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Supplemental information

Structural variants and modifications of hammerhead ribozymes targeting influenza A virus conserved structural motifs

Tomasz Czapik, Julita Piasecka, Ryszard Kierzek, and Elzbieta Kierzek

S1. (+)RNA5 A/California/04/2009 (H1N1) secondary structure.

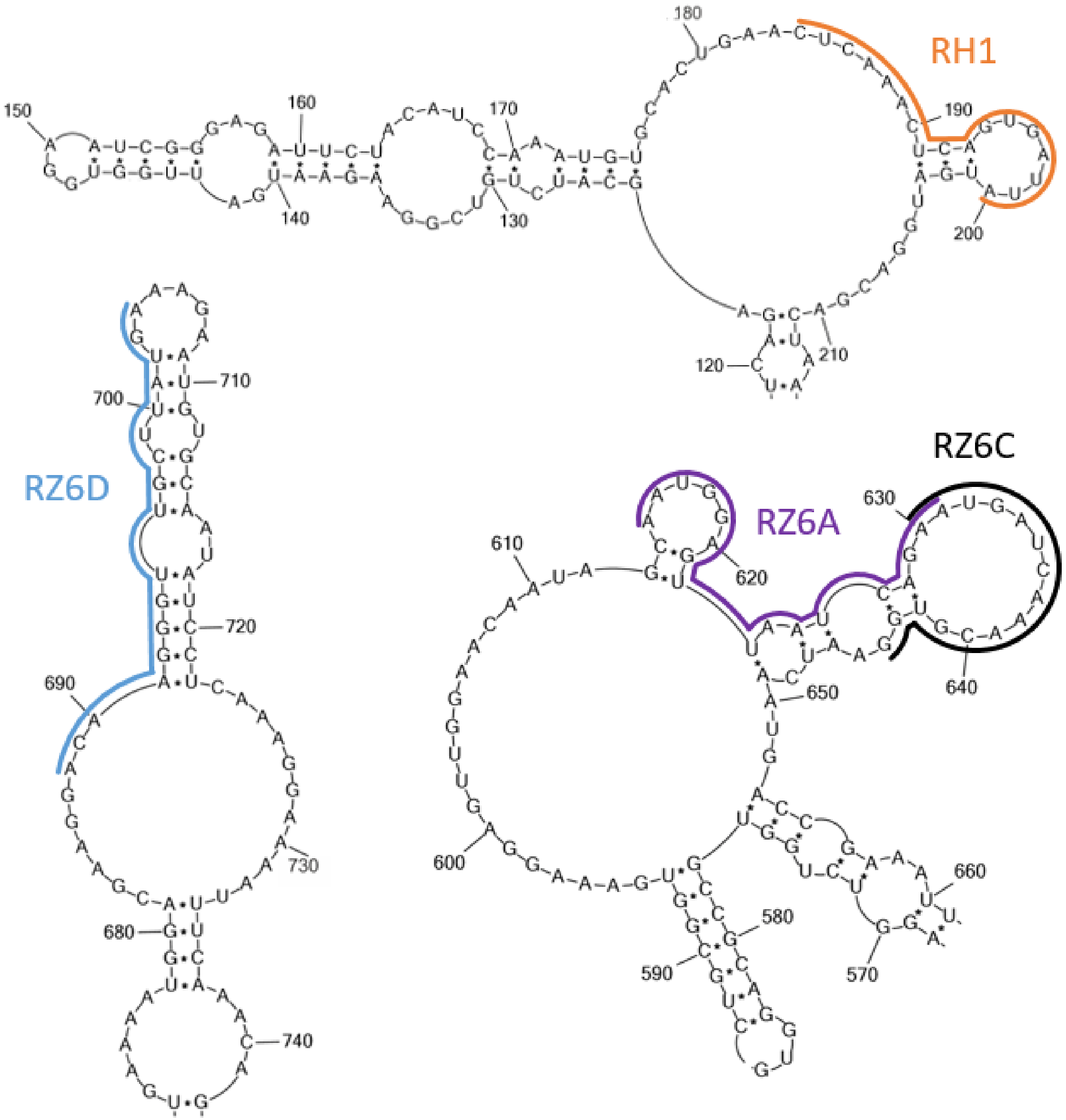
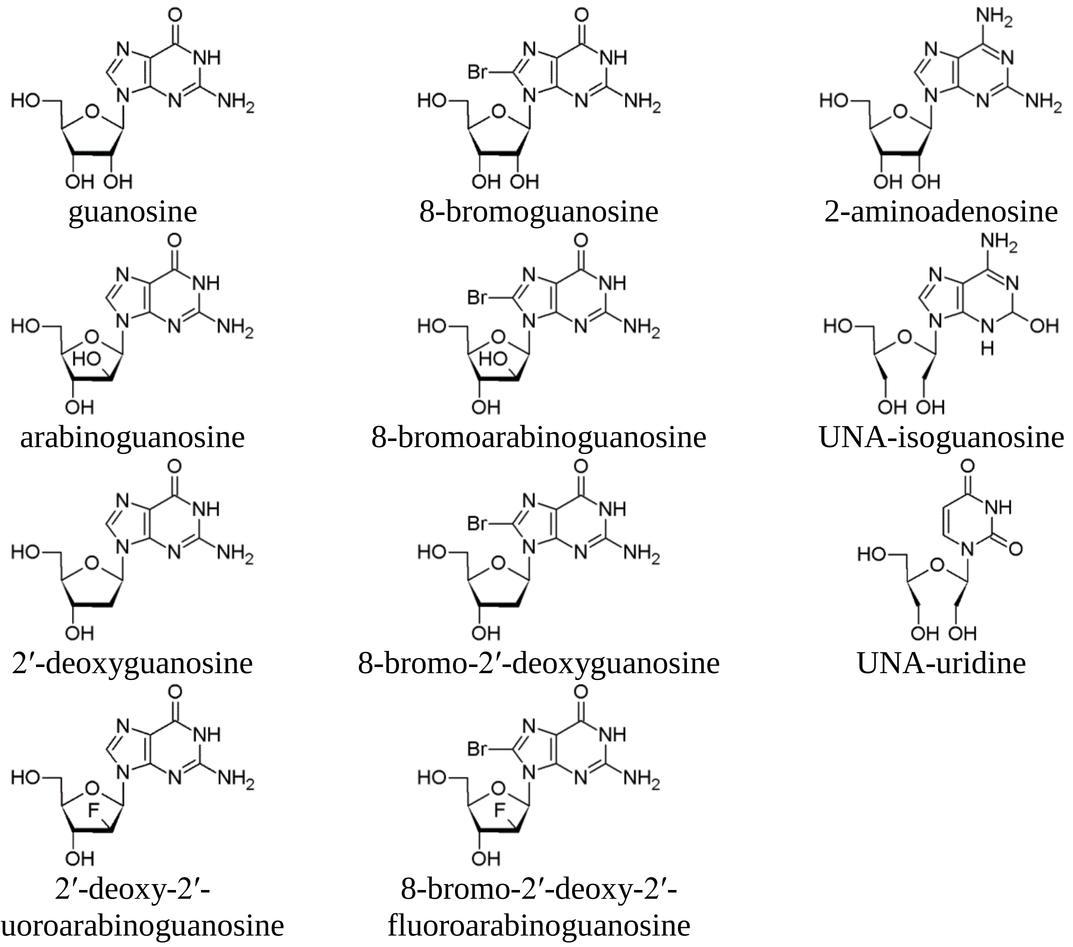


Figure S1. Target regions of hammerhead ribozymes in the (+)RNA5 A/California/04/2009 (H1N1) secondary structure. Secondary structure of (+)RNA5 A/California/04/2009 (H1N1) was previously published[1]. Target regions for analyzed hammerhead ribozymes are marked with colored lines.

S2. Modified nucleosides .



guanosine

8-bromoguanosine

2-aminoadenosine

arabinoguanosine

8-bromoarabinoguanosine

UNA-isoguanosine

2′-deoxyguanosine

8-bromo-2′-deoxyguanosine

UNA-uridine

2′-deoxy-2′-fluoroarabinoguanosine

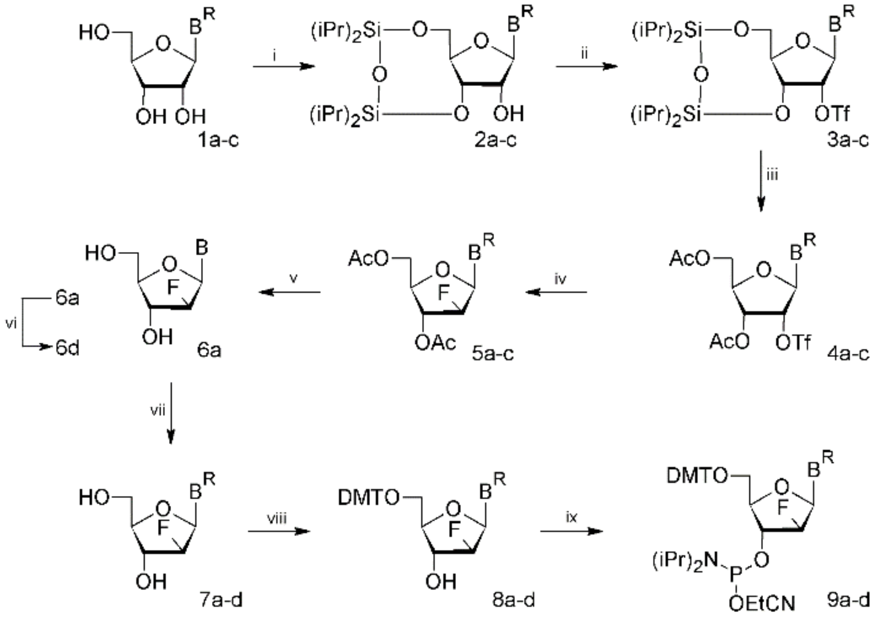
8-bromo-2′-deoxy-2′-fluoroarabinoguanosine

Figure S2. Structural formulas of guanosine derivatives.

S3. Nucleoside and Phosphoroamidite synthesis.

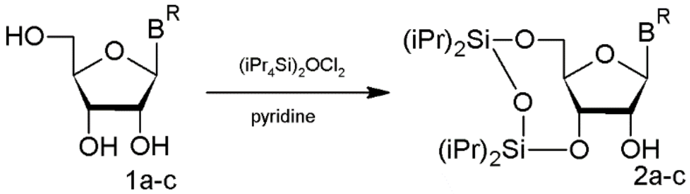
General. Guanosine, adenosine and 2,6-diaminopurine riboside used for synthesis and most other reagents were purchased from Sigma-Aldrich and Merck. Dimethoxytrityl chloride and 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite were purchased from ChemGenes. Solvents were used at the highest available purity and dried with molecular sieves, if necessary.

NMR spectra were acquired using a Bruker Avance II (400 MHz) or Avance III (500 MHz) or Avance III (700 MHz) spectrophotometer. For ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra TMS, H_3PO_4 and trifluoroacetic acid, respectively, were used as references.



Scheme S1. Synthesis of 2′-deoxy-2′-fluoropurinearabinosides marked as following: a – guanine, b – adenine, c – 2,6-diaminopurine, d – 8-bromoguanine, respectively. Where: i means 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane/pyridine; ii means triflate anhydride, DMAP/dichloromethane, pyridine; iii means triethylammonium fluoride (TEAHF), acetic anhydride/pyridine; iv means TEAHF/THF, pyridine; v means ammonia/methanol; vi means bromine/water; vii means dimethylformamide dimethyl acetal/methanol; viii means 4,4′-

dimethoxytrityl chloride/pyridine and iv means 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphoramidite, terazole/acetonitrile.



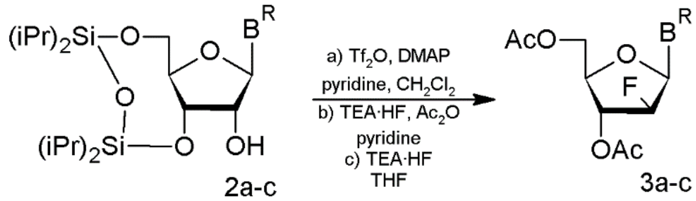
N-protected-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-purine riboside (step i). N-protected purine riboside (**1**) (1 equiv) suspension was evaporated twice with anhydrous pyridine. The dried residue was suspended in pyridine (5 ml per 1 mmol) and 1,1,3,3-tetraisopropyldisiloxane-1,3-dichloride (1.1 equiv) was added. After 3 h at room temperature (rt), the reaction was completed, worked up with aqueous solution of sodium bicarbonate, and extracted 3 times with dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, filtered and evaporated to obtain product (**2**) as white foam. Yield ca. 100 %.

N^2-(dimethylaminomethylene)-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-guanosine (**2a**). ^{1}H NMR (400 MHz, dmso-d_6), δ, ppm: 11.34 (s, 1H, NH), 8.54 (s, 1H, CH dmm), 7.85 (s, 1H, H-8), 5.79 (d, J_{H-H} = 3.92 Hz, 1H, H-1′), 5.68 (s, 1H, 2′-OH), 4.36 (m, 2H, H-3′, H-2′), 4.09-3.91 (m, 3H, H-5′, H-5″, H-4′), 3.14 (s, 3H, CH_3 dmm), 3.02 (s, 3H, CH_3 dmm), 0.99-1.04 (m, 28H, $(iPr)_4$); ^{13}C NMR (100 MHz, dmso-d_6), δ, ppm : 157.93, 157.54, 157.40, 149.32, 135.37, 119.65, 88.10, 80.95, 73.83, 69.67, 60.73, 17.27, 17.11, 17.08, 16.90, 16.84, 16.81, 16.74, 12.75, 12.40, 12.25, 11.98; HRMS (ESI): calcd for $C_{25}H_{44}N_6O_6Si_2$ $[M+H]^+$ 581.2934; found 581.2932;

N^6-benzoyl-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-adenosine (**2b**) ^{1}H NMR (400 MHz, dmso-d_6), δ, ppm: 11.24 (s, 1H, NH), 8.67 (s, 1H, H-2), 8.53 (s, 1H, H8), 8.06 - 7.53 (5H, H-

Ar), 6.01 (d, 1H, H-1′), 5.68 (d, 1H, 2′-OH), 4.84-4.81 (m, 1H, 2′), 4.65, (m, 1H, 3′) 4.09-3.93 (m, 3H, 4′, 5′, 5″), 1.06 - 0.94 (m, 28H, (iPr)$_4$); ^{13}C NMR (100 MHz, dmso-d$_6$), δ, ppm: 165.68, 151.41, 150.48, 143.07, 133.34, 132.43, 128.48, 128.43, 128.16, 127.41, 125.95, 89.52, 80.95, 73.38, 69.86, 60.72, 54.88, 17.32, 17.18, 17.17, 17.13, 17.01, 16.89, 16.81, 12.73, 12.42, 12.25, 12.05; HRMS (ESI): calcd for $C_{29}H_{43}N_5O_6Si_2$ $[M+H]^+$ 614.2825; found 614.2831;

N^2,N^6-diacetyl-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-2-aminoadenosine (**2c**) ^{1}H NMR (400 MHz, dmso-d$_6$): δ 10.56 (s, 1H, NH), 10.30 (s, 1H, NH), 8.32 (s, 1H, H-8), 5.89 (s, 1H, H-1′), 5.59 (s, 1H, 2′-OH), 4.55 - 4.50 (m, 2H, H-2′, H-3′), 4.09 - 3.92 (m, 3H, H-4′, H-5′, H-5″), 2.31 (s, 3H, Ac CH$_3$), 2.22 (s, 3H, Ac CH$_3$), 1.05-0.85 (m, (iPr)$_4$); ^{13}C NMR (176 MHz, dmso-d$_6$), δ, ppm: 169.88, 152.68, 152.40, 150.15, 141.30, 120.30, 89.09, 81.86, 73.87, 70.57, 61.51, 25.13, 24.98, 17.83, 17.67, 17.64, 17.57, 17.49, 17.41, 17.35, 13.18, 12.94, 12.68, 12.51; HRMS (ESI): calcd for $C_{26}H_{44}N_6O_7Si_2$ $[M-H]^-$ 607.2737; found 607.2747;



N-protected 3′,5′-di-O-acetyl-2′-deoxy-2′-fluoropurine arabinoside (step ii).

a) N-protected-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-purine riboside (**2**) (1 equiv) was dissolved in anhydrous dichloromethane (6 ml per 1 mmol) with the addition of pyridine (0.2 ml per 1 mmol) and 4-dimethylaminopyridine (DMAP) (5 equiv). The mixture was cooled in an ice bath for 30 min and trifluoromethanesulfonyl anhydride (7 equiv) was added dropwise over 15 min. Then, the reaction mixture was left at rt for 2 h to complete the reaction. The reaction mixture was worked up with an aqueous solution of sodium bicarbonate and extracted

3 times with dichloromethane. The organic layer was washed with an aqueous solution of dibasic sodium phosphate and the combined organic layers were dried with anhydrous sodium sulfate, filtered and evaporated.

b) Residue from previous reaction was dissolved in a 1 molar pyridine solution of triethylammonium fluoride (4 equiv), and acetic anhydride (8 equiv) was added. The mixture was left for 2 h at rt in a plastic container. After that time, the reaction was completed, and the reaction mixture was worked up with an aqueous solution of sodium bicarbonate and was extracted 3 times with dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, filtered and evaporated.

