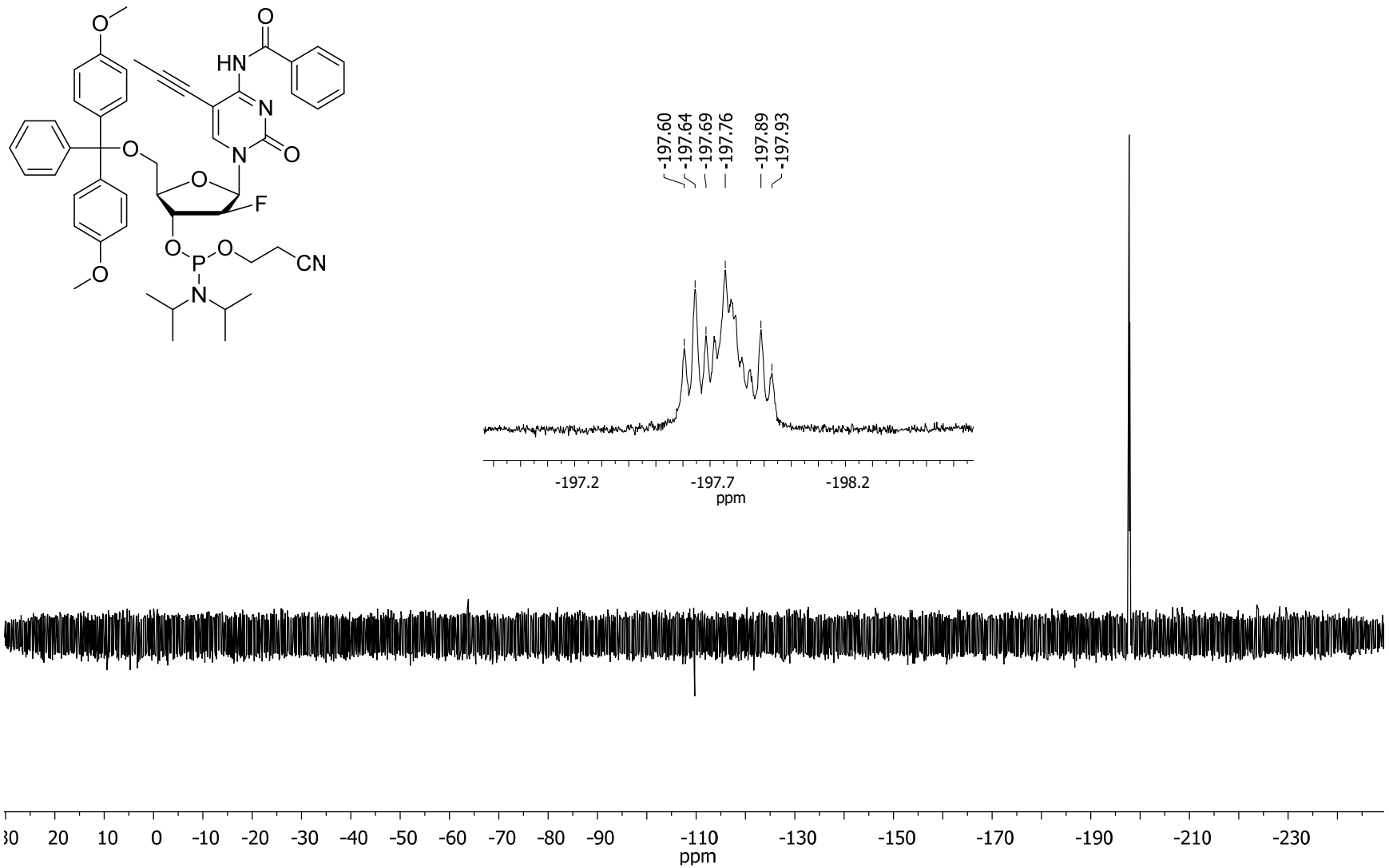


Fig. S24: 202.3 MHz ^{31}P NMR spectrum of compound **10** in acetone-d_6

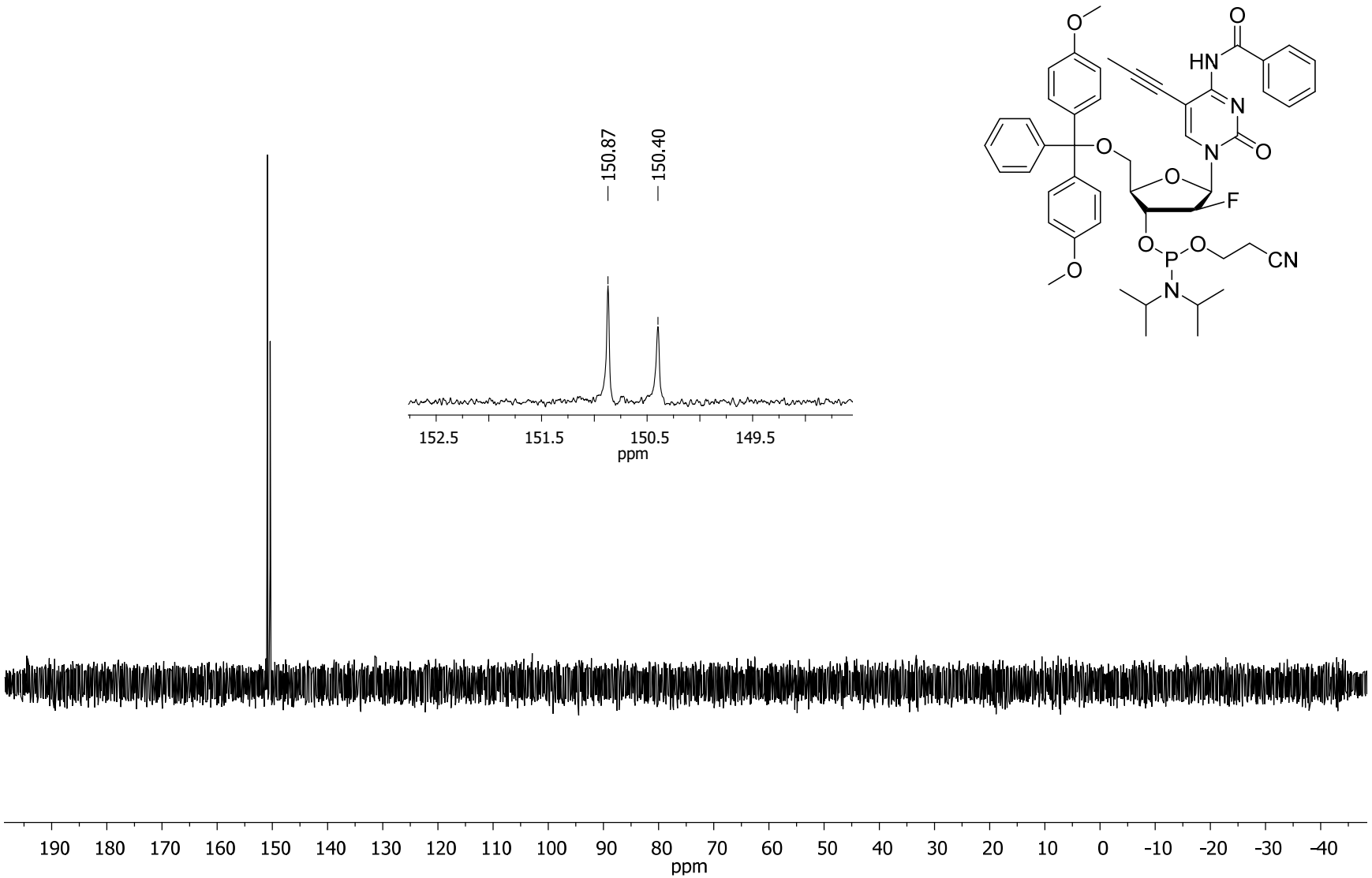


Table S1: List of modified oligonucleotides synthesized in this work.