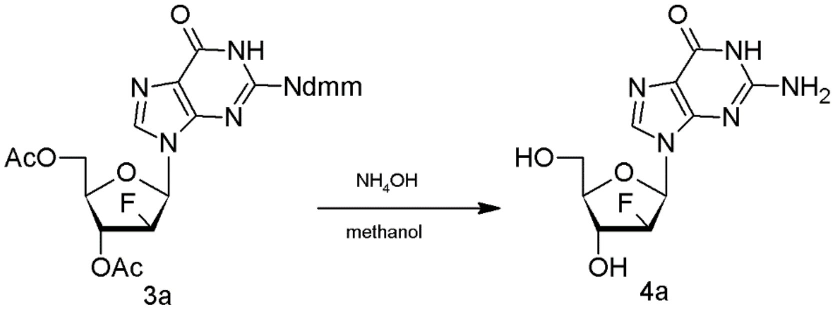
c) Prepared N-protected 2′-O-trifluoromethanesulfonyl-3′,5′-di-O-acetylpurine riboside without further purification was dissolved in anhydrous tetrahydrofuran (5 ml per 1 mmol), and a 1 molar pyridine solution of TEAHF (7 equiv) was added. The mixture was left for 64 h at 37° C. After that time, ^{19}F NMR signal at -74.57 ppm disappeared, and a new signal at -198.43 ppm was indicated. The reaction mixture was worked up with an aqueous solution of sodium bicarbonate and was extracted 3 times with dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, filtered and evaporated. Product (**3**) without purification was used for the next reaction.

3′,5′-di-O-acetyl-N^2-(dimethylaminomethylene)-2′-deoxy-2′-fluoroarabinoguanosine (**3a**). ^{1}H NMR (500 MHz, dmso-d$_6$), δ, ppm: 11.44 (s, 1H, NH), 8.62 (s, 1H, CH dmm), 7.87 (d, 1H, H-8), 6.34 (dd, 1H, H-1′, $J_{H\text{-}F}$ = 15.18 Hz), 5.74 (m, 1H, H-2′, $J_{H\text{-}F}$ = 19.30 Hz), 5.56 (m, 1H, H-3′, $J_{H\text{-}F}$ = -51.20 Hz), 4.41-4.23 (m, 3H, H-5′, H-5″, H-4′), 3.18 (s, 3H, CH_3 dmm), 3.05 (s, 3H, CH_3 dmm), 2.15 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C NMR (125 MHz, dmso-d$_6$), δ, ppm: 170.60, 170.32, 158.48, 157.98, 150.11, 138.12, 119.73, 93.76, 92.23, 81.89, 81.76, 77.84, 75.71, 75.49, 63.42, 41.09, 40.54-39.45, 35.18, 21.11, 20.93, 17.77, 13.08; ^{19}F NMR

(470 MHz, dmso-d_6), δ, ppm: -198.43 (ddd, 1F, $J_{H\text{-}F}$ = 17.36 Hz, $J_{H2'\text{-}F}$ = -51.58 Hz); HRMS (ESI): calcd for $C_{17}H_{21}FN_6O_6$ $[M+H]^+$ 425.1579; found 425.1573;

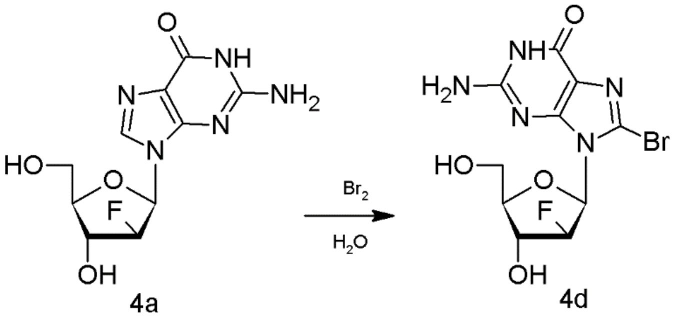
3',5'-di-O-acetyl-N^6-benzoyl-2'-deoxy-2'-fluoroarabinoadenosine (**3b**). ^{1}H NMR (500 MHz, dmso-d_6), δ, ppm: 11.27 (s, 1H, NH), 8.81 (s, 1H, H-2), 8.56 (s, 1H, H-8), 8.06-7.54 (5H, H-Ar), 6.64 (dd, 1H, H-1′, $J_{H\text{-}F}$ = 15.18 Hz), 5.71-5.57 (m, 2H), 4.44-4.33 (m, 3H), 2.16 (s, 3H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac); ^{13}C NMR (125 MHz, dmso-d_6), δ, ppm: 170.16, 169.63, 152.03, 151.91, 150.57, 143.24, 143.19, 133.24, 132.51, 128.51, 128.48, 93.68, 91.77, 81.96, 81.80, 78.17, 78.13, 75.41, 75.13, 63.00, 20.61, 20.52; ^{19}F NMR (470 MHz, dmso-d_6), δ, ppm: -197.90 (ddd, 1F, $J_{H\text{-}F}$ = 17.18 Hz, $J_{H2'\text{-}F}$ = -51.74 Hz); HRMS (ESI): calcd for $C_{21}H_{20}FN_5O_6$ $[M+H]^+$ 458.1470; found 458.1453;

N^2,N^6-diacetyl-3',5'-di-O-acetyl-2'-deoxy-2'-fluoro-2-aminoarabinoadenosine (**3c**). ^{1}H NMR (500 MHz, dmso-d_6), δ, ppm: 10.43 (s, 1H, NH), 8.36 (s, 1H, H8), 6.44 (dd, 1H, H-1′, $J_{H1'\text{-}F}$ = 16.73 Hz, $J_{H1'\text{-}H2'}$ = 3.96 Hz), 5.65-5.51 (m, 2H), 4.41-4.32 (m, 4H), 2.32 (s, 3H, NAc CH_3), 2.23 (s, 3H, NAc CH_3), 2.14 (s, 3H, OAc CH_3), 2.04 (s, 3H, OAc CH_3); ^{13}C NMR (125 MHz, dmso-d_6), δ, ppm: 170.65, 169.98, 169.92, 152.93, 152.79, 142.39, 119.51, 94.02, 92.49, 82.24, 82.10, 78.76, 75.93, 75.72, 63.61, 25.09, 25.06, 21.07, 21.00, 20.95, 20.81, 20.69, 19.00, 17.76, 13.08; ^{19}F NMR (470 MHz, dmso-d_6), δ, ppm: -197.75 (ddd, 1F, $J_{H\text{-}F}$ = 17.28 Hz, $J_{H2'\text{-}F}$ = -50.92 Hz); HRMS (ESI): calcd for $C_{18}H_{21}FN_6O_7$ $[M+H]^+$ 453.1529; found 453.1514;



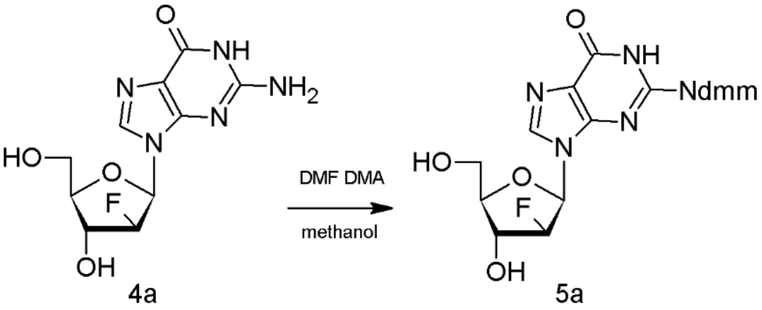
2′-deoxy-2′-fluoroarabinoguanosine (step iii). The residue from the previous reaction was dissolved in methanol (5 ml per 1 mmol) and 25 % aqueous ammonia (5 ml per 1 mmol) was added; the reaction was left in sealed vessel overnight at 55 °C. The mixture was cooled and evaporated twice with isopropanol to dryness. The yield for the two last steps was 60 %.

2′-deoxy-2′-fluoroarabinoguanosine (**4a**). ^{1}H NMR (500 MHz, dmso-d$_6$), δ, ppm: 10.94 (s, 1H, NH), 7.77 (s, 1H, H-8), 6.65 (s, 1H), 6.13 (dd, 1H, H-1′, $J_{H\text{-}F}$ = 16.19 Hz), 5.10 (m, 2H, H-2′, $J_{H2'\text{-}F}$ = -52.45 Hz, 2′-OH), 4.36 (dt, 1H, H-3′, $J_{H\text{-}F}$ = 17.87 Hz), 3.80 (m, 1H, H-4′), 3.40 (m, 2H, H-5′, H-5″); ^{13}C NMR (176 MHz, dmso-d$_6$), δ, ppm: 171.98, 157.10, 154.52, 151.31, 136.27, 136.24, 116.35, 96.12, 95.03, 84.14, 84.12, 81.73, 81.64, 73.18, 73.05, 60.90, 40.32-39.60, 22.97; ^{19}F NMR (376 MHz, dmso-d$_6$), δ, ppm: -198.09 (ddd, 1F, $J_{H\text{-}F}$ = 16.99 Hz, $J_{H2'\text{-}F}$ = -52.40 Hz); HRMS (ESI): calcd for $C_{10}H_{12}FN_5O_4$ [M+H]$^+$ 286.0946; found 286.0941;



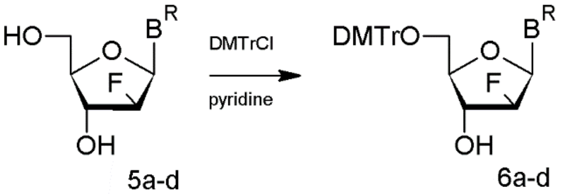
2′-deoxy-2′-fluoro-8-bromoarabinoguanosine (**4d**) (step iv). 2′-Deoxy-2′-fluoroarabinoguanosine (**4a**) was suspended in water and aqueous bromine was added dropwise until discoloration occurred. The product (**4d**), in the form of a white precipitate, was filtered and dried.

^{1}H NMR (500 MHz, dmso-d$_6$), δ, ppm: 10.83 (s, 1H, NH), 6.52 (s, 1H, 2′-OH), 6.27 (dd, 1H, H-1′, $J_{H\text{-}F}$ = 16.19 Hz), 5.23 (dt, 1H, H-2′, $J_{H2'\text{-}F}$ = -52.45 Hz), 4.68 (dt, 1H, H-3′), 3.78-3.71 (m, 3H, H-4′, H-5′, H-5″); ^{13}C NMR (125 MHz, dmso-d$_6$), δ, ppm: 155.45, 153.55, 152.35, 119.67, 116.97, 97.40, 95.45, 82.20, 82.11, 82.08, 81.90, 73.61, 73.40, 61.53; ^{19}F NMR (376 MHz, dmso-d_6), δ, ppm: -197.05 (dq, 1F, $J_{H\text{-}F}$ = 10.06 Hz, $J_{H\text{-}F}$ = 21.88 Hz, $J_{H2'\text{-}F}$ = -54.17 Hz); HRMS (ESI): calcd for $C_{10}H_{11}BrFN_5O_4$ $[M+H]^+$ 364.0051; found 364.0055



N^2-(dimethylaminomethylene)-2′-deoxy-2′-fluoroarabinoguanosine (**5a**) (step v). 2′-Deoxy-2′-fluoroarabinoguanosine (**4a**) was evaporated twice with methanol. The substrate was suspended in methanol (5 ml per 1 mmol) and N,N-dimethylformamide dimethyl acetal (DMF DMA) (5 equiv) was added and stirred for 6 h at 50 °C. Product (**5a**) was filtered, dried and used without further purification.

^{1}H NMR (500 MHz, dmso-d_6), δ, ppm: 8.59 (s, 1H, CH dmm), 7.89 (d, 1H, H-8), 6.29 (dd, 1H, H-1′, $J_{H\text{-}H}$ = 4.48 Hz, $J_{H\text{-}F}$ = 15.69 Hz), 5.16 (dt, 1H, H-2′, $J_{H2'\text{-}F}$ = -52.55 Hz), 4.41 (dt, 1H, H-3′, $J_{H\text{-}F}$ = 19.11 Hz), 4.09 (m, 1H, H-4′), 3.63 (m, 2H, H-5′, H5″), 3.16 (s, 3H, CH_3 dmm), 3.03 (s, 3H, CH_3 dmm); ^{13}C NMR (125 MHz, dmso-d_6), δ, ppm: 162.80, 151.70, 135.23, 116.68, 96.50, 94.98, 83.98, 83.95, 81.65, 81.52, 73.40, 73.21, 61.01, 36.25, 31.23; ^{19}F NMR (376 MHz, dmso-d_6), δ, ppm: -197.27 (ddd, 1F, $J_{H\text{-}F}$ = 17.26 Hz, $J_{H2'\text{-}F}$ = -52.45 Hz); HRMS (ESI): calcd for $C_{13}H_{17}FN_6O_4$ $[M+H]^+$ 341.1368; found 341.1366



N-protected 5′-O-(4,4′-dimethoxytrityl)-2′-deoxy-2′-fluoropurine arabinoside (step vi). Base-protected 2′-deoxy-2′-fluoropurine arabinoside (**5**) was evaporated twice with anhydrous pyridine and dissolved in pyridine (5 ml per 1 mmol). The dimethoxytrityl chloride (1.1 equiv) was added and the mixture was stirred at rt for 3 h. After reaction completion, the reaction mixture was worked up with an aqueous solution of sodium bicarbonate and extracted 3 times with dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, filtered and evaporated. Product (**6**) was purified by silica gel column chromatography using dichloromethane and methanol (linear gradient up to 7 %) as eluent. Yield: ca. 80 %.