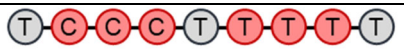
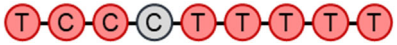
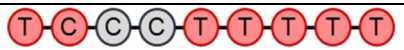
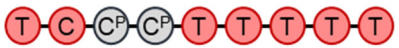
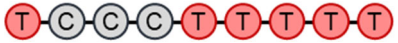
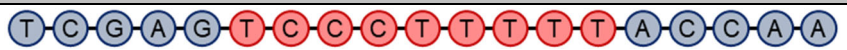
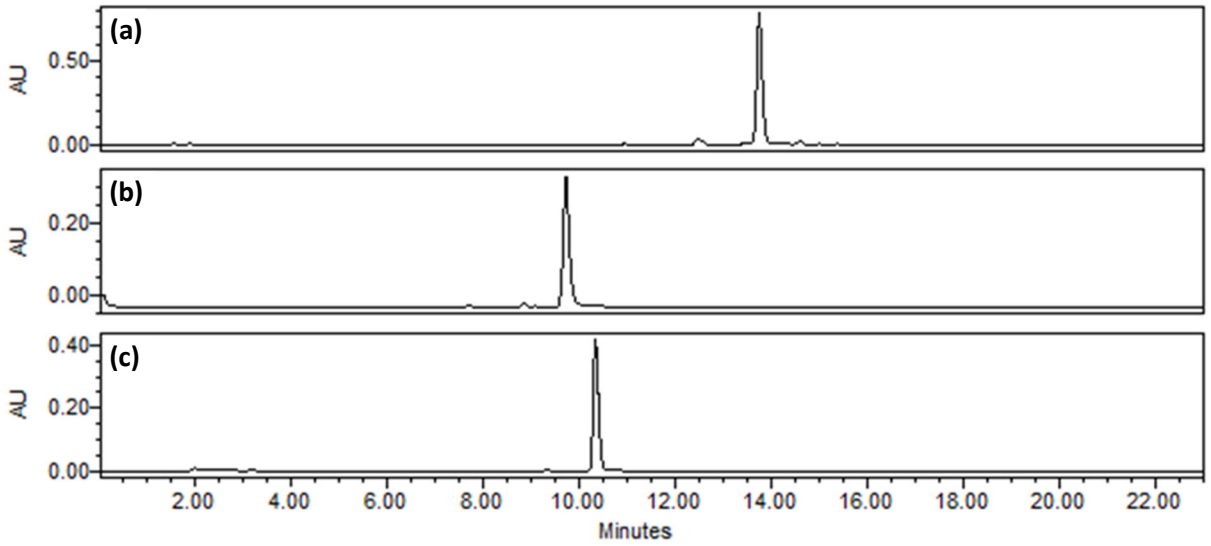
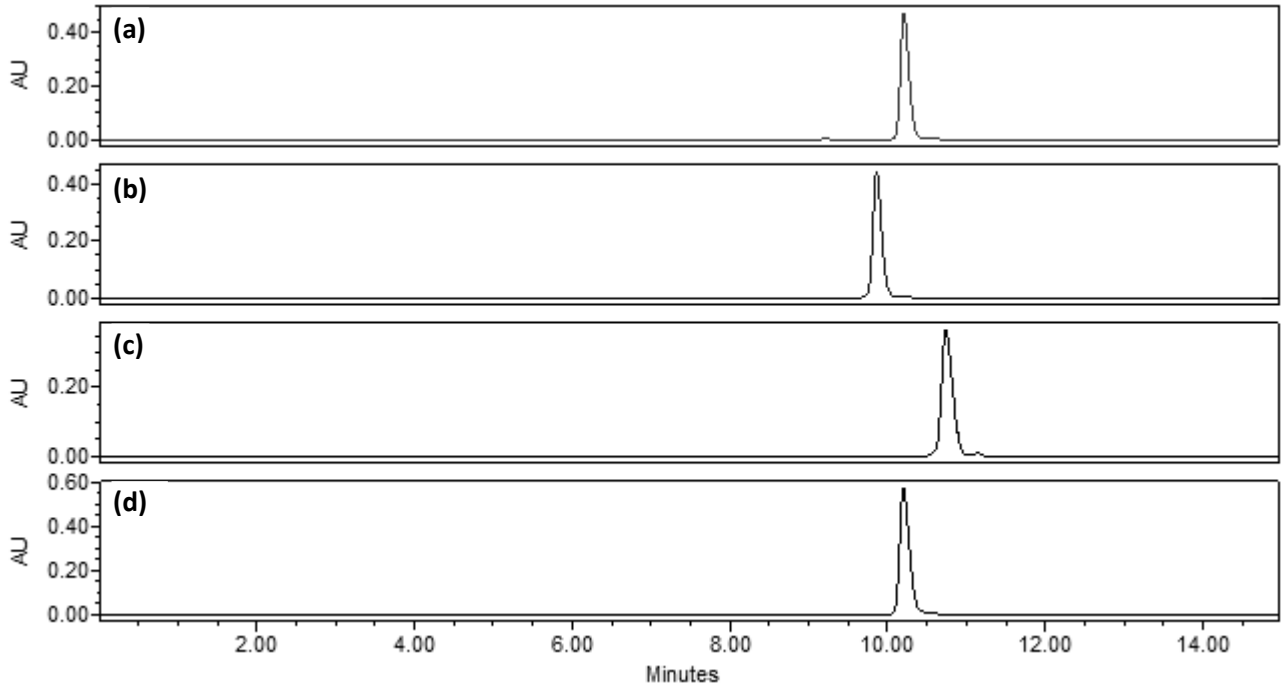
Identifier	Sequence (5' → 3')
Ctrl DNA	
ON 1a	
ON 1b	
ON 2a	
ON 2b	
ON 3a	
ON 3b	
ON 4a	
ON 4b	
ON 5a	
ON 5b	
ON 6a	
ON 6b	
ON 7a	
ON 7b	
Gapmers	
G-DNA	
G-FANA	
G-FANA^{P}	
G-ANA^{P}	

Fig. S25: SAX-HPLC chromatograms of purified (**a**) target 9mer RNA**,** (**b**) target 9mer DNA and (**c**) **Ctrl DNA**.



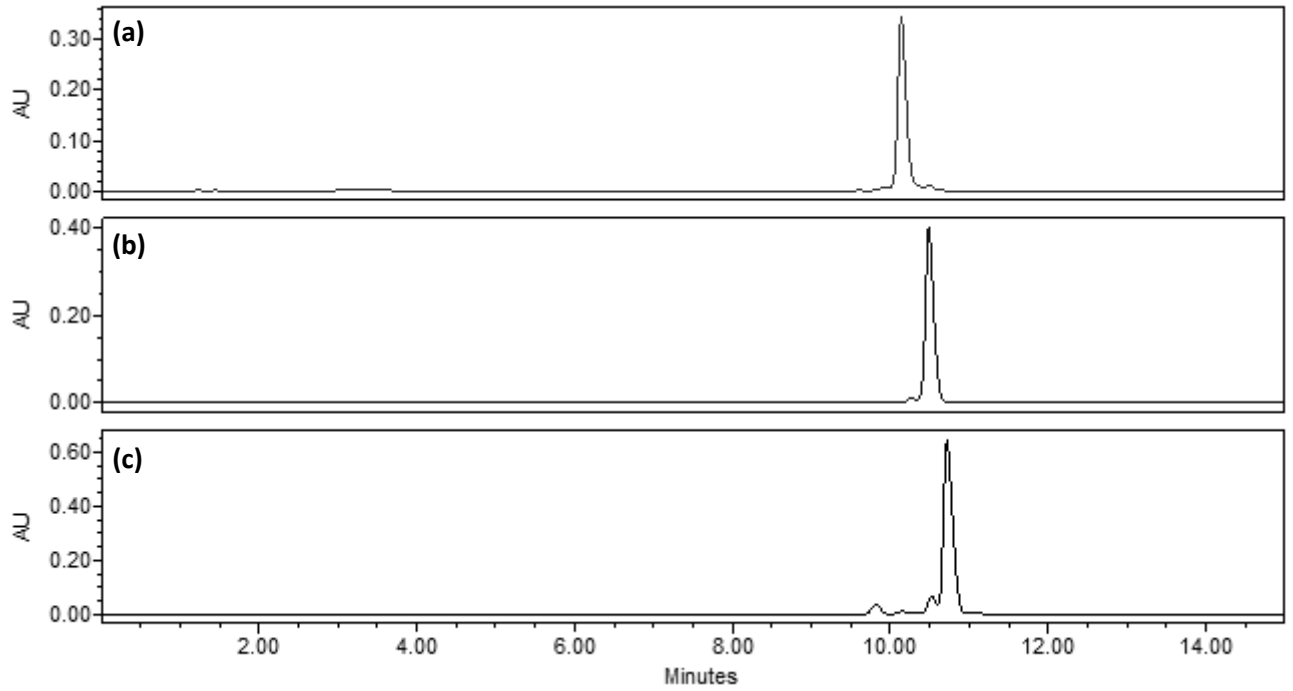
SAX-HPLC chromatograms of synthesized ONs after purification. See experimental section for synthesis and purification procedures. Elution gradient: 0 – 48 %B over 22 minutes. Buffer A: 10 % ACN (v/v), 0.1 M Tris, pH 7.4. Buffer B: 10 % ACN (v/v), 0.1 M Tris, 1.0 M NaCl, pH 7.4.

Fig. S26: SAX-HPLC chromatograms of purified (**a**) **ON 1a**, (**b**) **ON 2a**, (**c**) **ON 3a** and (**d**) **ON 4a.**



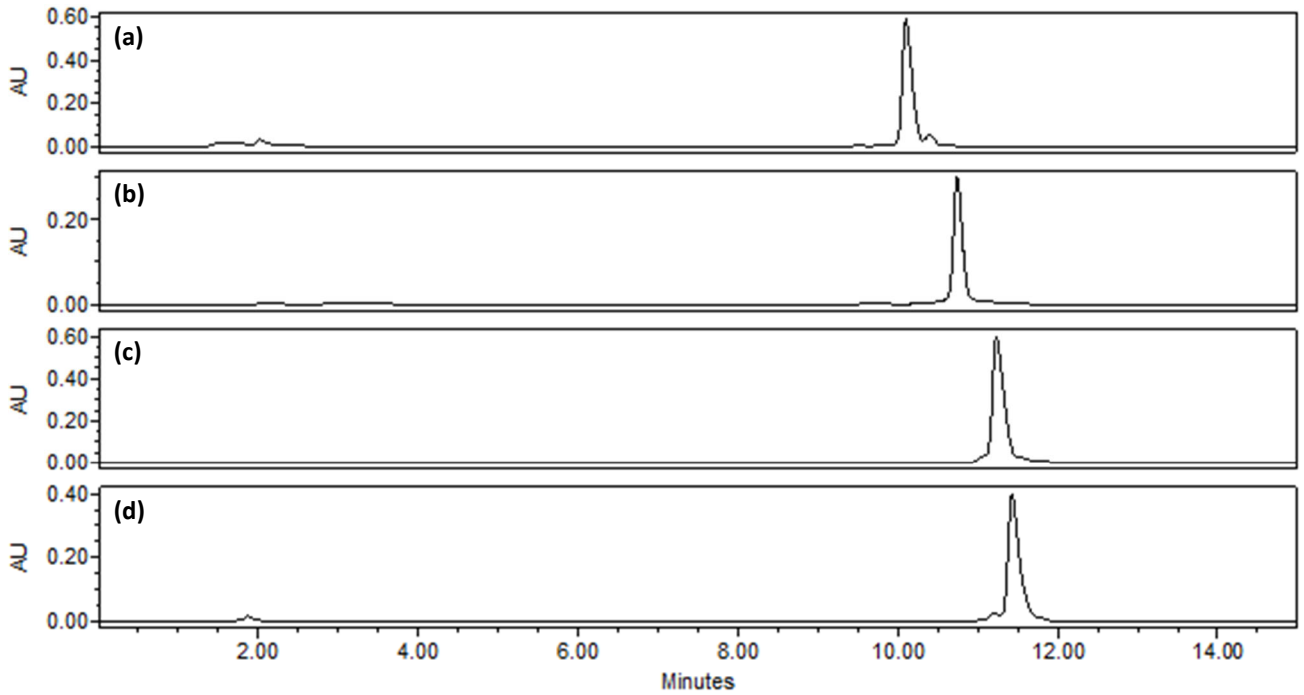
Pure SAX-HPLC chromatograms of synthesized ONs after purification. See experimental section for synthesis and purification procedures. Elution gradient: 0 – 30 %B over 14 minutes. Buffer A: 10 % ACN (v/v), 0.1 M Tris, pH 7.4. Buffer B: 10 % ACN (v/v), 0.1 M Tris, 1.0 M NaCl, pH 7.4.

Fig. S27: SAX-HPLC chromatograms of purified (**a**) **ON 5a**, (**b**) **ON 6a** and (**c**) **ON 7a**.



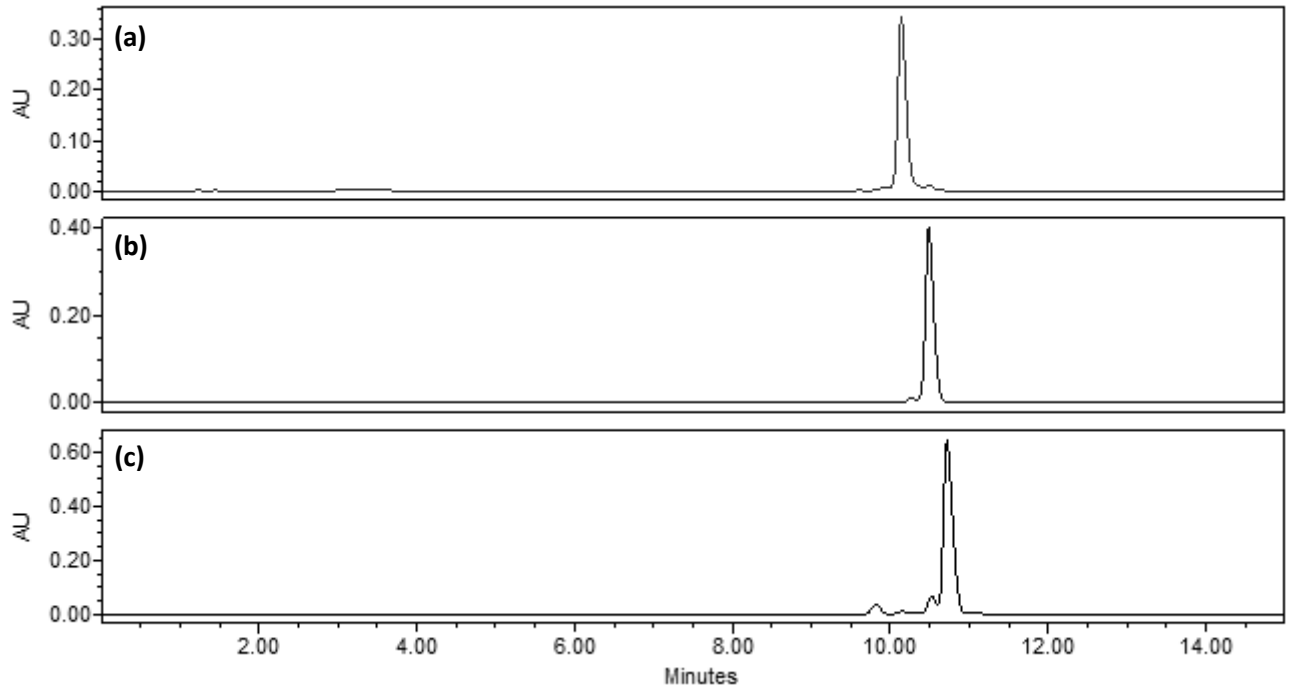
SAX-HPLC chromatograms of synthesized ONs after purification. See experimental section for synthesis and purification procedures. Elution gradient: 0 – 30 %B over 14 minutes. Buffer A: 10 % ACN (v/v), 0.1 M Tris, pH 7.4. Buffer B: 10 % ACN (v/v), 0.1 M Tris, 1.0 M NaCl, pH 7.4.

Fig. S28: SAX-HPLC chromatograms of purified (**a**) **ON 1b**, (**b**) **ON 2b**, (**c**) **ON 3b** and (**d**) **ON 4b.**



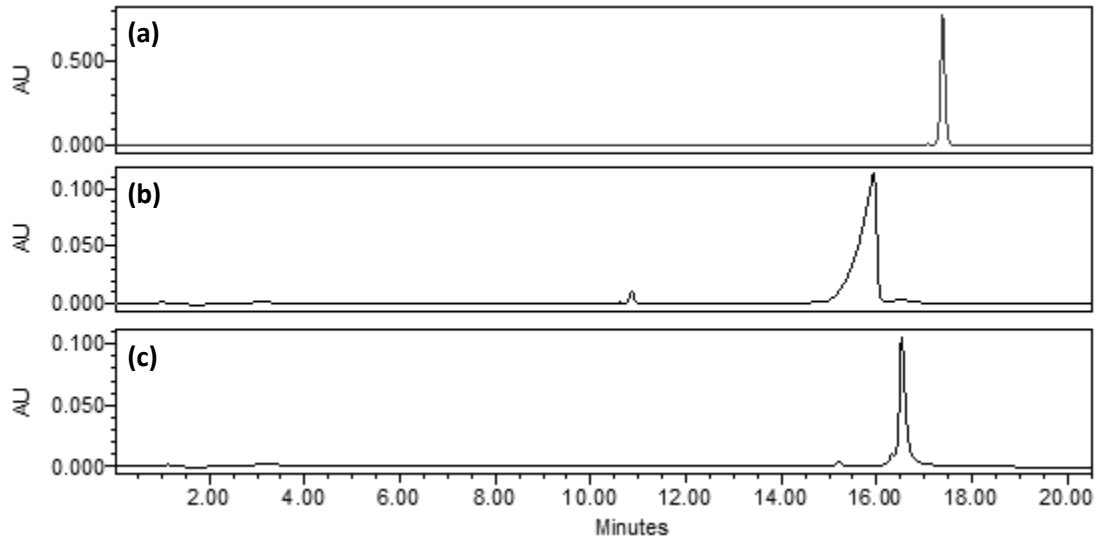
SAX-HPLC chromatograms of synthesized ONs after purification. See experimental section for synthesis and purification procedures. Elution gradient: 0 – 30 %B over 14 minutes. Buffer A: 10 % ACN (v/v), 0.1 M Tris, pH 7.4. Buffer B: 10 % ACN (v/v), 0.1 M Tris, 1.0 M NaCl, pH 7.4.

Fig. S29: SAX-HPLC chromatograms of purified (**a**) **ON 5b**, (**b**) **ON 6b** and (**c**) **ON 7b**.



SAX-HPLC chromatograms of synthesized ONs after purification. See experimental section for synthesis and purification procedures. Elution gradient: 0 – 30 %B over 14 minutes. Buffer A: 10 % ACN (v/v), 0.1 M Tris, pH 7.4. Buffer B: 10 % ACN (v/v), 0.1 M Tris, 1.0 M NaCl, pH 7.4.

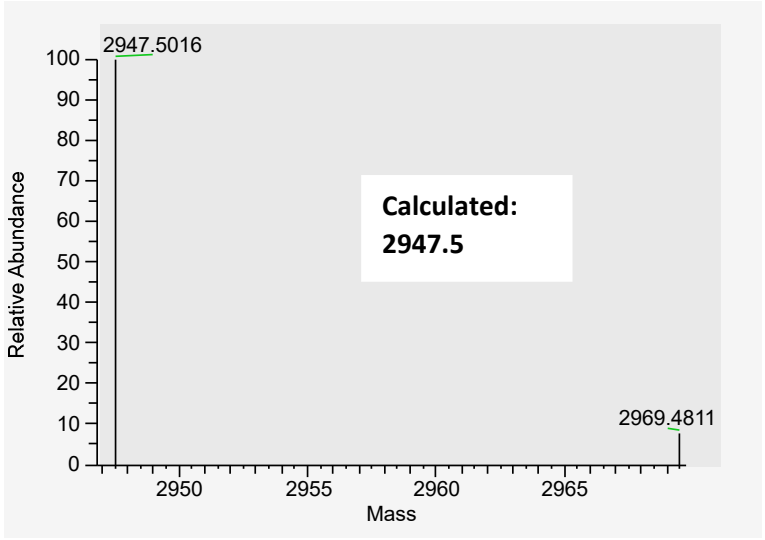
Fig. S30: SAX-HPLC chromatograms of purified (**a**) **G-DNA**, (**b**) **G-FANA** and (**c**) **G-FANA^{P}**.