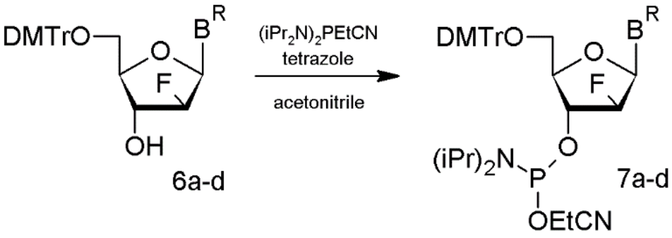
5′-O-(4,4′-dimethoxytrityl)-N^2-(dimethylaminomethylene)-2′-deoxy-2′-fluoroarabinoguanosine (**6a**). ^{1}H NMR (500 MHz, dmso-d_6), δ, ppm: 11.37 (s, 1H, NH), 8.53 (s, 1H, CH dmm), 7.92 (d, 1H, H-8), 7.74-6.83 (Ar-DMT), 6.84 (dd, 1H, H-1′, $J_{H1'-F}$ = 16.39 Hz, $J_{H1'-H2'}$ = 4.38 Hz), 6.12 (s, 1H, 3′-OH), 5.19 (dt, 1H, H-2′, $J_{H2'-F}$ = -52.19 Hz), 4.43 (m, 1H, H-3′), 3.99 (m, 1H, H-4′), 3.71 (s, 6H, CH_3-DMT), 3.32-3.20 (m, 2H, H-5′, H-5″), 3.10 (s, 3H, CH_3 dmm), 3.04 (s, 3H, CH_3 dmm); ^{13}C NMR (125 MHz, dmso-d_6), δ, ppm: 162.98, 158.55, 158.13, 158.03, 150.25, 145.15, 137.61, 137.57, 135.91, 135.84, 130.13, 128.30, 128.09, 127.24, 119.28, 113.61, 96.43, 94.90, 85.97, 82.15, 82.11, 81.61, 81.48, 74.29, 74.10, 63.84, 55.48, 41.15, 40.22, 40.05, 39.97, 39.88, 39.80, 39.71, 39.55, 39.38, 39.21, 36.30, 35.15, 31.27; ^{19}F NMR (470 MHz, dmso-d_6), δ, ppm: -197.28 (ddd, 1F, J_{H-F} = 17.52 Hz, $J_{F-H2'}$ = -53.34 Hz); HRMS (ESI): calcd for $C_{34}H_{35}FN_6O_6$ $[M+H]^+$ 643.2675; found 643.2676

N^6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxy-2′-fluoroarabinoadenosine (**6b**). ^{1}H NMR (500 MHz dmso-d_6), δ, ppm: 11.27 (s, 1H, NH), 8.03-6.83 (m, Bz-Ar, DMT-Ar), 6.61 (dd, 1H, $J_{H1'-F}$ = 13.9 Hz, $J_{H1'-H2'}$ = 4.74 Hz), 6.04 (d, 3′-OH), 5.32 (dt, 1H, H-2′, $J_{H2'-F}$ = -52.44 Hz), 4.57-4.51 (m, 1H, H-3′), 4.07 (m, 1H, H-4′), 3.72 (s, 6H, CH_3-DMT), 3.41-3.37, 3.28-3.26 (m, 2H, H-5′, H-5″); ^{13}C NMR (125 MHz dmso-d_6), δ, ppm: 167.90, 165.65, 158.10, 151.92, 150.50, 144.74, 142.90, 135.53, 135.39, 134.25, 133.30, 132.48, 131.19, 129.71, 129.69, 128.51,

128.47, 128.30, 128.18, 127.81, 127.68, 127.44, 126.72, 125.08, 113.18, 96.33, 94.42, 85.55, 81.74, 81.66, 81.49, 73.69, 73.45, 63.38, 55.01, 39.50; ^{19}F NMR (470 MHz, dmso-d_6), δ, ppm: -197.68 (ddd, 1F, $J_{H\text{-}F}$ = 17.11 Hz, $J_{H2'\text{-}F}$ = -53.14 Hz); HRMS (ESI): calcd for $C_{38}H_{34}FN_5O_6$ $[M+H]^+$ 676.2566; found 676.2572

N^2,N^6-diacetyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxy-2′-fluoro-2-aminoarabinoadenosine (**6c**). ^{1}H NMR (400 MHz, dmso-d_6), δ, ppm: 10.60, 10.35 (2xs, 2H, NH), 8.22 (1H, H-8), 7.38 - 6.79 (m, 9H, Ar-DMT), 6.43 (dd, 1H, H-1′, $J_{H1'\text{-}F}$ = 13.36 Hz, $J_{H1'\text{-}H2'}$ = 4.84 Hz), 5.96 (d, 1H, 3′-OH), 5.27 (dt, 1H, H-2′, $J_{H\text{-}F}$ = -52.47 Hz), 4.65 (m, 1H, H-3′), 4.04 (m, 1H, H-4′), 3.72 (s, 6H, CH_3-DMT), 3.48-3.44, 3.24-3.21 (m, 2H, H-5′, H-5″), 2.32 (s, 3H, Ac CH_3), 2.20 (s, 3H, Ac CH_3); ^{13}C NMR (100 MHz, dmso-d_6), δ, ppm: 162.79, 158.35, 157.93, 157.83, 150.06, 144.96, 135.71, 135.65, 129.93, 128.10, 127.90, 127.04, 119.08, 113.42, 96.23, 94.71, 85.77, 81.95, 81.91, 81.42, 81.28, 74.09, 73.90, 63.64, 55.28, 40.95 - 39.02, 36.11, 34.95, 31.07; ^{19}F NMR (376 MHz, dmso-d_6): δ -198.05 (ddd, 1F, $J_{F\text{-}H2'}$ = = -52.10 Hz, $J_{F\text{-}H}$ = 16.09 Hz); HRMS (ESI): calcd for $C_{35}H_{35}FN_6O_7$ $[M+H]^+$ 671.2624; found 671.2634;

5′-O-(4,4′-dimethoxytrityl)-N^2-(dimethylaminomethylene)-2′-deoxy-2′-fluoro-8-bromoarabinoguanosine (**6d**). ^{1}H NMR (400 MHz, dmso-d_6), δ, ppm: 11.54 (s, 1H, NH), 8.23 (s, 1H, CH dmm), 7.35 - 6.77 (m, Ar-DMT), 6.48 (dd, 1H, H-1′, $J_{H\text{-}F}$ = 10.42 Hz, $J_{H1'\text{-}H2'}$ = 6.38 Hz), 5.97 (s, 1H, 3′-OH), 5.34 (dt, 1H, H-2′), 4.72-4.65 (m, 1H, H-3′), 3.96 (m, 1H, H-4′), 3.72 (s, 6H, CH_3-DMT), 3.55 (m, 1H, H-5″), 3.24-3.17 (m, 1H, H-5′), 3.00 (s, 3H, CH_3 dmm), 2.98 (s, 3H, CH_3 dmm); ^{13}C NMR (100 MHz, dmso-d_6), δ, ppm: 158.50, 158.43, 157.97, 157.52, 156.80, 151.49, 145.23, 135.89, 135.83, 130.13, 130.01, 128.17, 128.12, 127.09, 121.83, 120.46, 113.53, 113.49, 97.39, 95.83, 85.94, 82.23, 82.09, 79.89, 79.80, 74.52, 74.35, 64.21, 55.48, 55.45, 49.07, 41.24, 40.57-39.47, 35.12; ^{19}F NMR (376 MHz, dmso-d6): δ -197.31 (ddd, 1F, $J_{F\text{-}H2'}$ = -52.28 Hz, $J_{F\text{-}H}$ = 17.52 Hz); HRMS (ESI): calcd for $C_{34}H_{34Br}FN_6O_6$ $[M+H]^+$ 721.1780; found 721.1773;



N-protected [5′-O-(dimethoxytrityl)-2′-deoxy-2′-fluoroarabinofuranosyl]purine 3′-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (step vii). Dimethoxytrityl and base-protected purine riboside (**6**) (1 equiv) and tetrazole (1 equiv) was vacuum-dried for 8 h. Substrates were dissolved in anhydrous acetonitrile (5 ml per 1 mmol) and 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite (1.1 equiv) was added. The mixture was stirred at rt overnight. The reaction mixture after completion was worked up with an aqueous solution of sodium bicarbonate and extracted 3 times with dichloromethane containing 1 % of triethylamine (TEA). The combined organic layers were dried with sodium sulfate and evaporated. Product was purified using silica gel column chromatography starting with hexane, and ethyl acetate was used to form a gradient. During purification, the eluent contained 1 % TEA. Product (**7**) was lyophilized from benzene. Yield: 92 % (after purification).

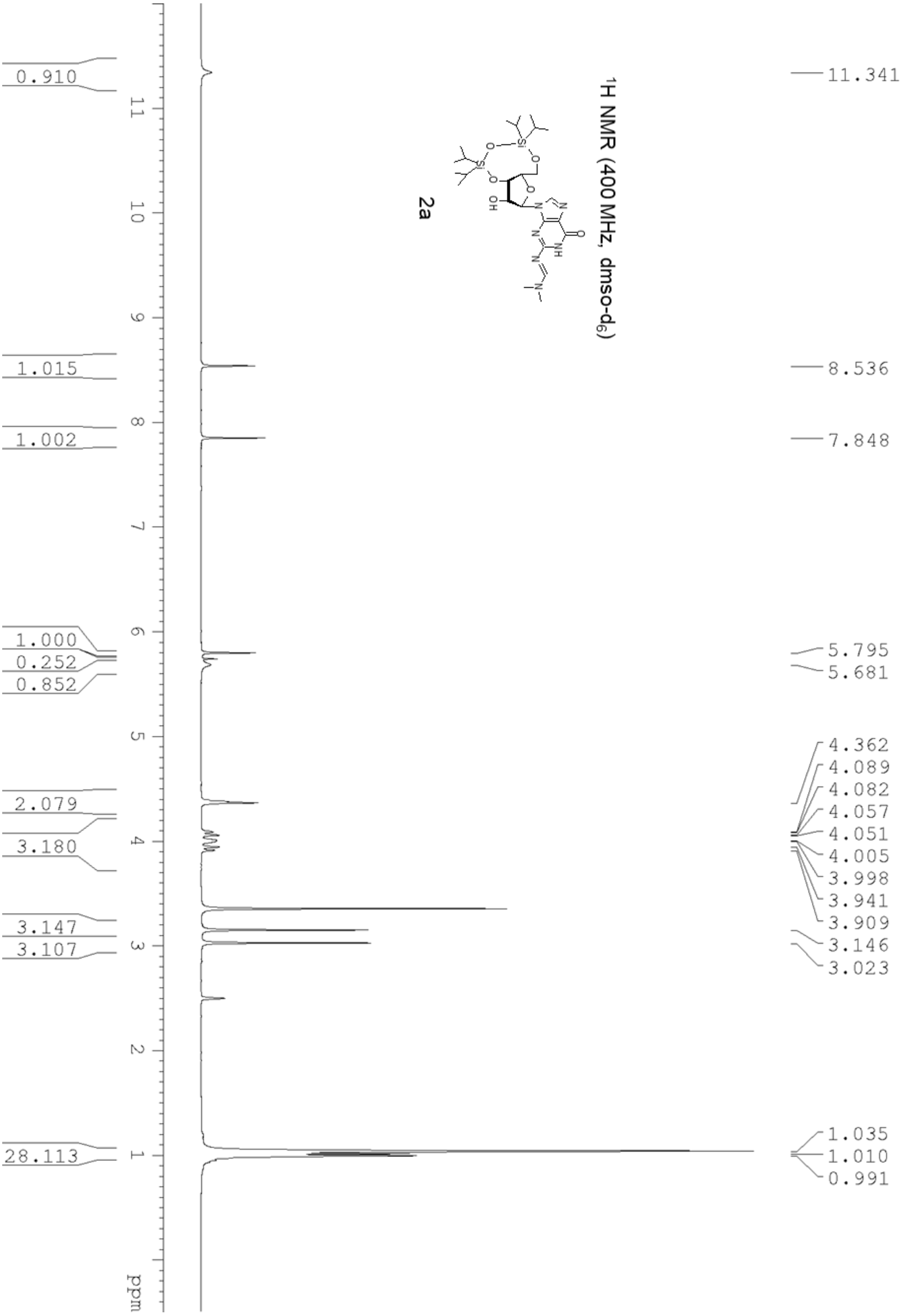
5′-O-(dimethoxytrityl)-N^2-(dimethylaminomethylene)-2′-deoxy-2′-fluoroarabinoguanosine 3′-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (**7a**). ^{1}H NMR (400 MHz, CD_3CN-d_3), δ, ppm: 9.49 (s, 1H, NH), 8.60 (s, 1H, CH dmm), 7.70 (s, 1H, H-8), 7.47-6.84 (13H, Ar-DMT), 6.34 (dd, 1H, H-1′), 5.23 (m, 1H, H-2′), 4.80-4.65 (m, 1H, H-3′), 4.15 (m, 1H, H-4′),3.76 (s, 6H, CH_3-DMT), 3.67-3.60 (m, 4H, O-CH_2 CEO, 2x CH iPr), 3.40-3.36 (m, 2H, H-5′, 5″), 3.10 (s, 3H, CH_3 dmm), 3.05 (s, 3H, CH_3 dmm), 2.62 (t, 1H, CH_2 CEO), 2.52 (t, 1H, CH_2 CEO), 1.18-1.07 (13H, CH_3 iPr); ^{13}C NMR (100 MHz, CD_3CN-d_3), δ, ppm: 159.68, 159.33, 159.26, 158.78, 158.66, 158.62, 151.26, 151.24, 145.95, 145.92, 138.10, 138.06, 138.04, 138.01, 136.73, 136.68, 131.00, 130.96, 130.94, 129.27, 128.97, 128.90, 128.83, 127.87, 127.84, 120.43, 120.36, 119.39, 119.28, 118.27, 114.06, 96.67, 96.63, 96.61, 96.59, 94.75, 94.71, 94.69, 94.67, 87.13, 87.10, 83.24, 83.10, 83.07, 82.99, 82.93, 82.90, 82.82, 82.78, 82.74, 77.77, 77.60, 77.51, 77.34, 77.00, 76.82, 76.74, 76.57, 63.94, 63.72, 59.94, 59.77, 59.75, 59.58, 59.16, 59.11, 55.89, 55.88, 45.99, 45.92, 44.27, 44.20, 44.15,44.07, 41.65, 35.30, 24.94, 24.90, 24.87, 24.83, 24.80, 24.73, 23.16, 23.14, 23.09, 23.07, 20.99, 20.94, 20.92, 20.87, 20.61, 20.53, 1.30; ^{19}F NMR (470 MHz, CD$_3$CN-d$_3$), δ, ppm: -197.17 (dddd, 1F, $J_{H2'-F}$ = -52.46 Hz, J_{H-F} = 18.83 Hz, J_{P-F} = 78,49 Hz); ^{31}P NMR (162 MHz CD$_3$CN-d$_3$): δ, ppm: 151.27, 150.82; HRMS (ESI): calcd for $C_{43}H_{52}FN_8O_7P$ [M+H]$^+$ 843.3753; found 843.3741;

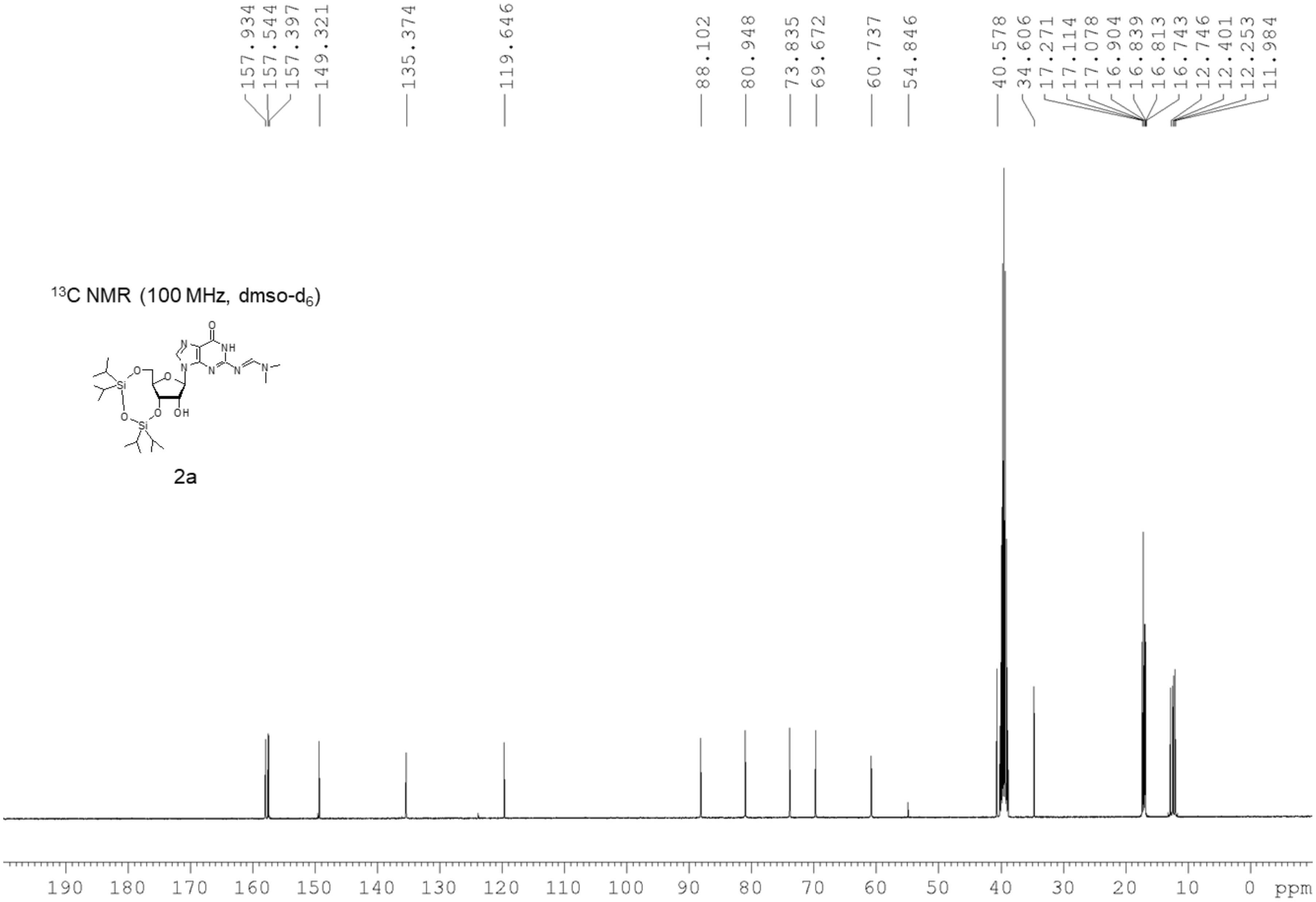
5′-O-(dimethoxytrityl)-N^6-benzoyl-2′-deoxy-2′-fluoroarabinoadenosine 3′-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (**7b**). ^{1}H NMR (500 MHz, CD$_3$CN-d$_3$), δ, ppm: 9.51 (s, 1H, NH), 8.67 - 8.66 (1H, H-2), 8.29-8.27 (1H, H-8), 8.03-6.85 (18H, Bz-Ar, DMT-Ar), 6.61-6.57 (m, 1H, H-1′), 5.47 – 5.32 (m, 1H, H-2′), 4.93-4.82 (m, 1H, H-3′), 4.27-4.25 (m, 1H, H-4′), 4.14-3.63 (m, 4H, H-5′, H-5″), 3.77 (s, 6H, CH$_3$-DMT), 2.66 (t, 1H, CH$_2$ CEO), 2.56 (t, 1H, CH$_2$ CEO), 1.26-1.07 (13H, CH$_3$ iPr); ^{13}C NMR (125 MHz, CD$_3$CN-d$_3$), δ, ppm: 159.29, 159.28, 145.53, 145.51, 143.19, 136.32, 136.30, 136.27, 133.14, 130.60, 129.22, 128.73, 128.42, 117.89, 113.64,96.22, 96.19, 96.07, 94.68, 94.65, 94.53, 86.76, 83.15, 83.02, 82.93, 82.79, 82.70, 82.49, 77.11, 76.98, 76.91, 76.78, 76.28, 76.15, 76.08, 75.95, 63.49, 63.28, 59.47, 59.35, 59.32, 59.19, 55.48, 55.47, 45.58, 45.53, 43.83, 43.77, 43.74, 43.67, 24.51, 24.44, 24.42, 24.39, 24.36, 24.33, 22.76, 22.74, 22.69, 22.67, 20.57, 20.52, 20.47, 1.39, 1.23, 1.06-0.40; ^{19}F NMR (470 MHz, CD$_3$CN-d$_3$), δ, ppm: -197.12 (dddd, 1F, $J_{H2'-F}$ = -51.78 Hz, J_{H-F} = 17.52 Hz, J_{P-F} = 96,71 Hz); ^{31}P NMR (202 MHz, CD$_3$CN-d$_3$), δ, ppm: 151.03, 150.95; HRMS (ESI): calcd for $C_{43}H_{52}FN_8O_7P$ [M+H]$^+$ 876.3655; found 876.3641;

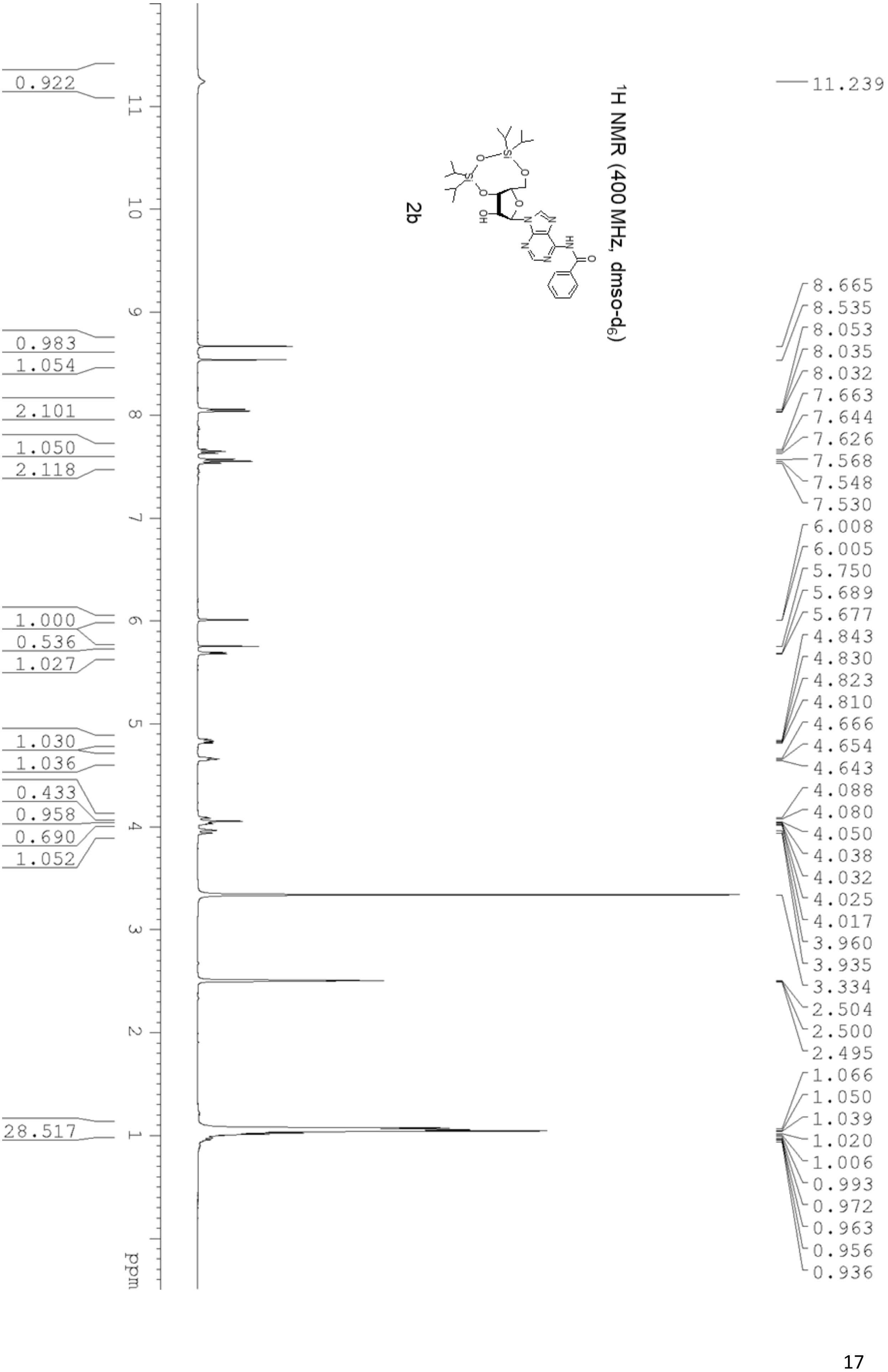
N^2,N^6-diacetyl-5′-O-(dimethoxytrityl)-2′-deoxy-2′-fluoro-2-aminoarabinoadenosine 3′-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (**7c**). ^{1}H NMR (400 MHz, CD$_3$CN-d$_3$), δ, ppm: 8.74, 8.64 (2xs, 2H, NH), 8.07 (m, 1H, H-8), 7.43 - 6.78 (14H, Ar-DMT), 6.41-6.36 (m, 1H, H-1′), 5.43-5.25 (m, 1H, H-2′), 5.01-4.83 (m, 1H, H-3′), 4.19 (m, 1H, H-4′), 4.14-3.80 (m, 2H, H-5′, H-5″), 3.74 (s, 6H, CH_3-DMT), 3.68-3.37 (m, 4H, O-CH_2 CEO, 2x CH iPr), 3.37 (m, 2H, H-5′, 5″), 2.75 (t, 1H, CH_2 CEO), 2.63 (t, 1H, CH_2 CEO), 2.31 (1H, CH_3 Ac), 2.16 (1H, CH_3 Ac), 1.24-1.07 (12H, CH_3 iPr); ^{13}C NMR (100 MHz, CD_3CN-d_3), δ, ppm: 170.98, 159.64, 159.62, 159.60, 153.03, 150.62, 145.97, 136.79, 136.71, 136.69, 136.65, 131.05, 131.00, 130.94, 128.99, 128.91, 128.72, 127.79, 119.28, 118.25, 113.93, 87.06, 83.59, 83.42, 83.27, 83.19, 83.10, 64.12, 59.74, 59.65, 59.55, 59.46, 59.12, 59.07, 55.84, 45.91, 44.24, 44.19, 44.11, 44.07, 25.79, 25.29, 24.89, 24.84, 24.81, 24.77, 24.71, 24.68, 23.14, 23.12, 23.06, 23.05, 20.87, 1.89, 1.68, 1.48, 1.27, 1.06, 0.86, 0.65; ^{19}F NMR (376 MHz, CD_3CN-d_3), δ, ppm: -197.79 - -198.16 (m); ^{31}P NMR (162 MHz, CD_3CN-d_3), δ, ppm: 151.12, 150.96; HRMS (ESI): calcd for $C_{43}H_{52}FN_8O_7P$ $[M+H]^+$ 871.3703; found 871.3711;

5′-O-(dimethoxytrityl)-N^2-(dimethylaminomethylene)-2′-deoxy-2′-fluoro-8-bromoarabinoguanosine 3′-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (**7d**). ^{1}H NMR (500 MHz CD_3CN-d_3), δ, ppm: 9.77 (s, 1H, NH), 8.28, 8.25 (2xs, 1H, H-8), 7.41-6.72 (14H, Ar-DMT), 6.52-6.47 (m, 1H, H-1′), 5.51-5.35 (m, 1H, H-2′), 5.25-5.02 (m, 1H, H-3′), 4.15 (m, 1H, H-4′),3.75 (s, 6H, CH_3-DMT), 3.72-3.50 (m, 4H, O-CH_2 CEO, 2x CH iPr), 3.37 (m, 2H, H-5′, 5″), 3.09 (s, 3H, CH_3 dmm), 3.04 (s, 3H, CH_3 dmm), 2.62 (t, 1H, CH_2 CEO), 2.52 (t, 1H, CH_2 CEO), 1.18-1.07 (12H, CH_3 iPr); ^{13}C NMR (400 MHz, CD_3CN-d_3), δ, ppm: 159.61, 159.54, 158.58, 158.51, 158.10, 158.03, 157.49, 152.53, 152.48, 146.01, 145.91, 136.70, 136.57, 136.53, 136.50, 130.99, 130.93, 130.77, 129.27, 128.90, 128.79, 128.77, 128.74, 127.79, 127.73, 122.56, 121.58, 119.32, 119.18, 118.27, 113.99, 113.97, 96.83, 95.25, 87.06, 87.03, 83.65, 83.50, 83.40, 83.26, 80.18, 80.14, 80.10, 80.06, 77.33, 77.16, 76.98, 64.61, 64.31, 59.99, 59.85, 59.56, 59.41, 55.89, 55.86, 44.35, 44.26, 44.18, 44.08, 41.81, 41.77, 35.35, 24.88, 24.82, 24.53, 24.47, 20.88, 20.82, 1.79, 1.62, 1.46, 1.29, 1.13, 0.96, 0.80 ; ^{19}F NMR (470 MHz, CD_3CN-d_3), δ, ppm: -197.77 - -197.97 (m); ^{31}P NMR (202 MHz, CD_3CN-d_3), δ, ppm: 152.21, 151.22; HRMS (ESI): calcd for $[M+H]^+$ 921.2858; found 921.2865;

S4. NMR Spectra.