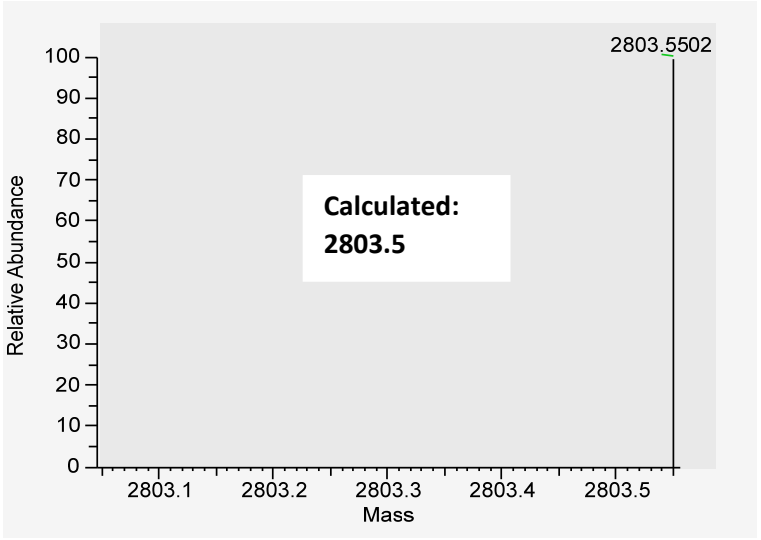
SAX-HPLC chromatograms of synthesized gapmers after purification. See experimental section for synthesis and purification procedures. Elution gradient: 0 – 48%B over 22 minutes. Buffer A: 10 % ACN (v/v), 0.1 M Tris, pH 7.4. Buffer B: 10 % ACN (v/v), 0.1 M Tris, 1.0 M NaCl, pH 7.4.

Fig. S31: Deconvoluted ESI MS of (**a**) target 9mer RNA, (**b**) target 9mer DNA and (**c**) **Ctrl DNA**.

(a)



(b)



(c)

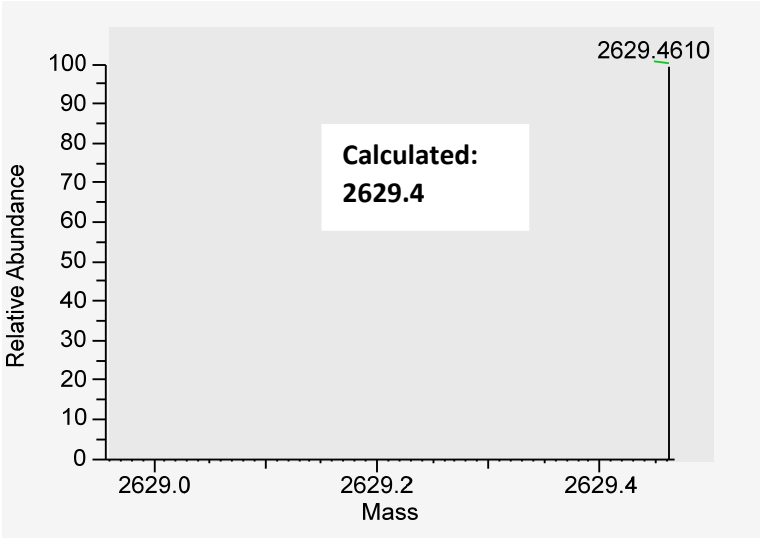
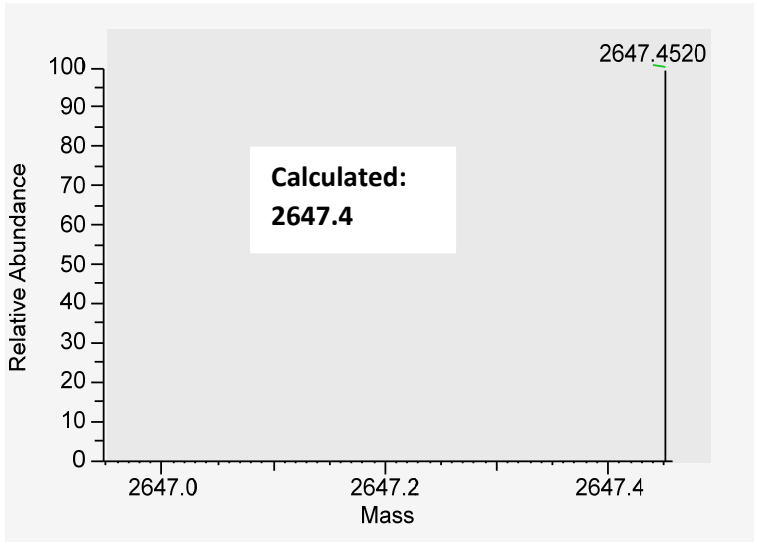
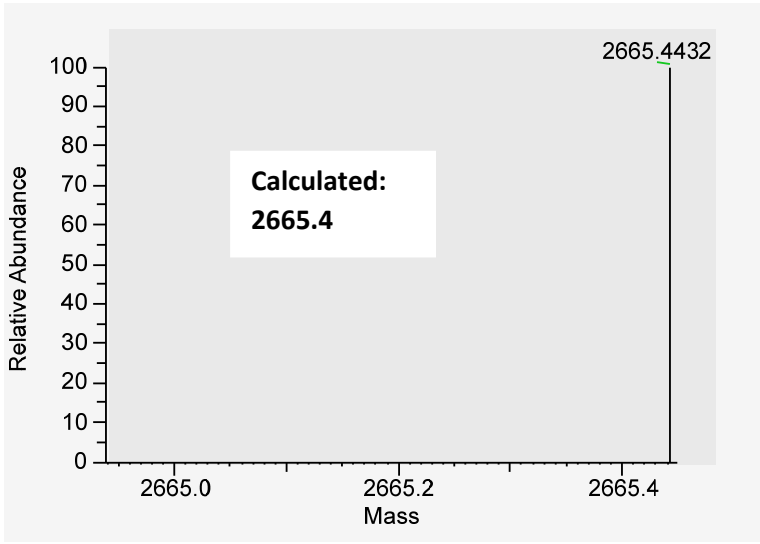


Fig. S32: Deconvoluted ESI MS of (**a**) **ON 1a**, (**b**) **ON 2a**, (**c**) **ON 3a** and (**d**) **ON 4a**.

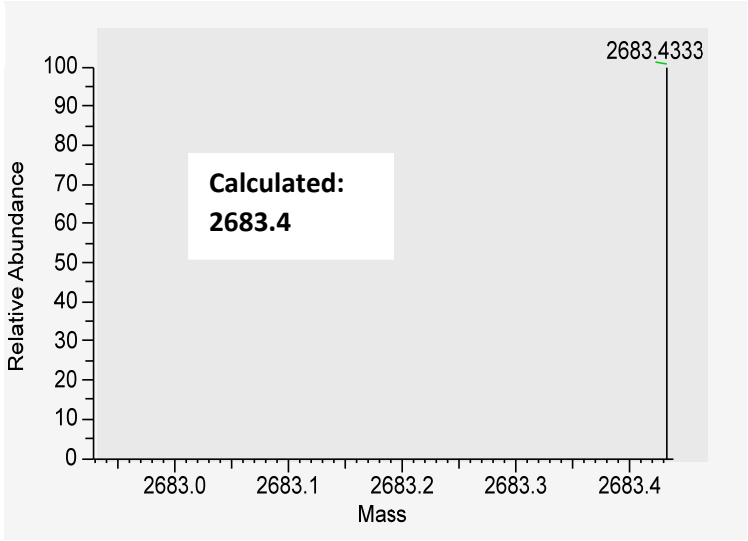
(a)



(b)



(c)



(d)

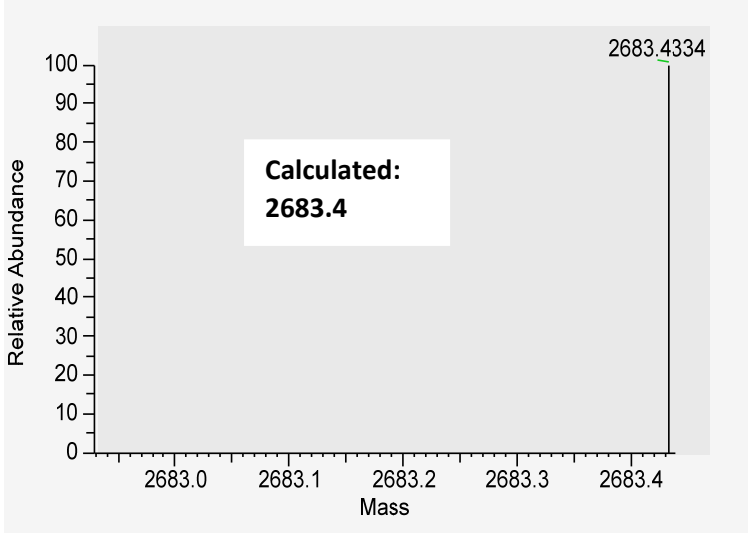
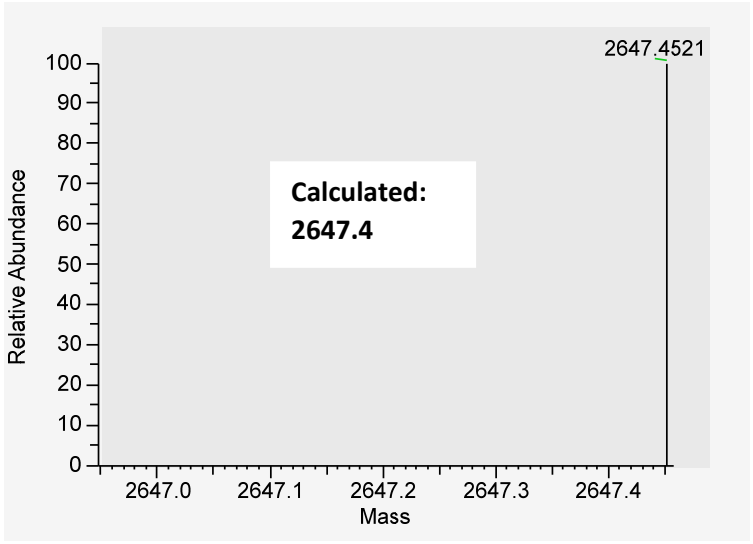
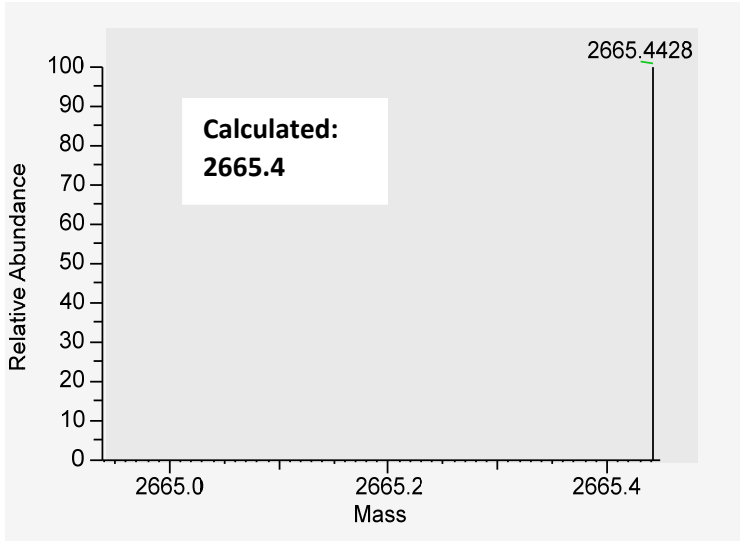


Fig. S33: Deconvoluted ESI MS of (**a**) **ON 5a**, (**b**) **ON 6a**, (**c**) **ON 7a** and (**d**) **G-FANA**.

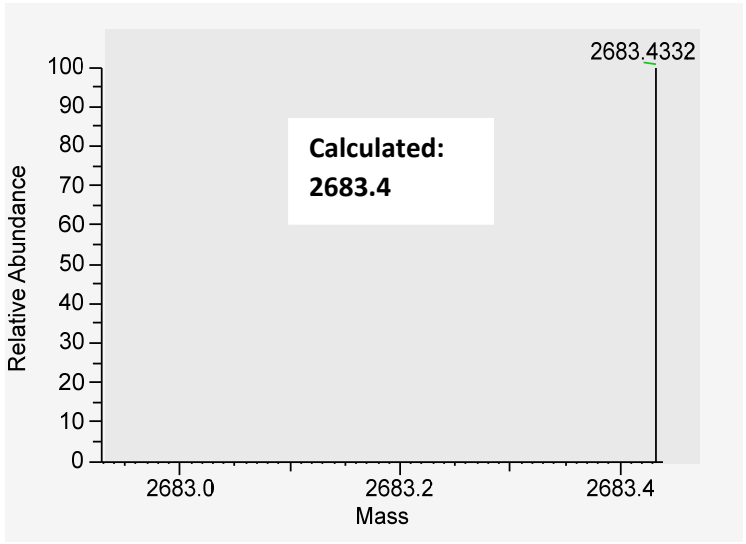
(a)



(b)



(c)



(d)

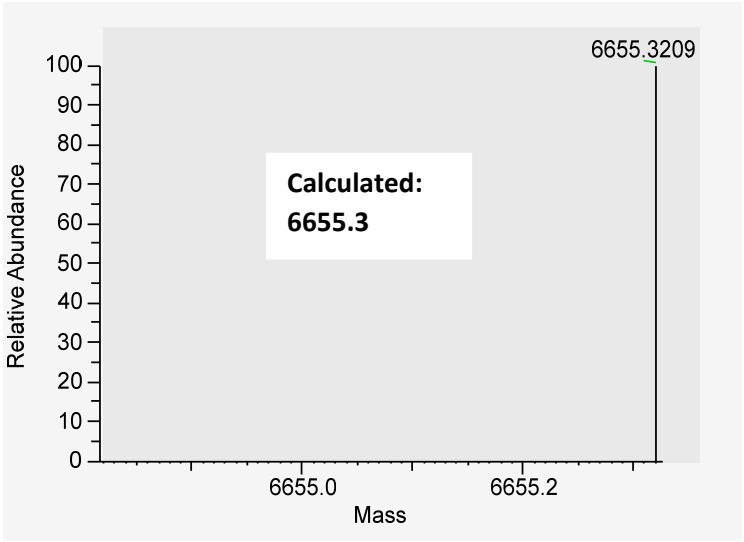
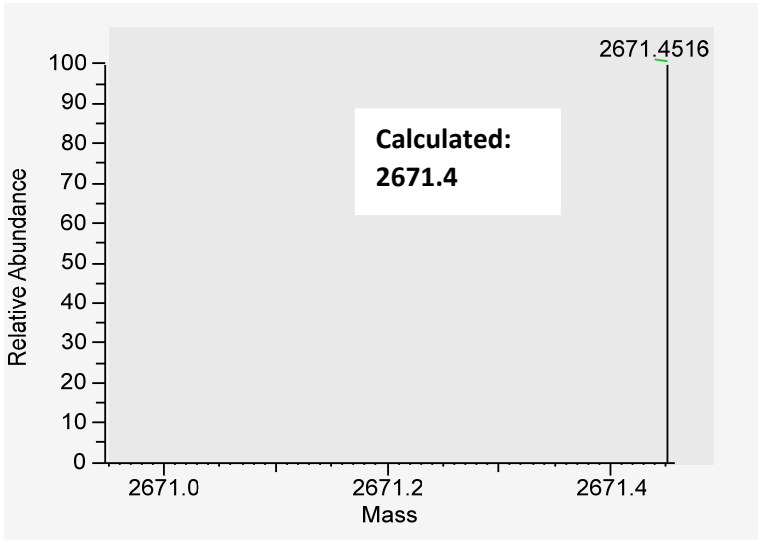
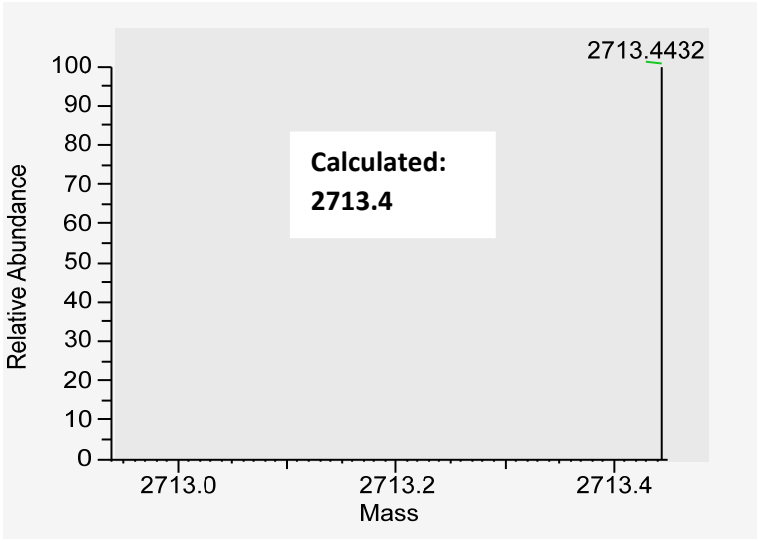


Fig. S34: Deconvoluted ESI MS of (**a**) **ON 1b**, (**b**) **ON 2b**, (**c**) **ON 3b** and (**d**) **ON 4b**.

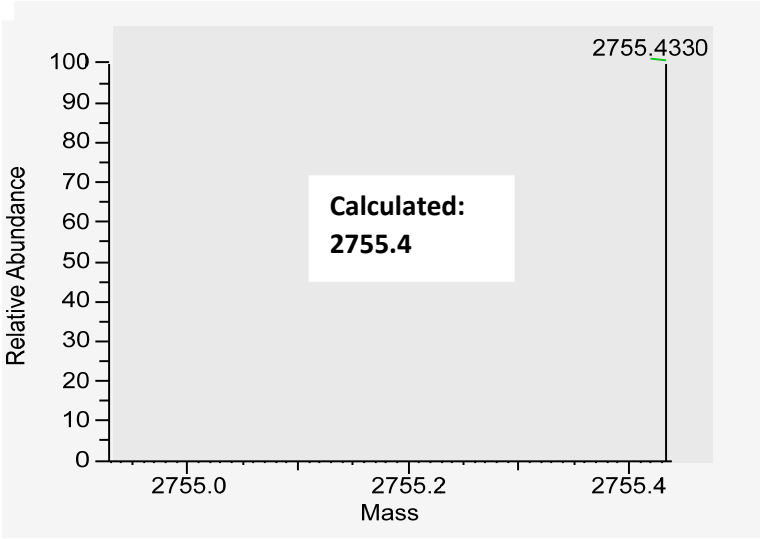
(a)



(b)



(c)



(d)

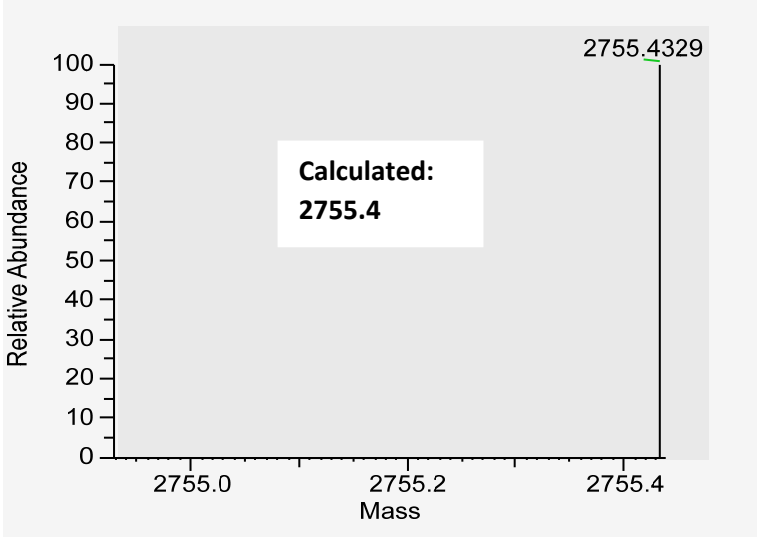


Fig. S35: Deconvoluted ESI MS of (**a**) **ON 5b**, (**b**) **ON 6b**, (**c**) **ON 7b**, and (**d**) **G-FANA^{P}**.