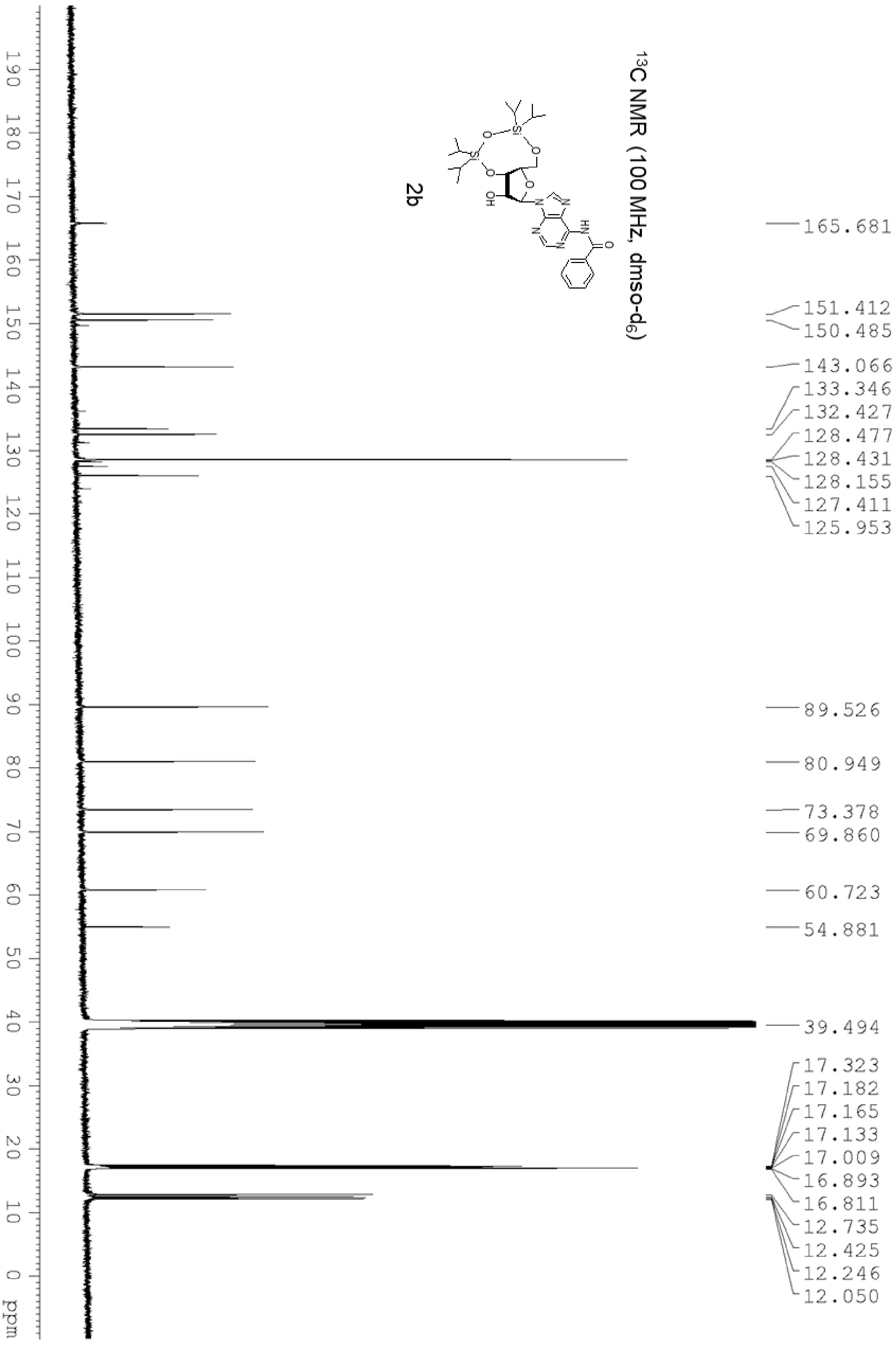


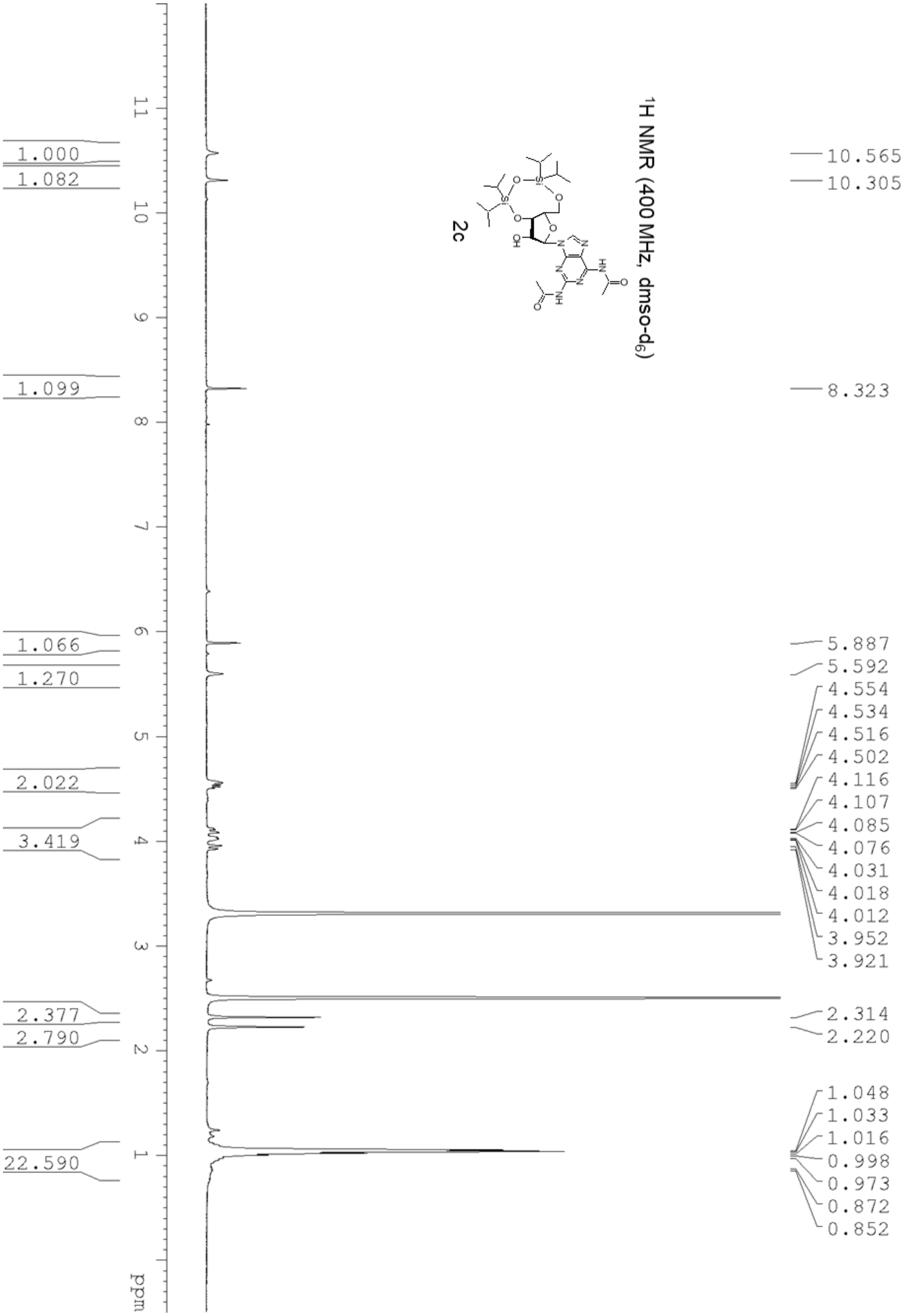

^{13}C NMR (100 MHz, dmso-d$_6$)
O
N
NH
N
N
N
N
O
O
Si
O
O
Si
O
OH
2a
157.934
157.544
157.397
149.321
135.374
119.646
88.102
80.948
73.835
69.672
60.737
54.846
40.578
34.606
17.271
17.114
17.078
16.904
16.839
16.813
16.743
12.746
12.401
12.253
11.984
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm



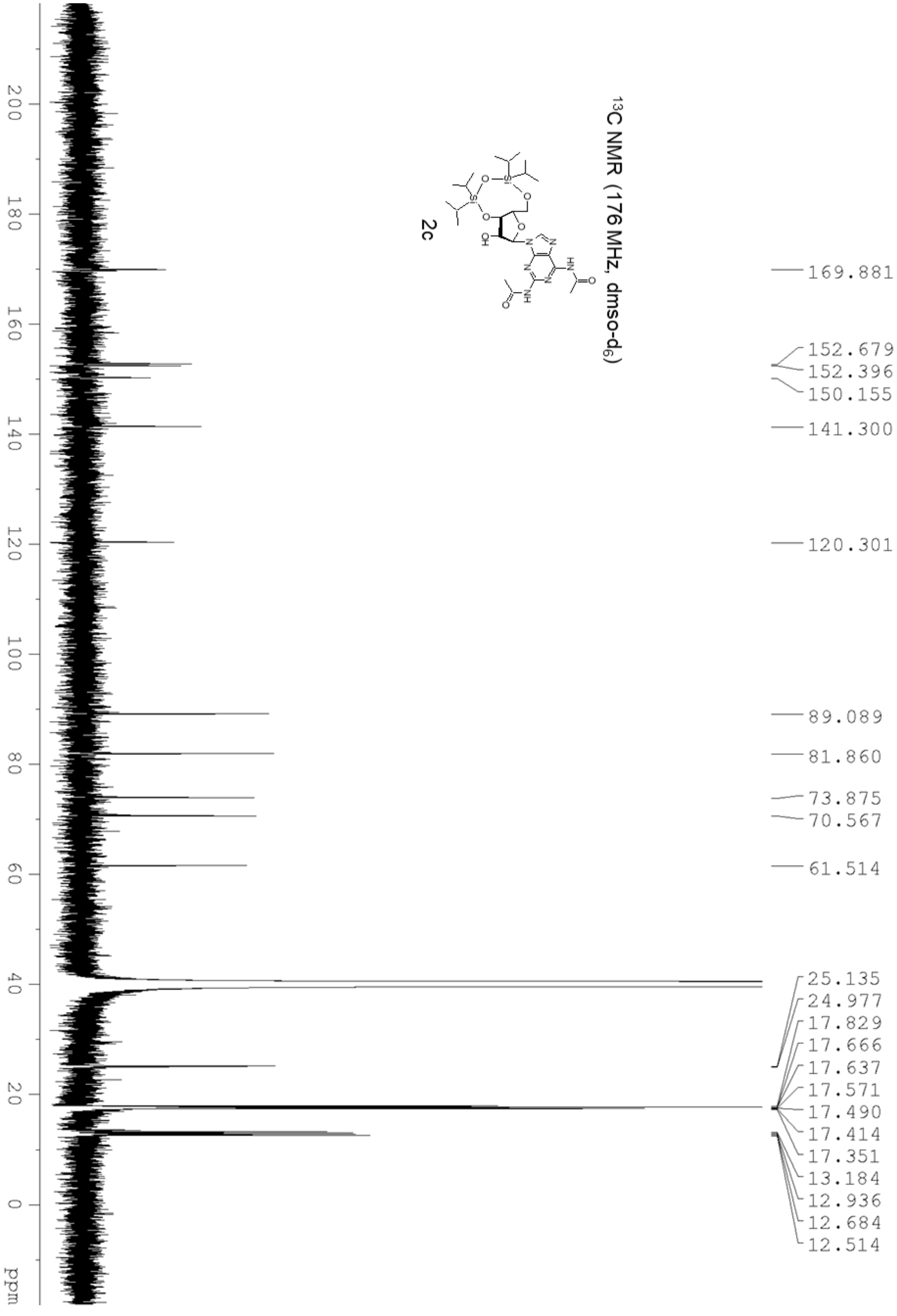
^{1}H NMR (400 MHz, dmso-d_6)

2b


13C NMR (100 MHz, dmso-d6)
2b
165.681
151.412
150.485
143.066
133.346
132.427
128.477
128.431
128.155
127.411
125.953
89.526
80.949
73.378
69.860
60.723
54.881
39.494
17.323
17.182
17.165
17.133
17.009
16.893
16.811
12.735
12.425
12.246
12.050
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

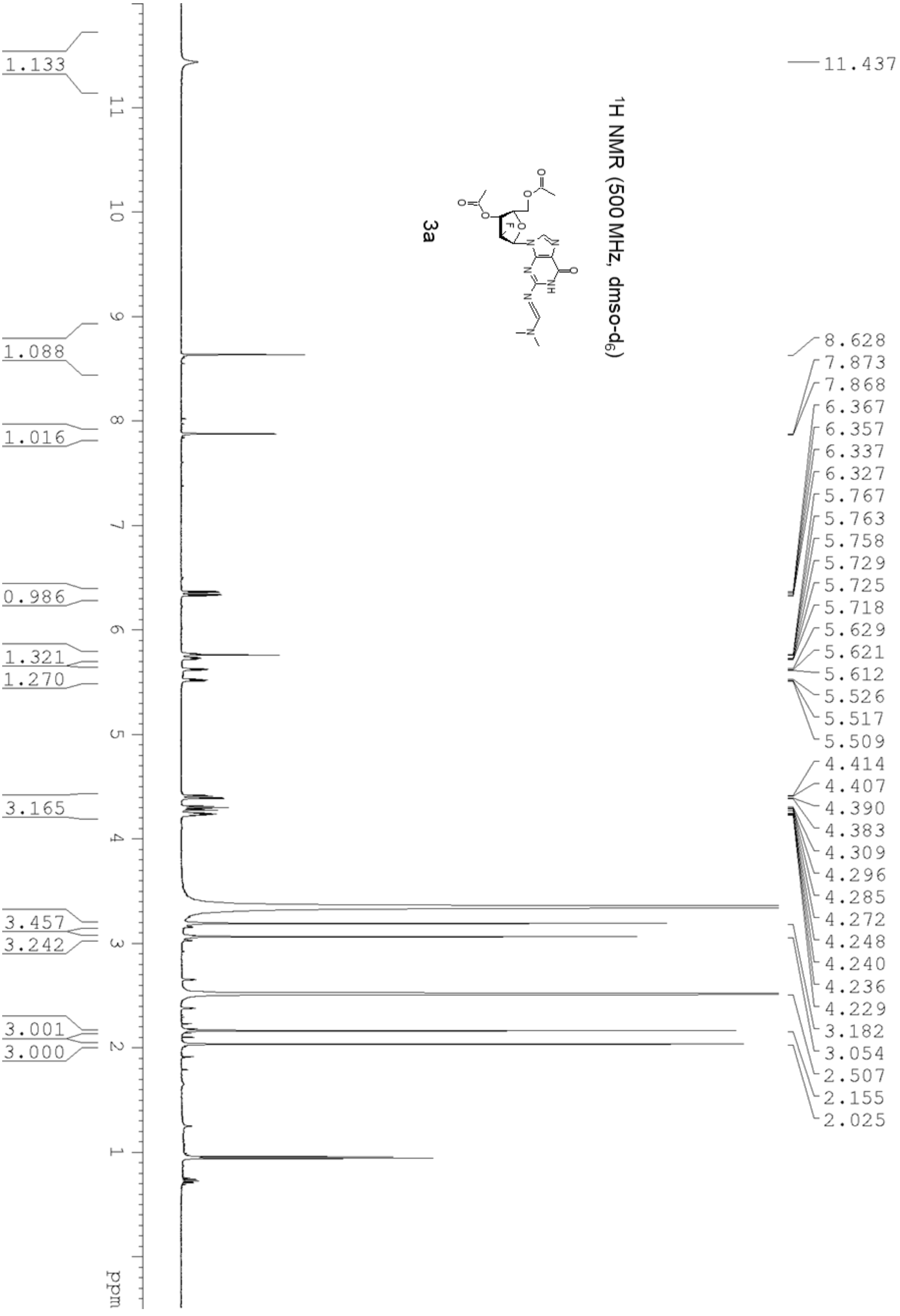


¹H NMR (400 MHz, dmso-d$_6$)
2c
10.565
10.305
8.323
5.887
5.592
4.554
4.534
4.516
4.502
4.116
4.107
4.085
4.076
4.031
4.018
4.012
3.952
3.921
2.314
2.220
1.048
1.033
1.016
0.998
0.973
0.872
0.852
1.000
1.082
1.099
1.066
1.270
2.022
3.419
2.377
2.790
22.590
11
10
9
8
7
6
5
4
3
2
1
ppm



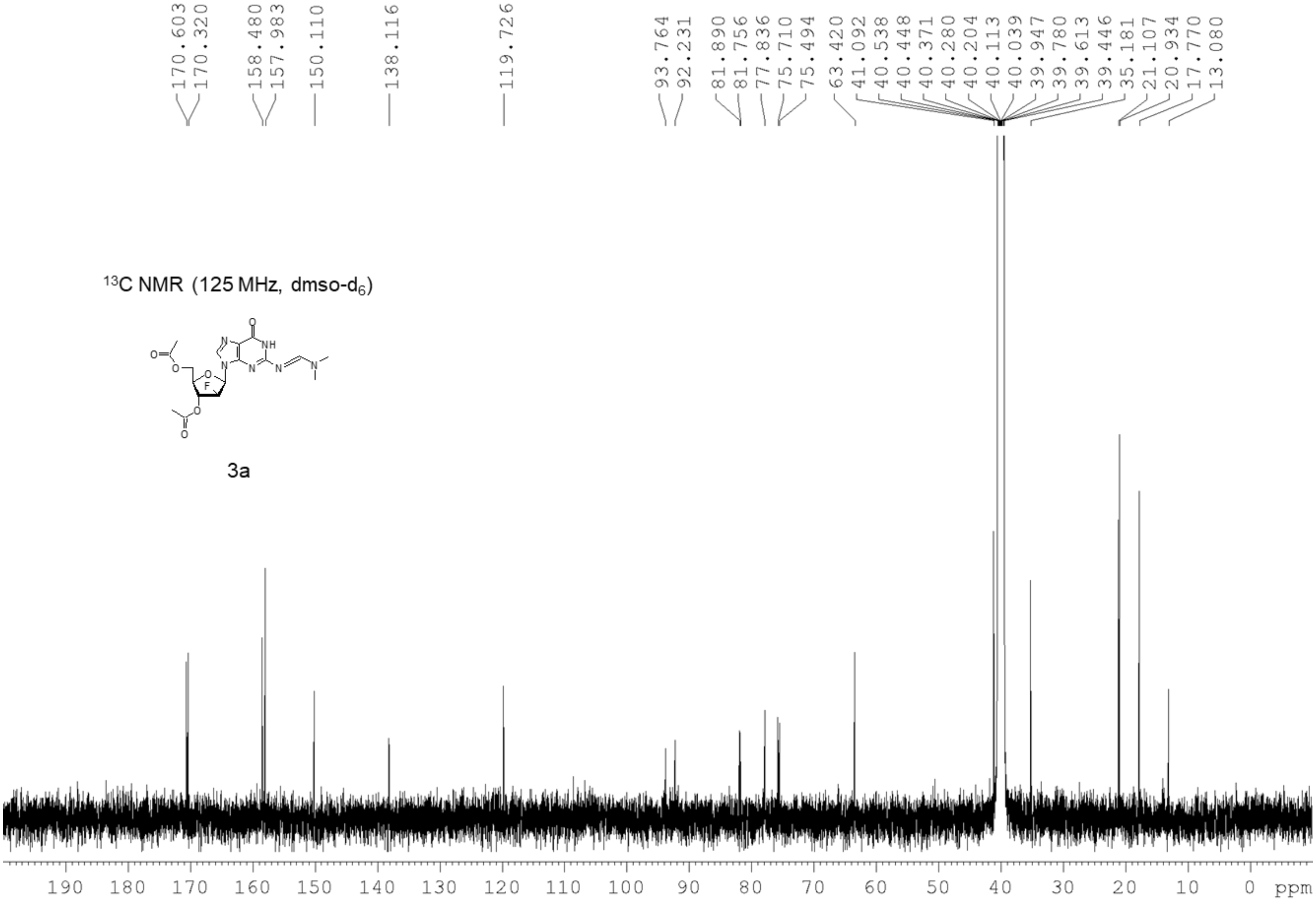
^{13}C NMR (176 MHz, dmso-d$_6$)

2c

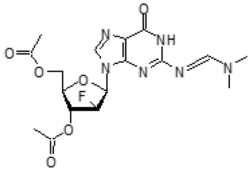


^{1}H NMR (500 MHz, dmso-d$_6$)