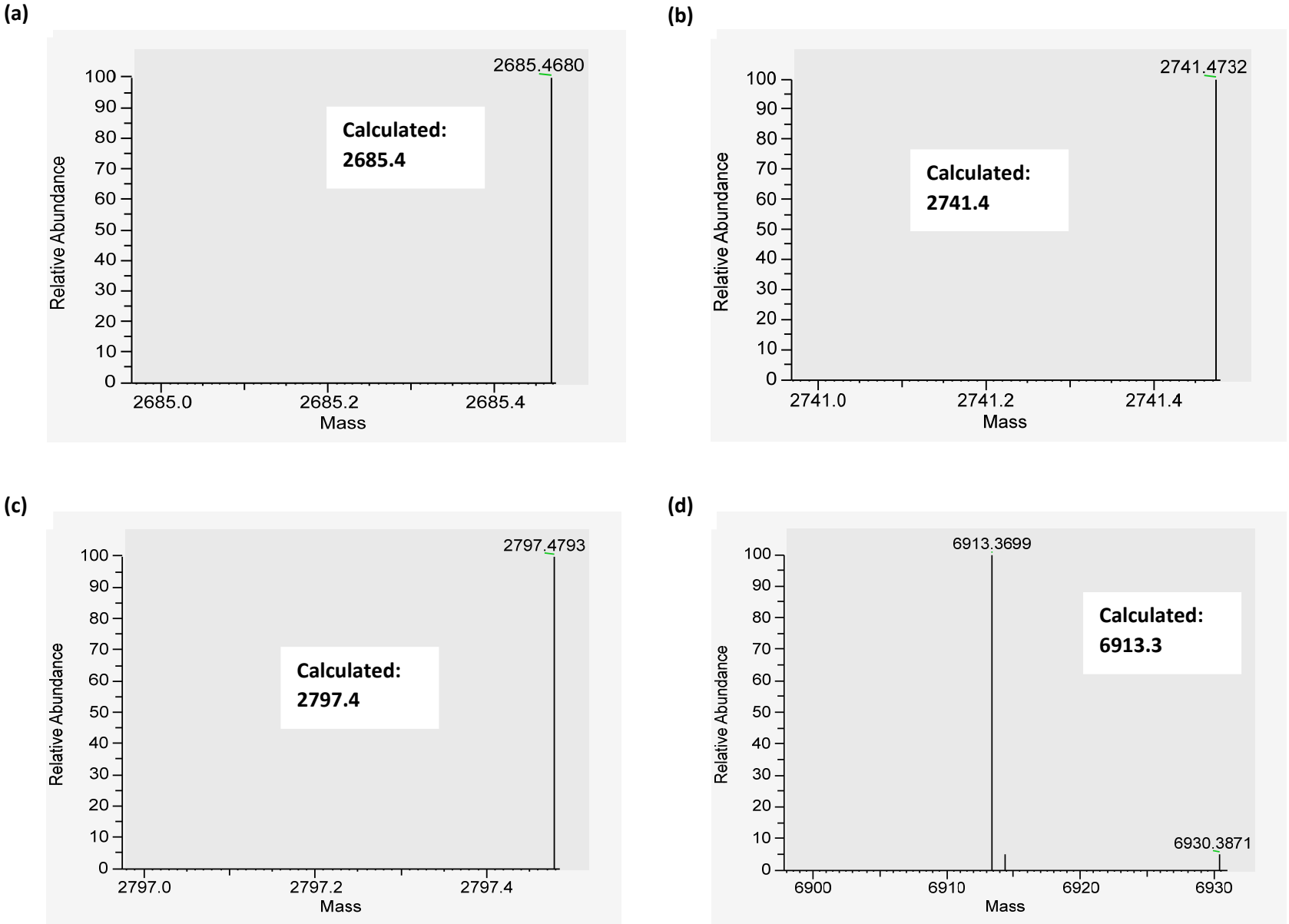
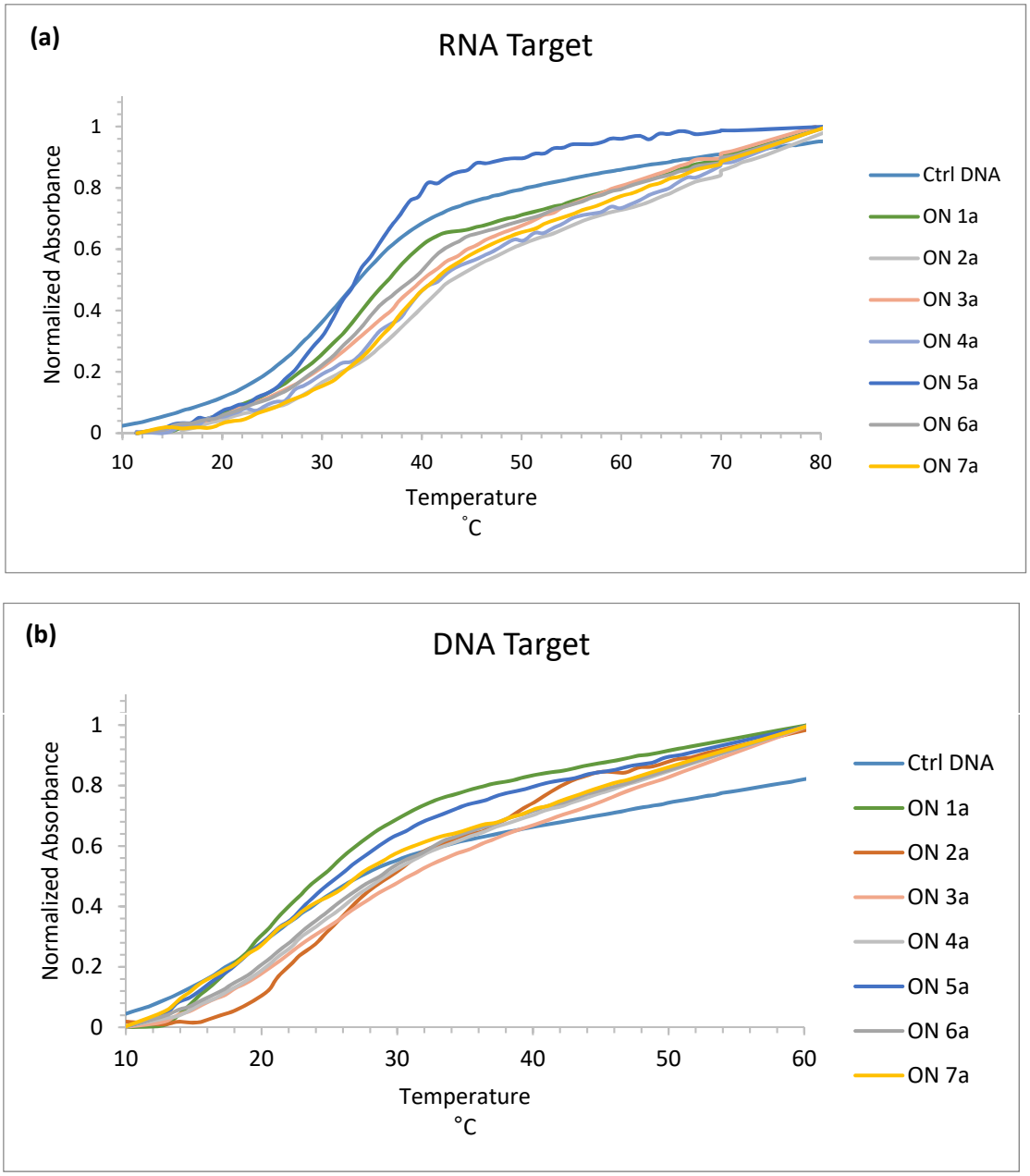
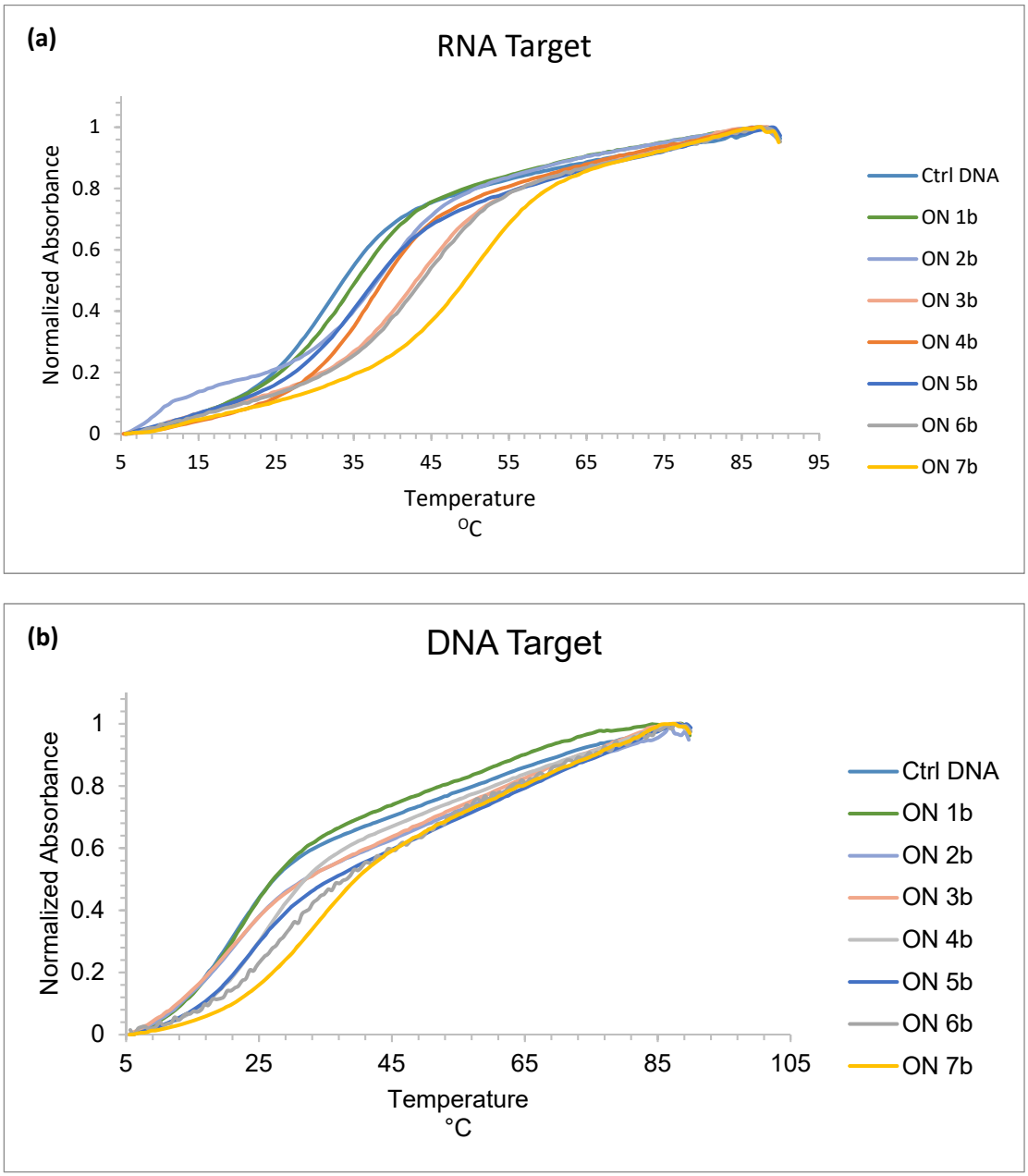