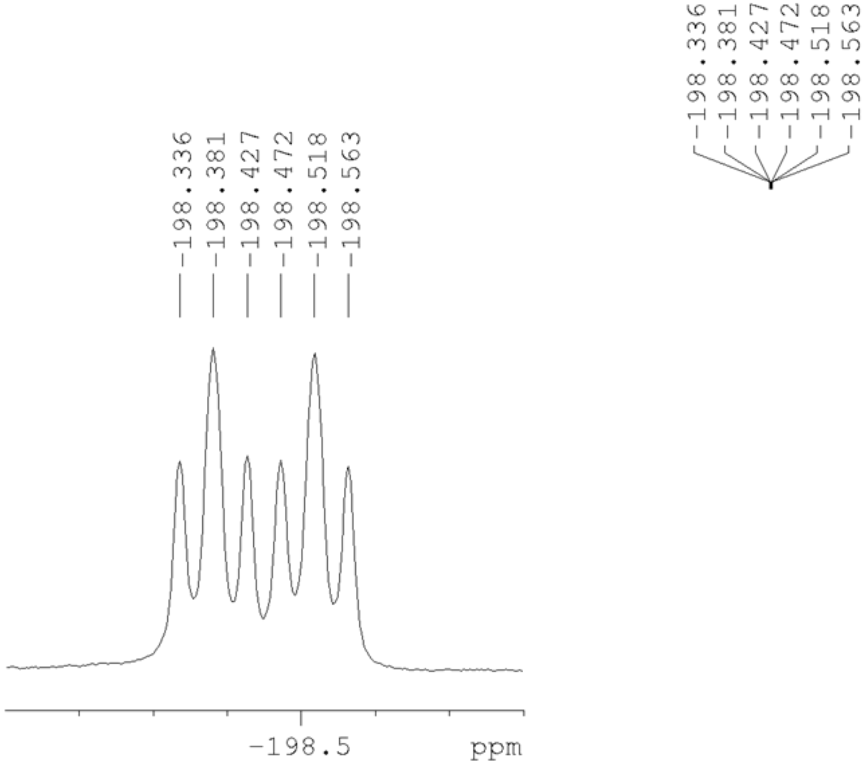
3a

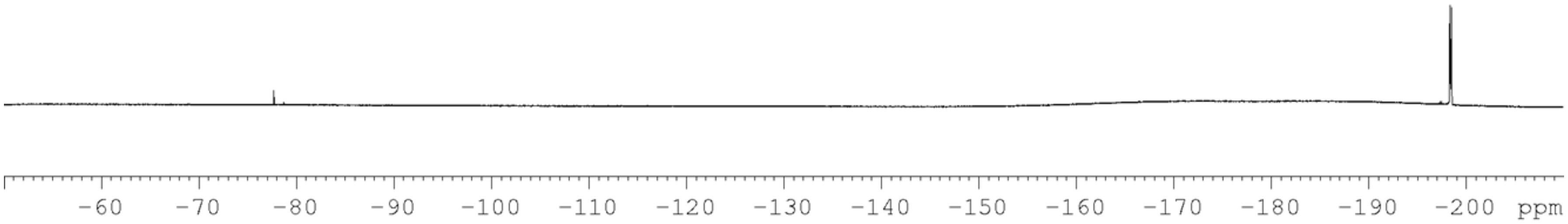

^{13}C NMR (125 MHz, dmso-d$_6$)
3a
O
NH
N
F
170.603
170.320
158.480
157.983
150.110
138.116
119.726
93.764
92.231
81.890
81.756
77.836
75.710
75.494
63.420
41.092
40.538
40.448
40.371
40.280
40.204
40.113
40.039
39.947
39.780
39.613
39.446
35.181
21.107
20.934
17.770
13.080
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

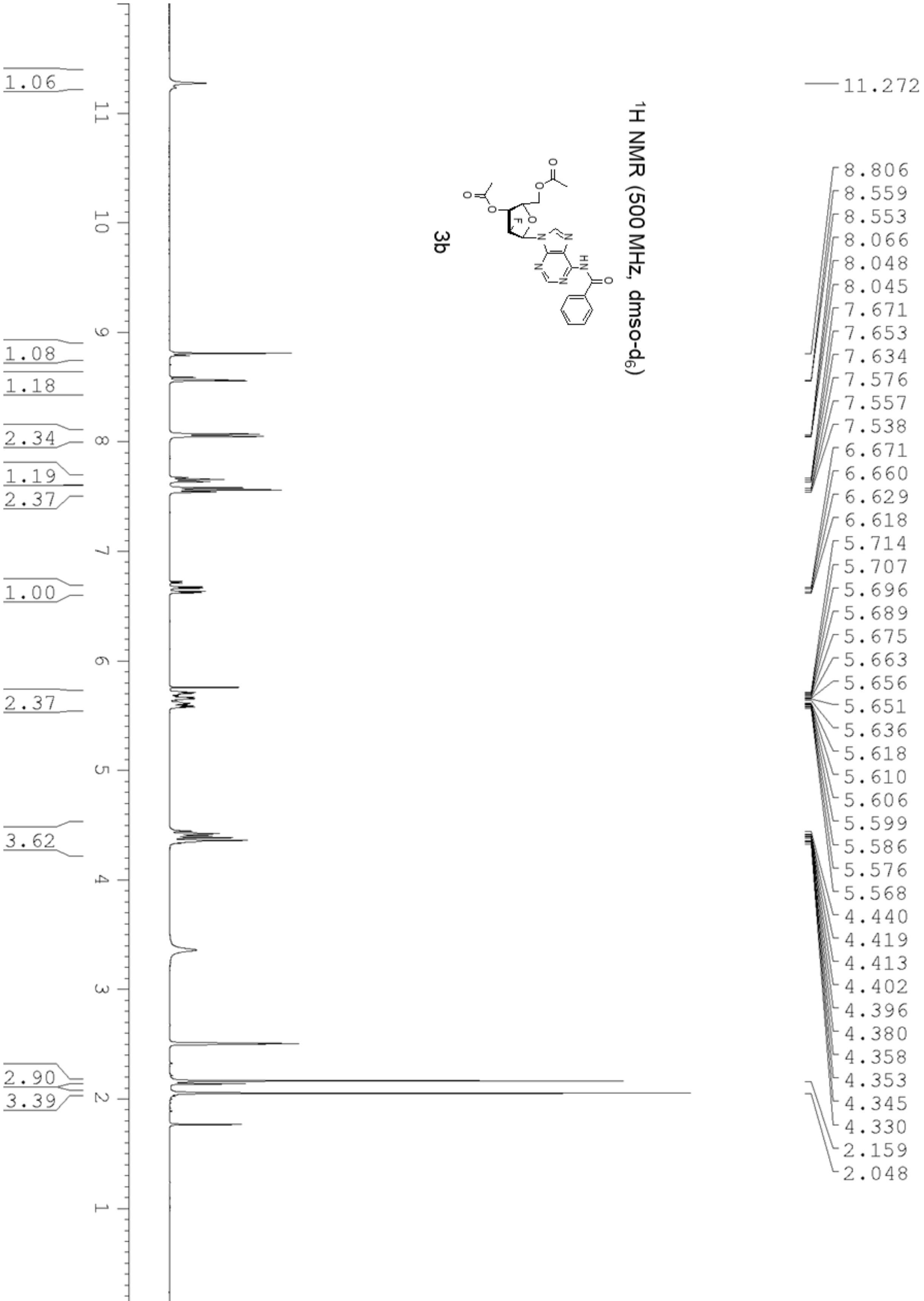
^{1}F NMR (470 MHz, dmso-d_6)



3a

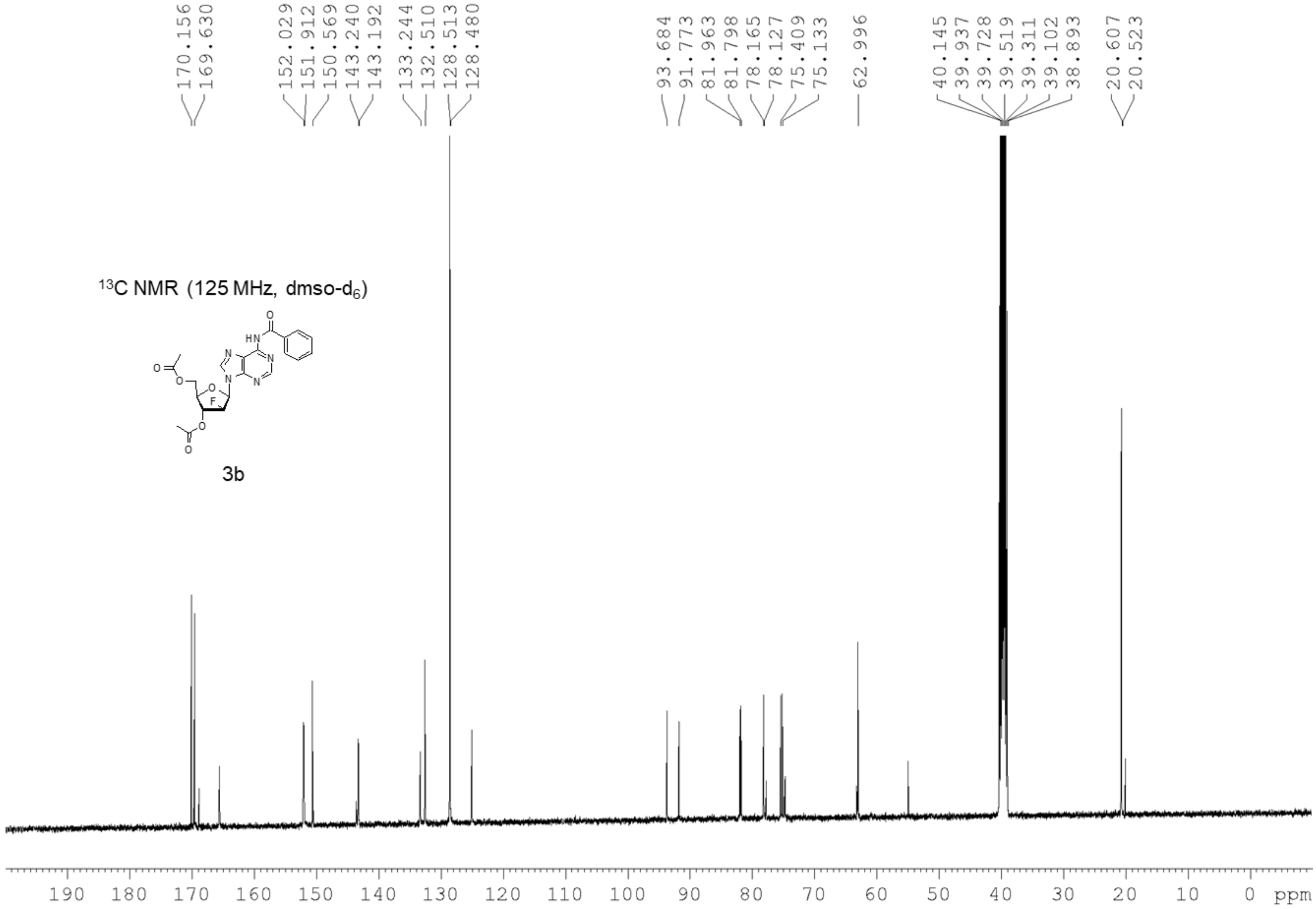




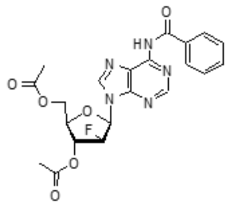


^{1}H NMR (500 MHz, dmso-d$_6$)

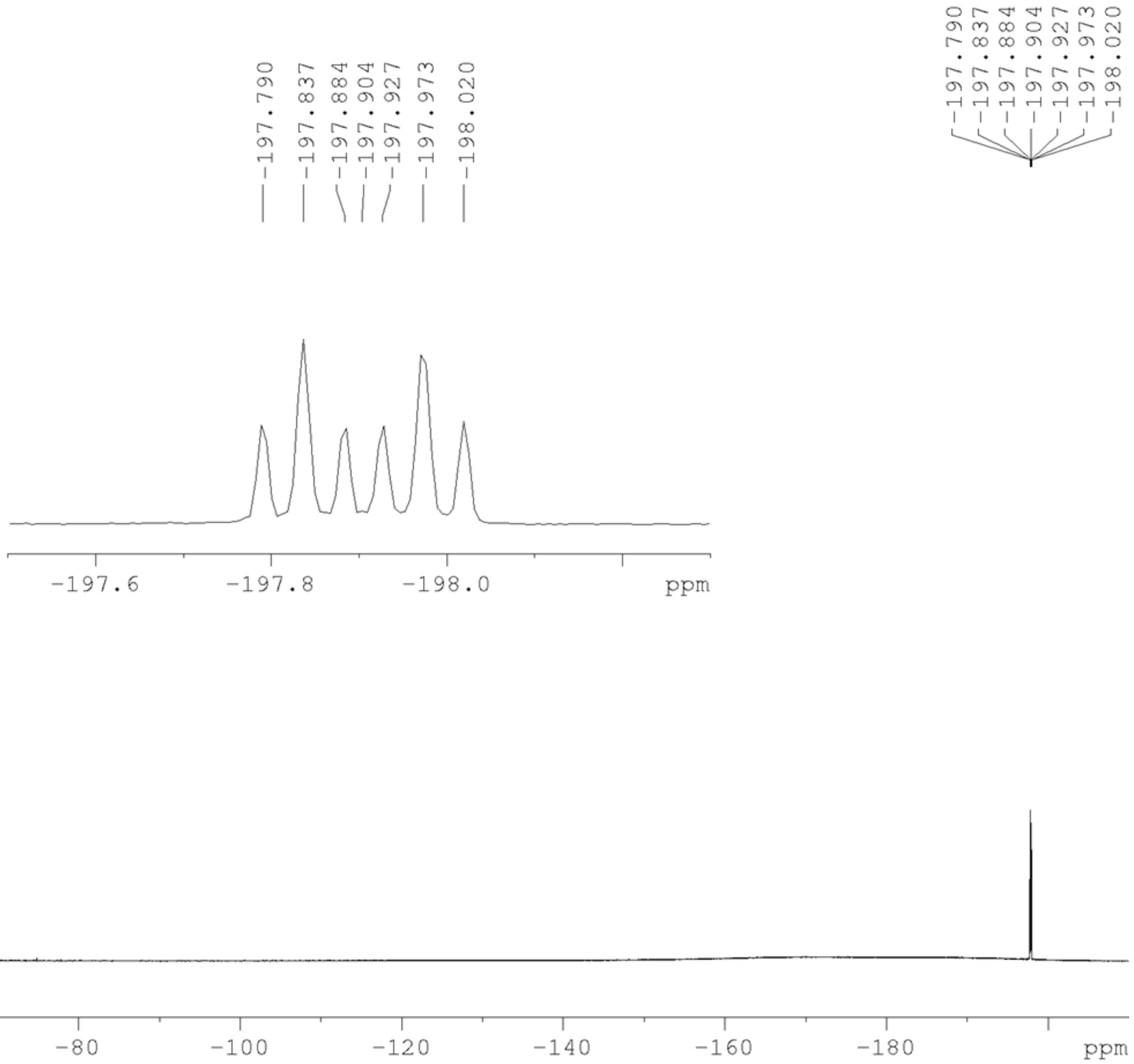
3b

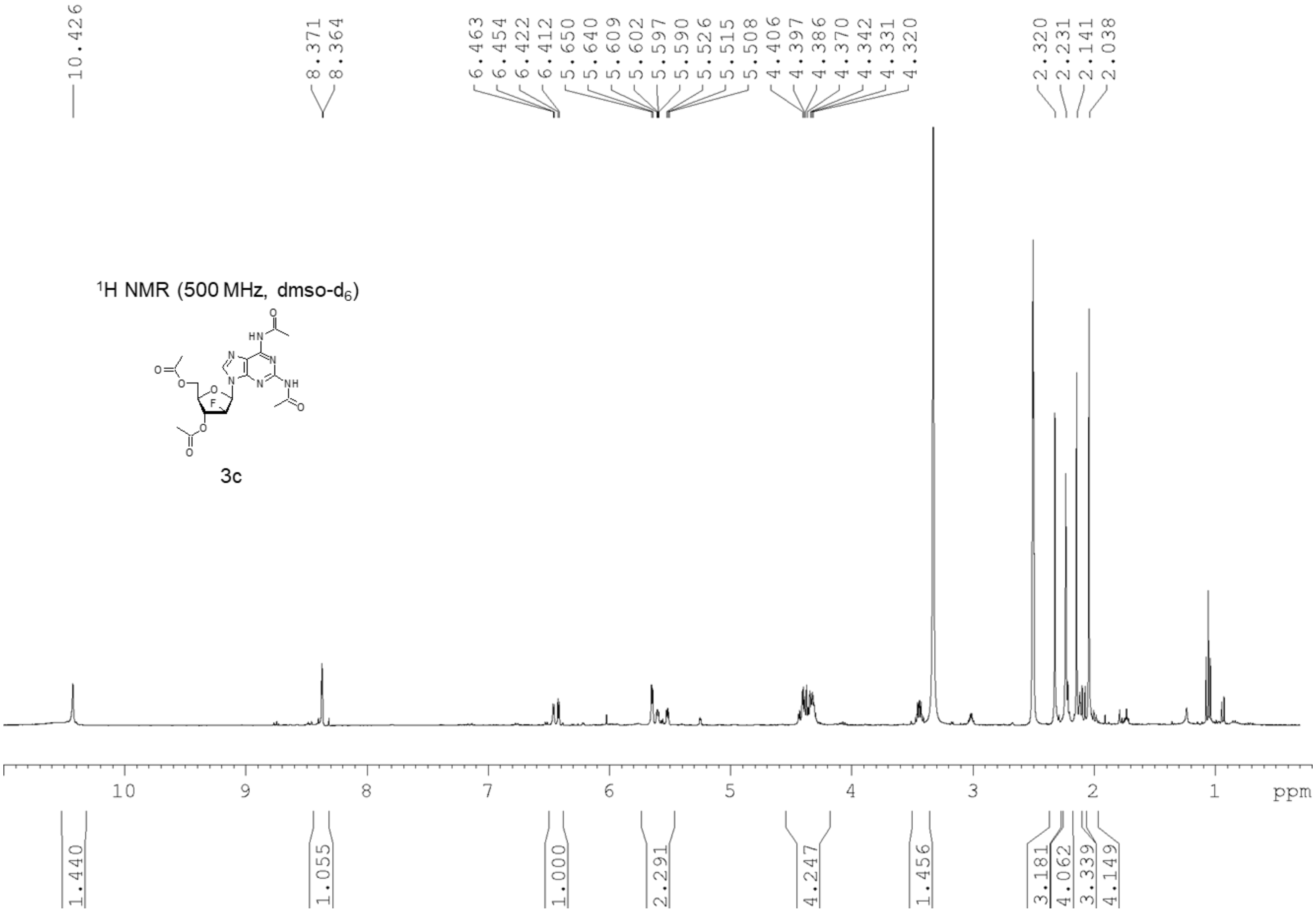

170.156
169.630
152.029
151.912
150.569
143.240
143.192
133.244
132.510
128.513
128.480
93.684
91.773
81.963
81.798
78.165
78.127
75.409
75.133
62.996
40.145
39.937
39.728
39.519
39.311
39.102
38.893
20.607
20.523
^{13}C NMR (125 MHz, dmso-d$_6$)
HN
O
N
F
3b
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