Fig. S36: UV thermal denaturation profiles of **Ctrl DNA** and **ON 1a-7a** with (**a**) target RNA (**b**) and target DNA.



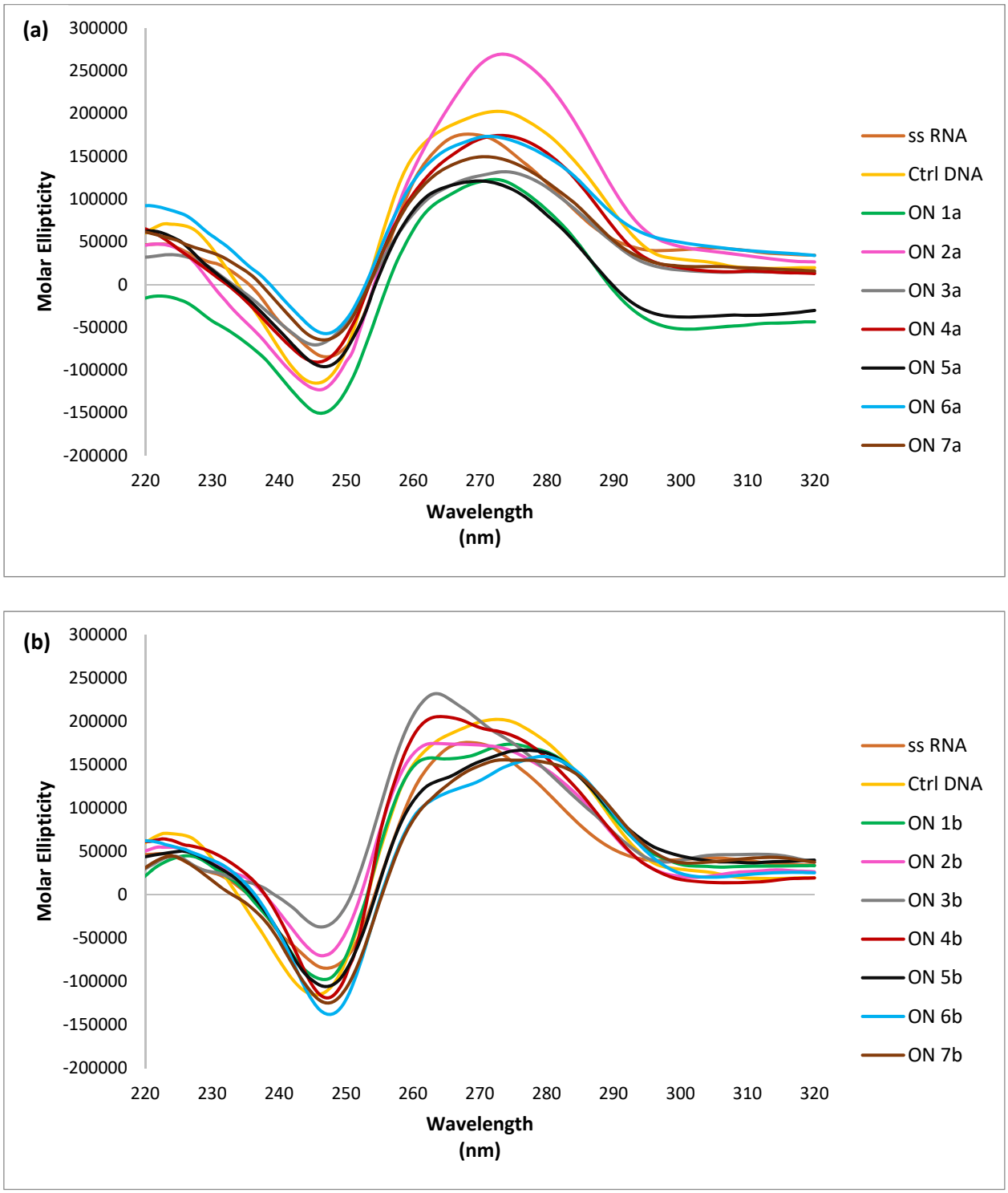
UV thermal denaturation was performed on duplexes at 260 nm in 90 mM sodium chloride, 10 mM sodium phosphate, 1 mM EDTA buffer (pH 7.0) at a final duplex concentration of 2.5 µM.

Fig. S37: UV thermal denaturation profiles of **Ctrl DNA** and **ON 1b-7b** with (**a**) target RNA and (**b**) target DNA.



UV thermal denaturation was performed on duplexes at 260 nm in 90 mM sodium chloride, 10 mM sodium phosphate, 1 mM EDTA buffer (pH 7.0) at a final duplex concentration of 2.5 µM.

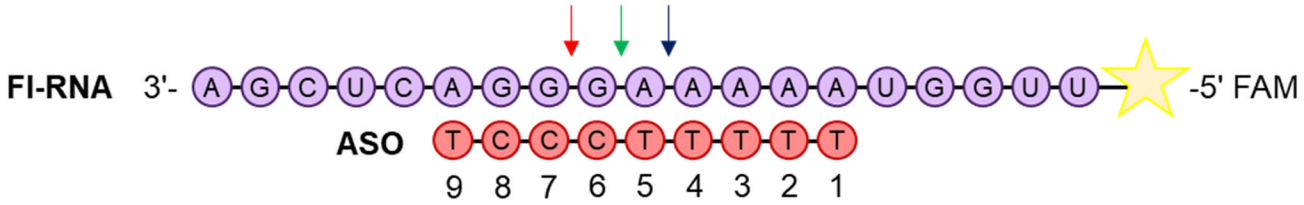
Fig. S38: Circular dichroism spectra of (**a**) **ON 1a-7a** and (**b**) **ON 1b-7b** with target RNA.



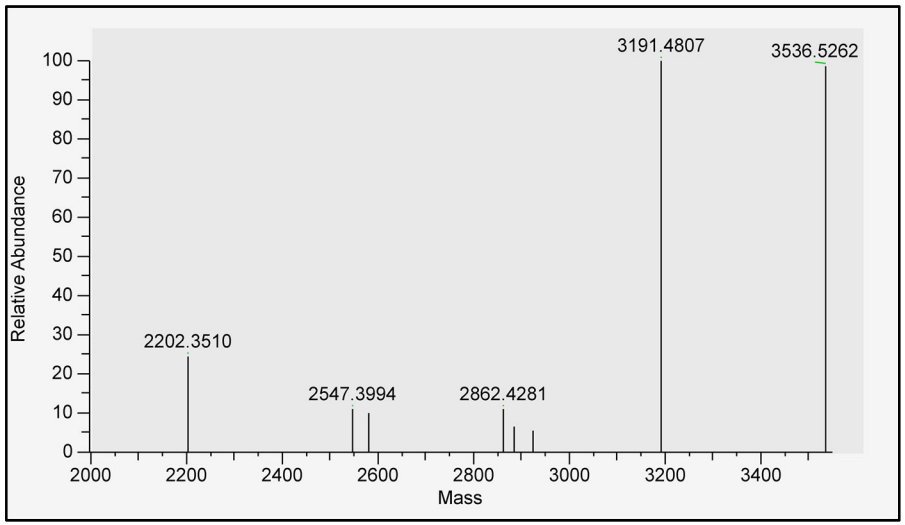
Circular dichroism was performed on duplexes in 90 mM sodium chloride, 10 mM sodium phosphate, 1 mM EDTA buffer (pH 7.0) at a final duplex concentration of 2.5 µM

Fig. S39: ESI-MS of products from *E. coli* RNase H cleavage of target RNA by (**a**) 9mer DNA Control (**Ctrl DNA**) and (**b**) **G-DNA**.