^{19}F NMR (470MHz, dmso-d_6)



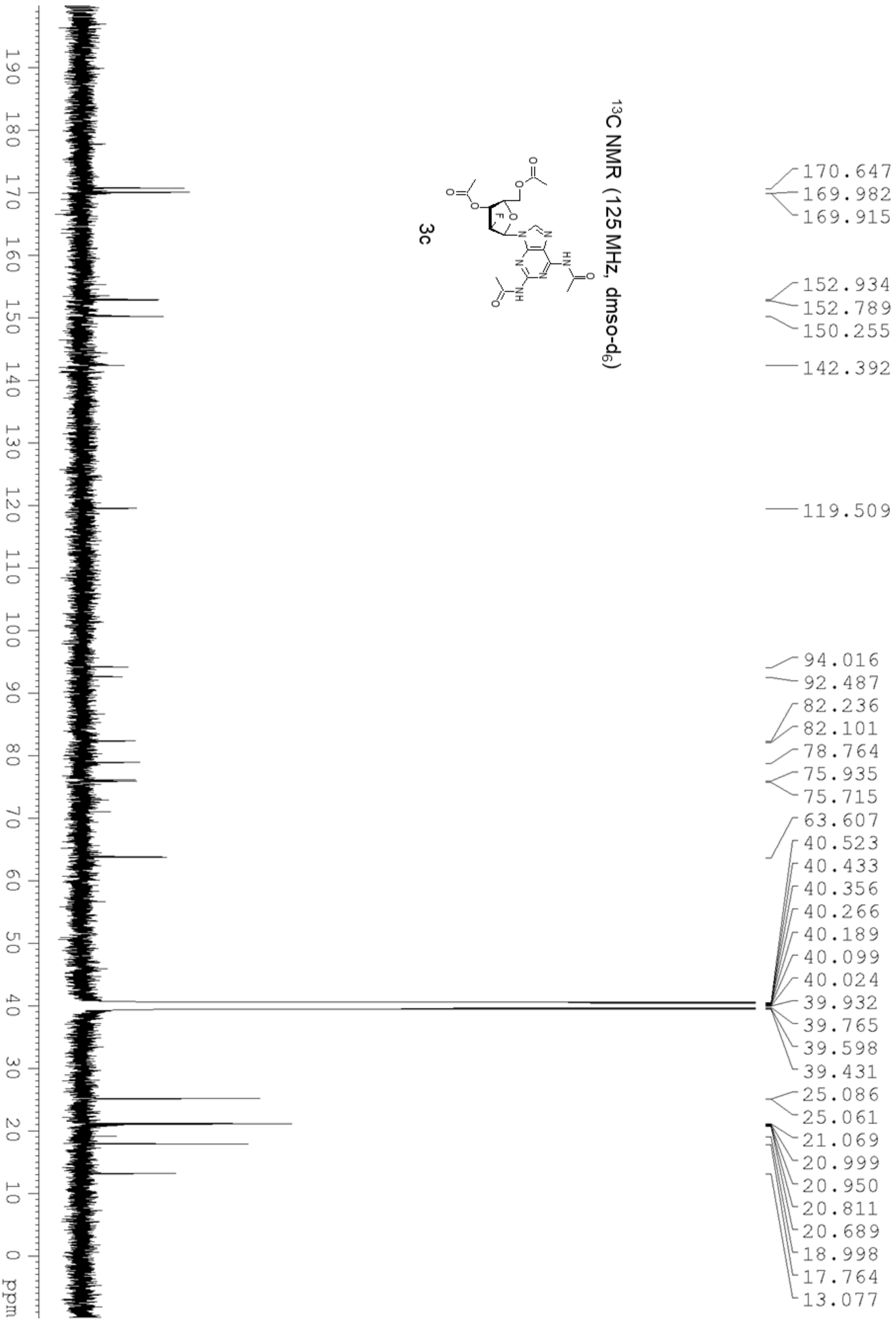
3b





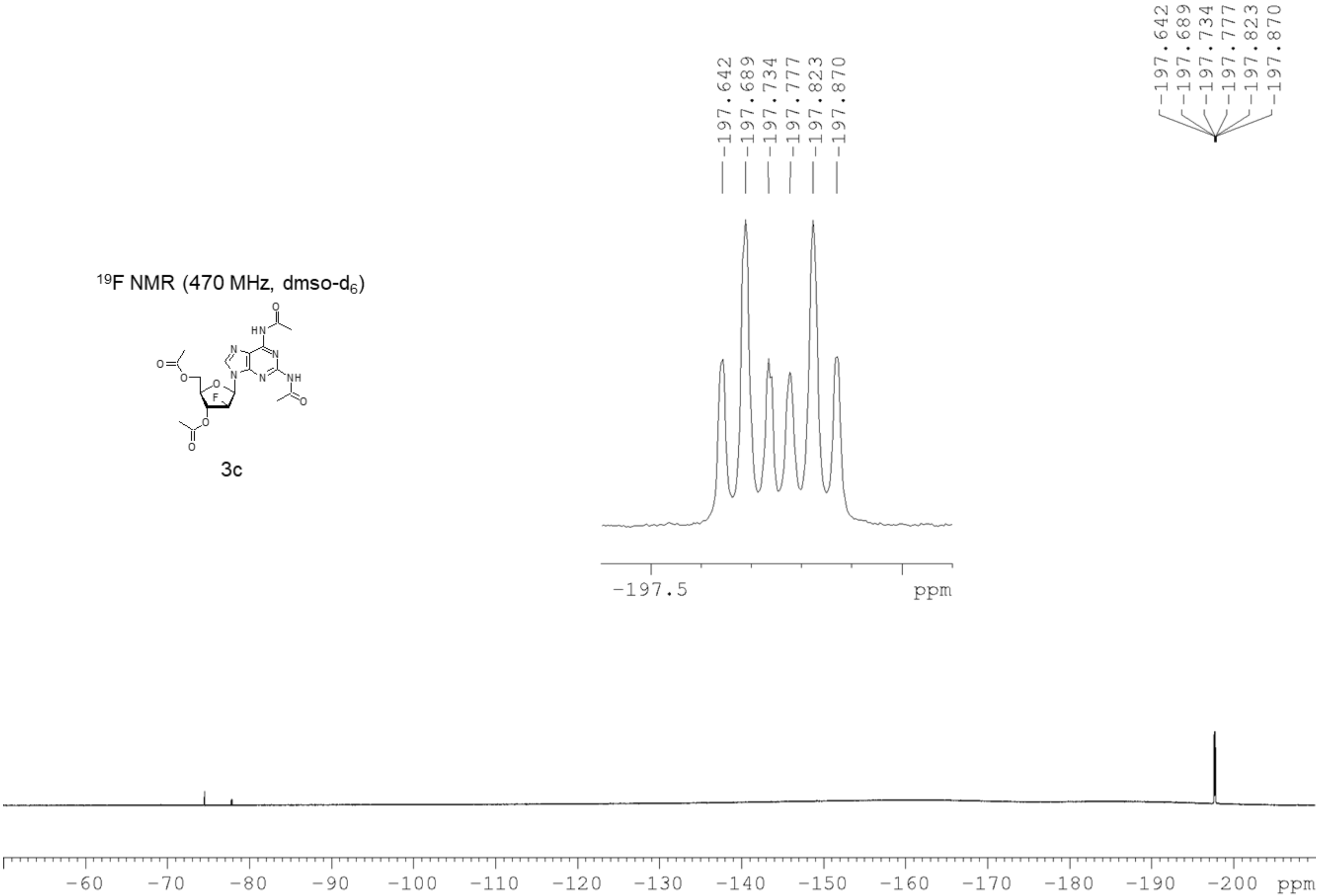
^{1}H NMR (500 MHz, dmso-d_6)

3c



^{13}C NMR (125 MHz, dmso-d$_6$)

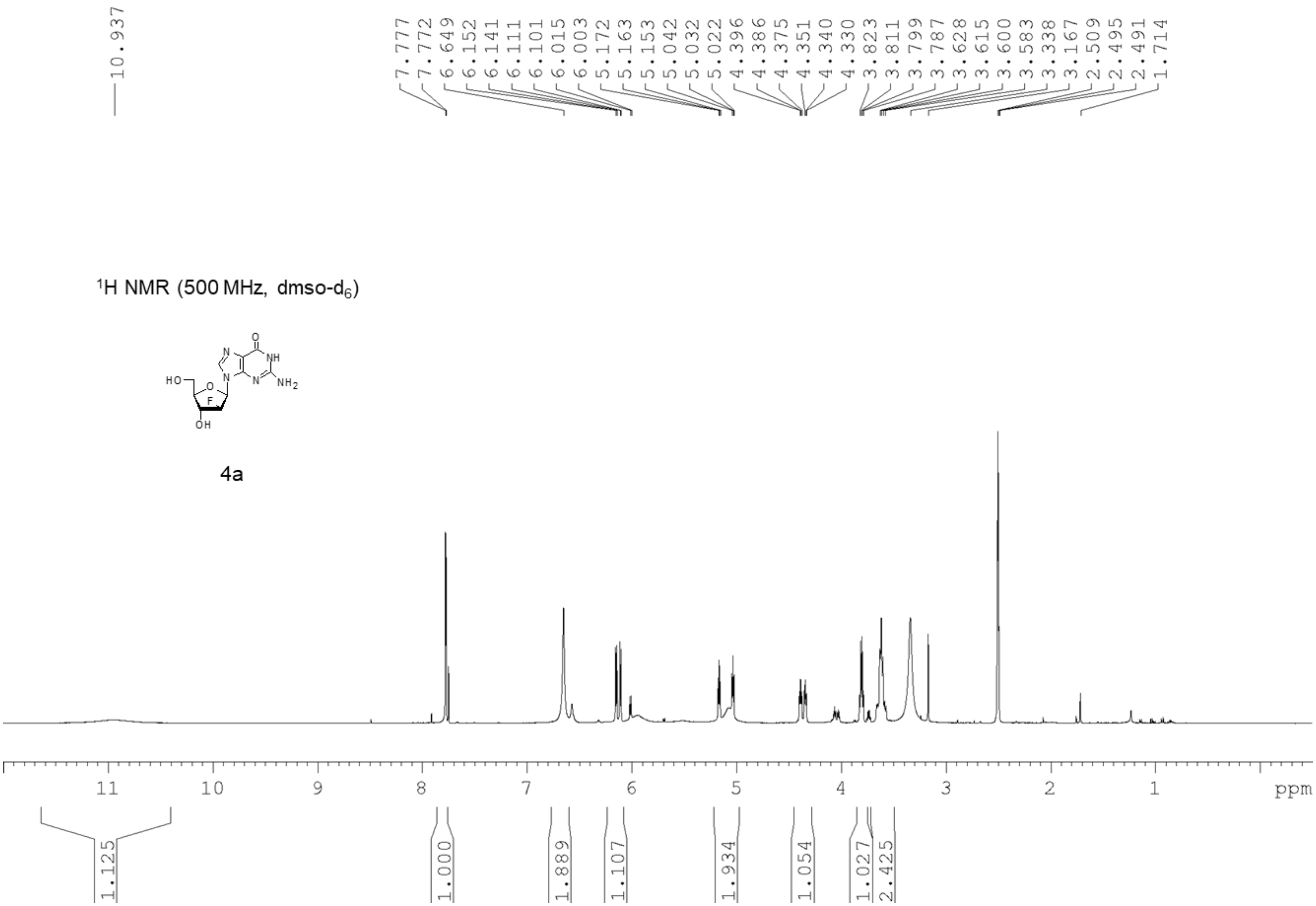
3c



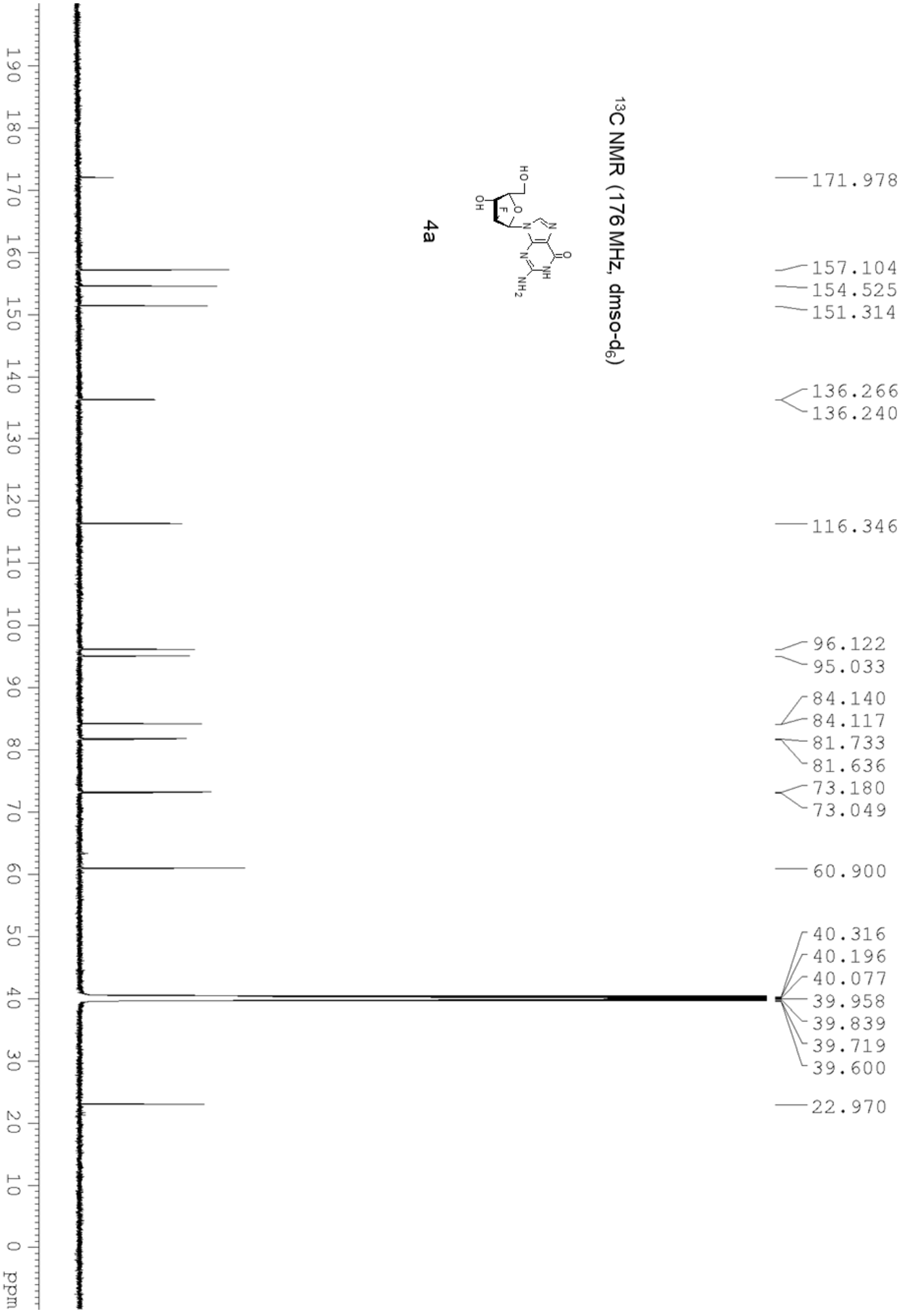
^{19}F NMR (470 MHz, dmso-d$_6$)