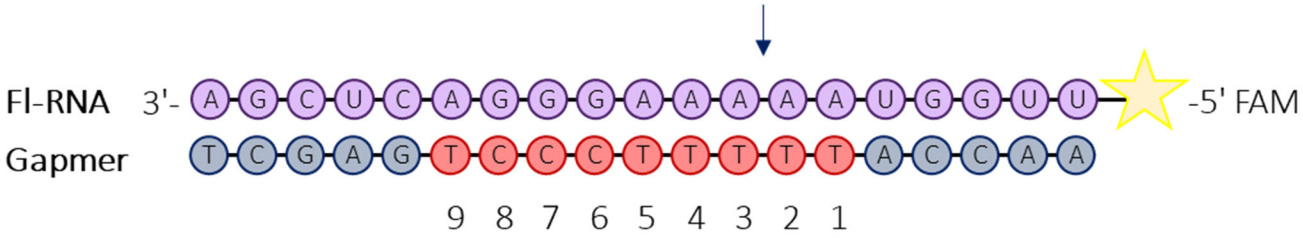
(a)



Position	5' Product (3' → 5')	Mass
9-8	G G G A A A A A U G G U U	4226
8-7	G G A A A A A U G G U U	3881
7-6	G A A A A A U G G U U	3536
6-5	A A A A A U G G U U	3191
5-4	A A A A U G G U U	2862
4-3	A A A U G G U U	2533
3-2	A A U G G U U	2204
2-1	A U G G U U	1875



(b)



Position	5' Product (3' → 5')	Mass
9-8	G G G A A A A A U G G U U	4226
8-7	G G A A A A A U G G U U	3881
7-6	G A A A A A U G G U U	3536
6-5	A A A A A U G G U U	3191
5-4	A A A A U G G U U	2862
4-3	A A A U G G U U	2533
3-2	A A U G G U U	**2204**
2-1	A U G G U U	1875

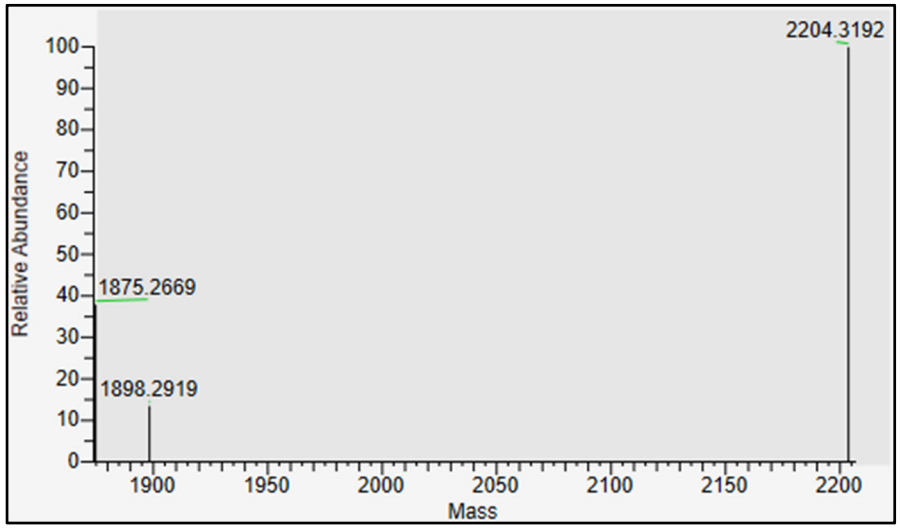
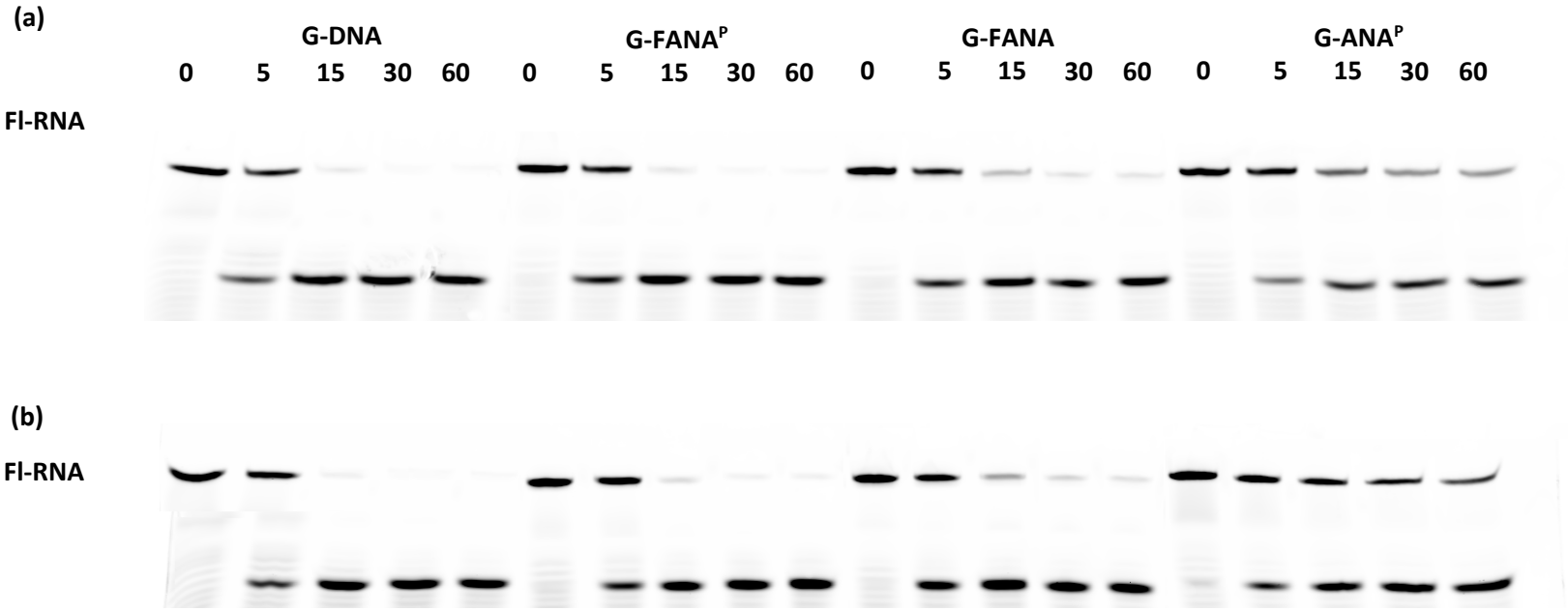
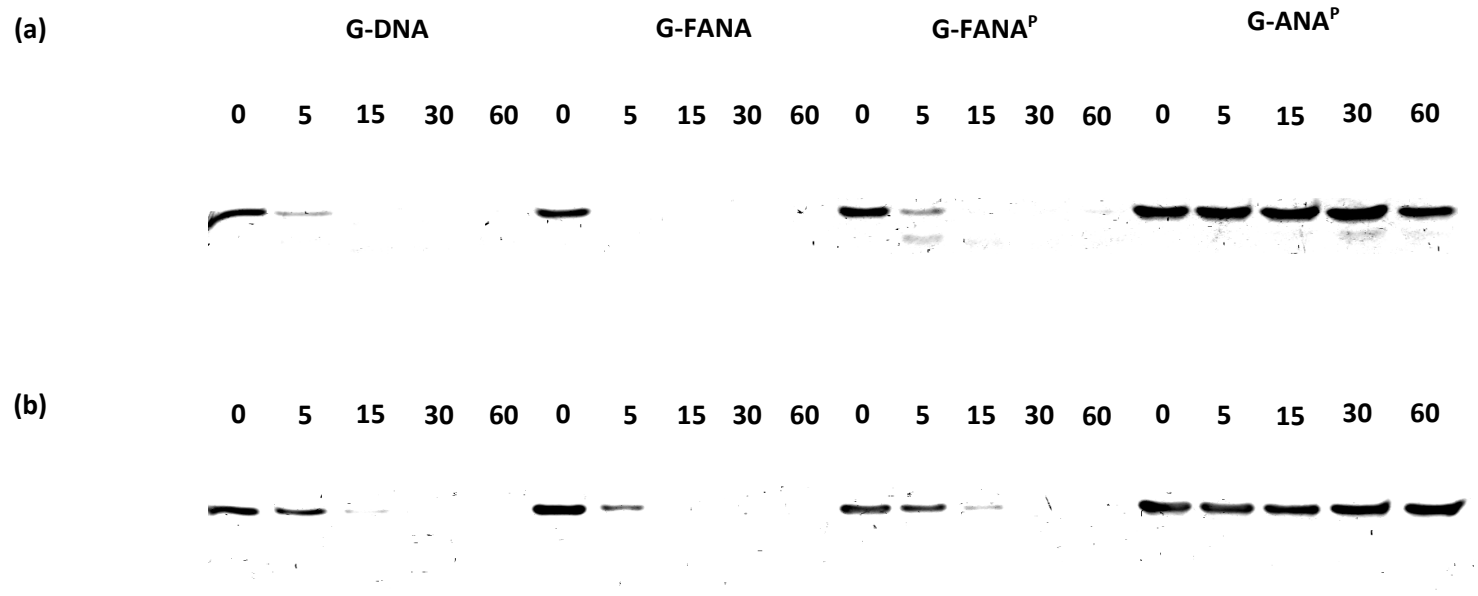


Fig. S40: Time dependent *E. coli* RNase H cleavage of target Fl-RNA by (**a**) gapmer ONs and (**b**) with replicate.



20% denaturing PAGE of *E. coli* mediated degradation of target Fl-RNA (25 pmol) by gapmer ONs (25 pmol). Gels were run using 1x TBE running buffer, at 350 V for 2 hours and visualized.

Fig. S41: Time dependent Nuclease S1 digest of (**a**) gapmer ONs and (**b**) with replicate.



20% denaturing PAGE of nuclease S1 digest of gapmer ONs (100 pmol). Gels were run using 1x TBE running buffer, at 350 V for 2 hours and visualized.