3c

^1H NMR (500 MHz, dmso-d_6)

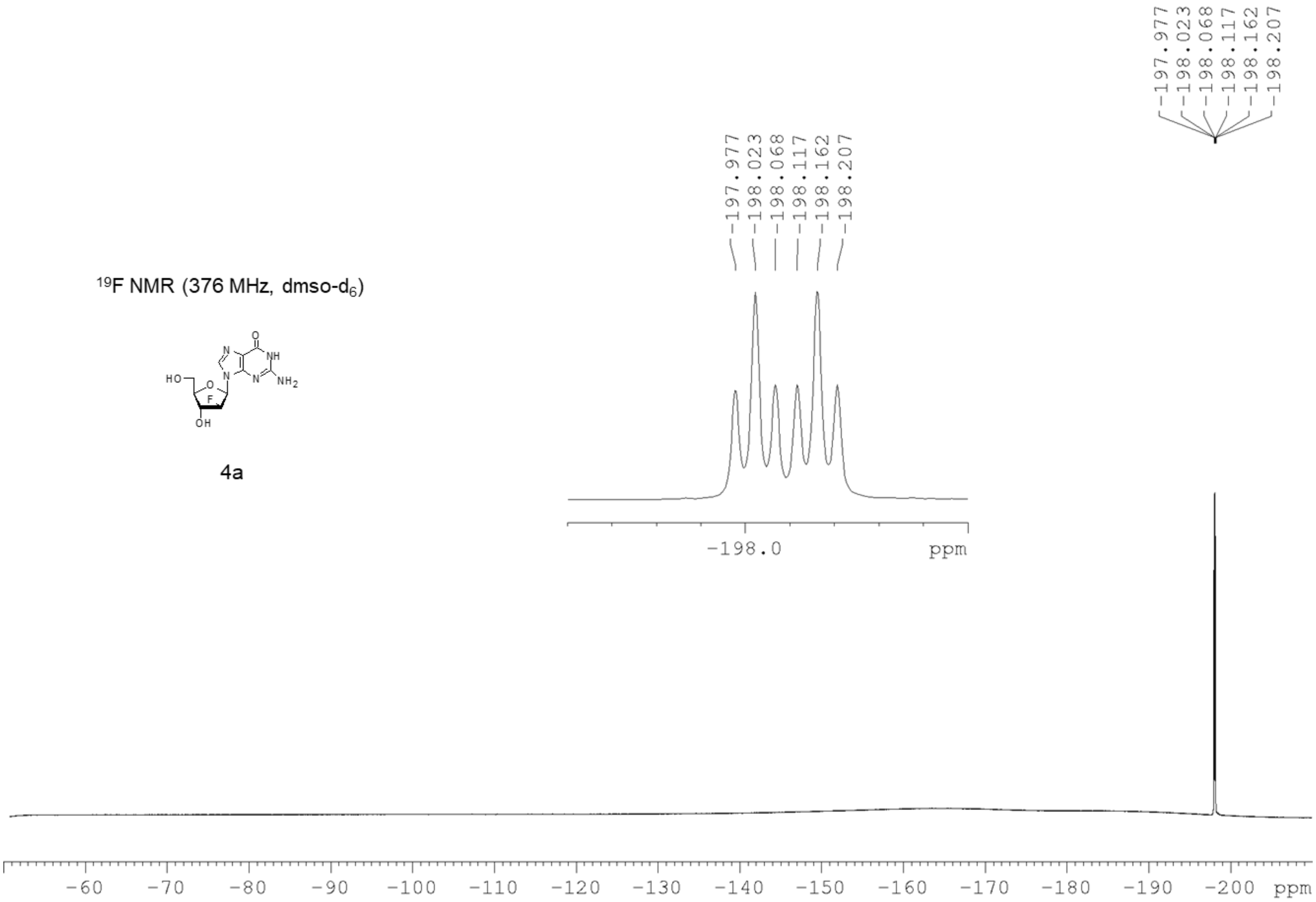


4a



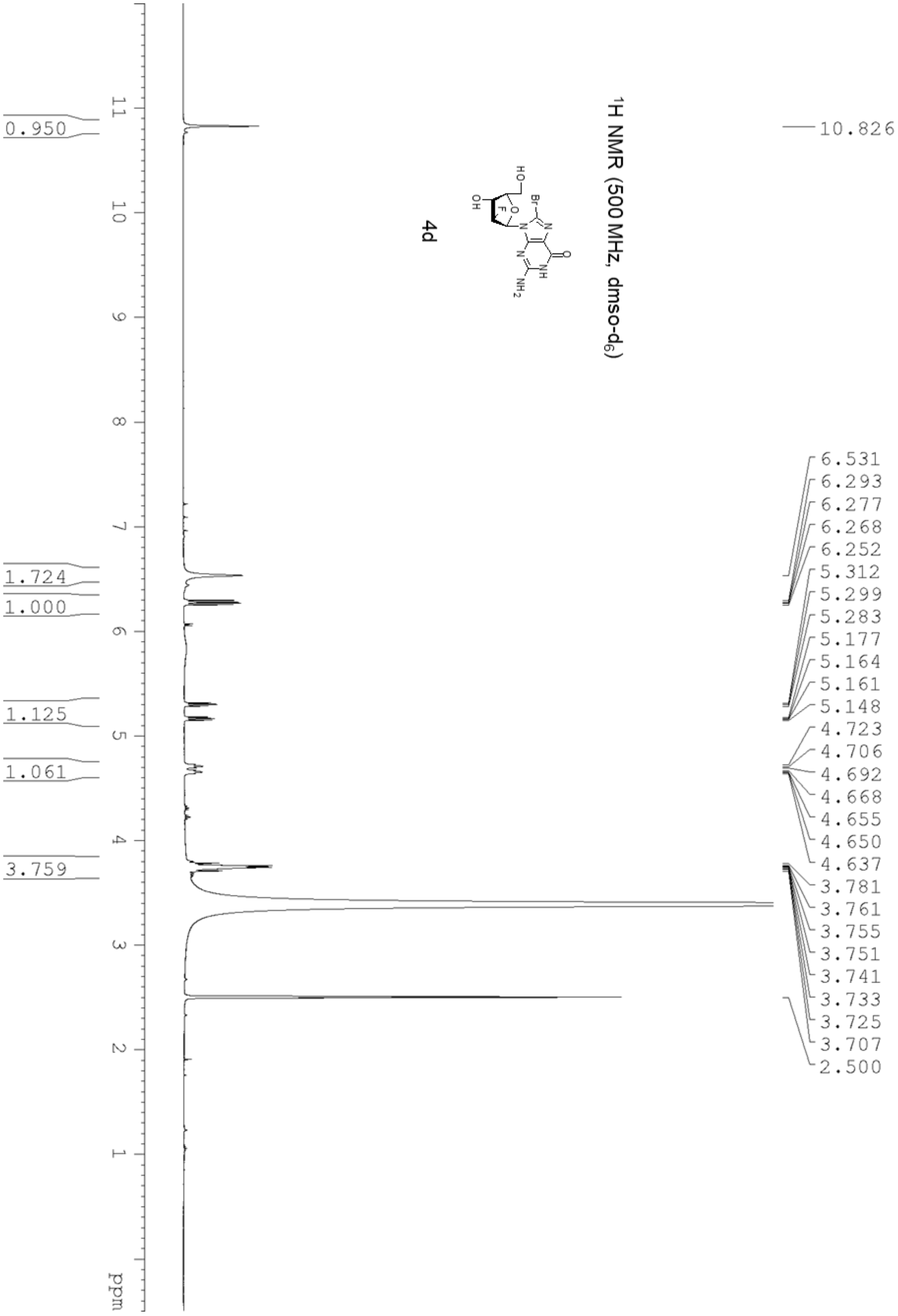
^{13}C NMR (176 MHz, dmso-d$_6$)

4a



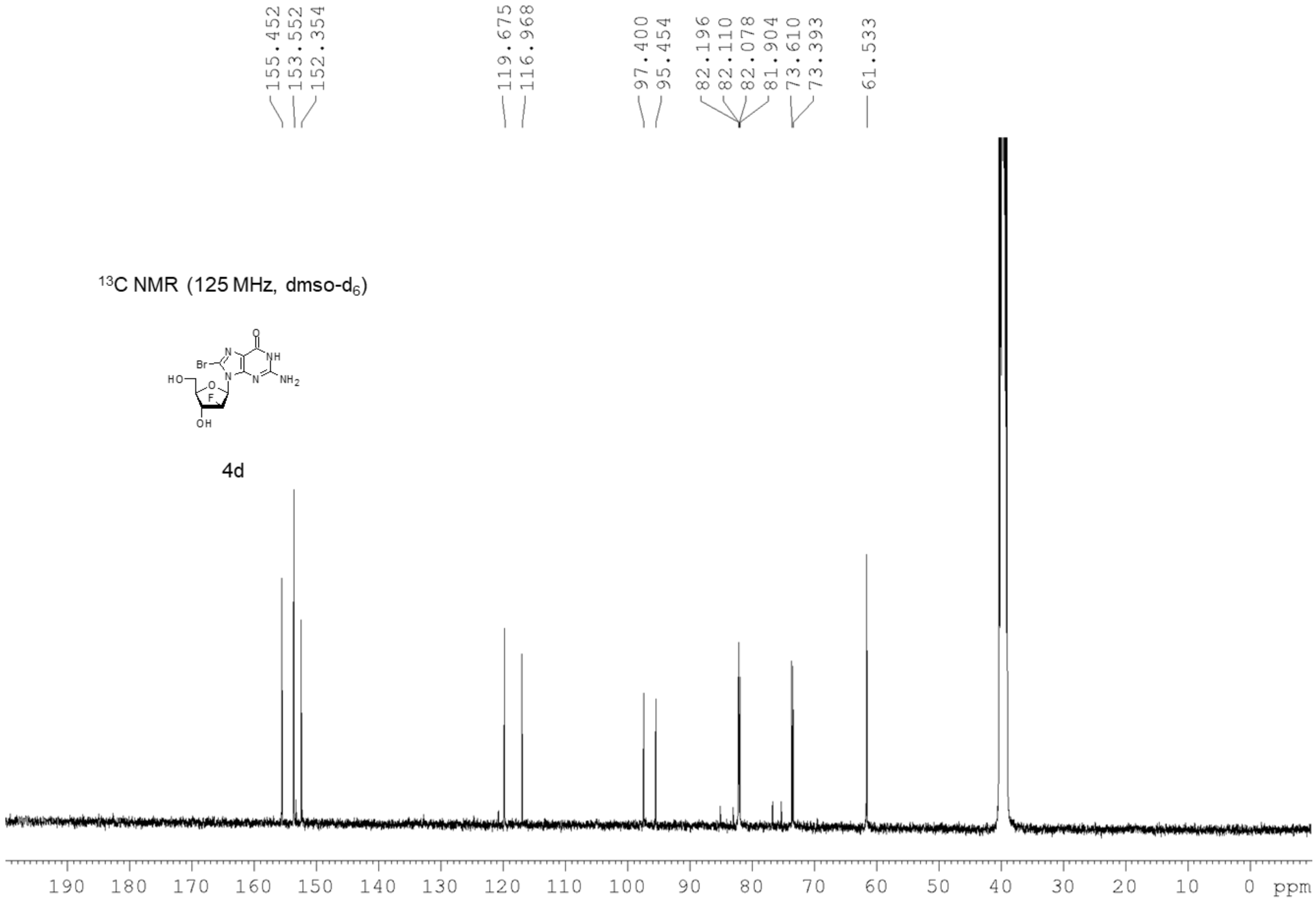
^{19}F NMR (376 MHz, dmso-d_6)

4a



^1H NMR (500 MHz, dmso-d_6)

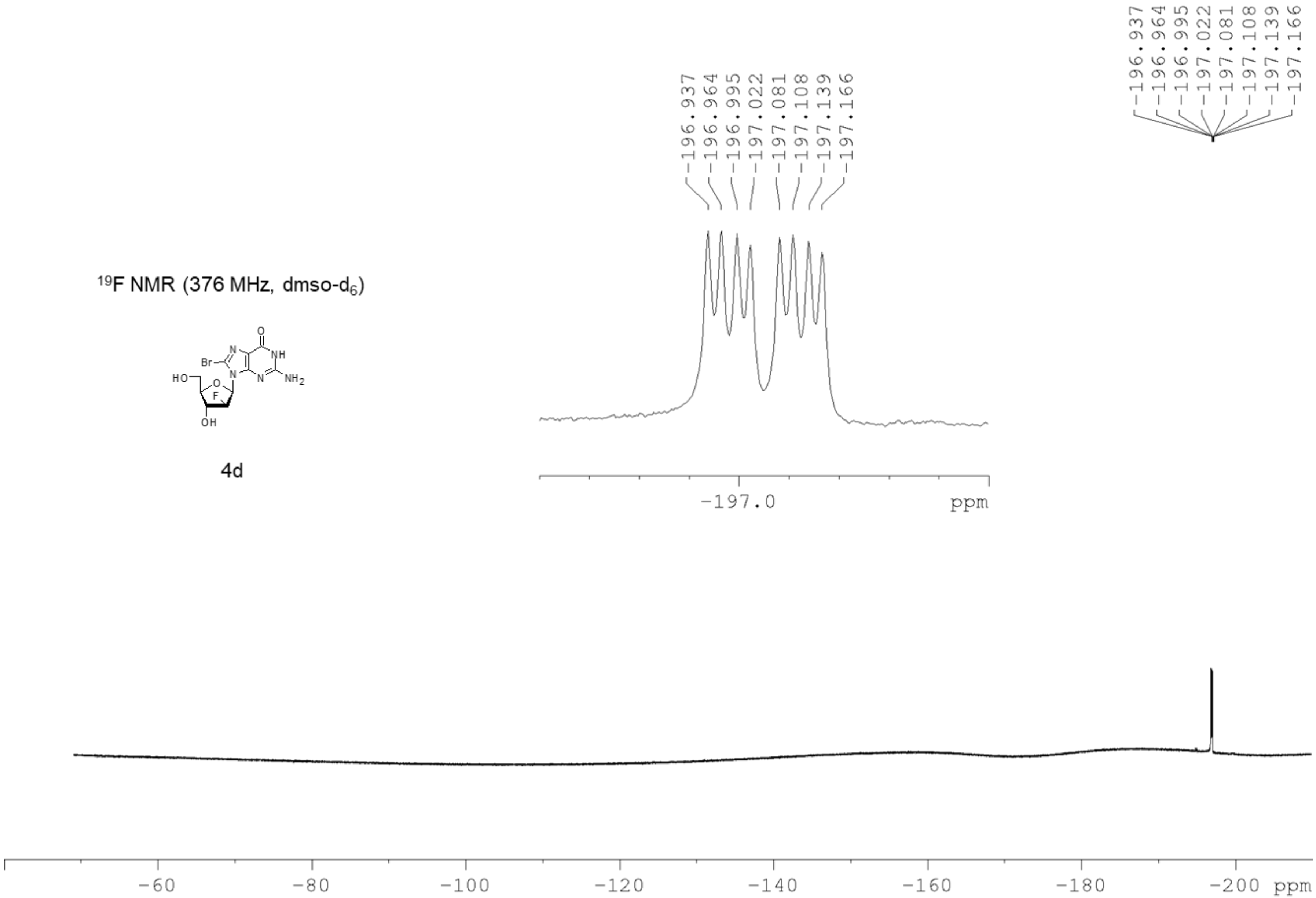
4d

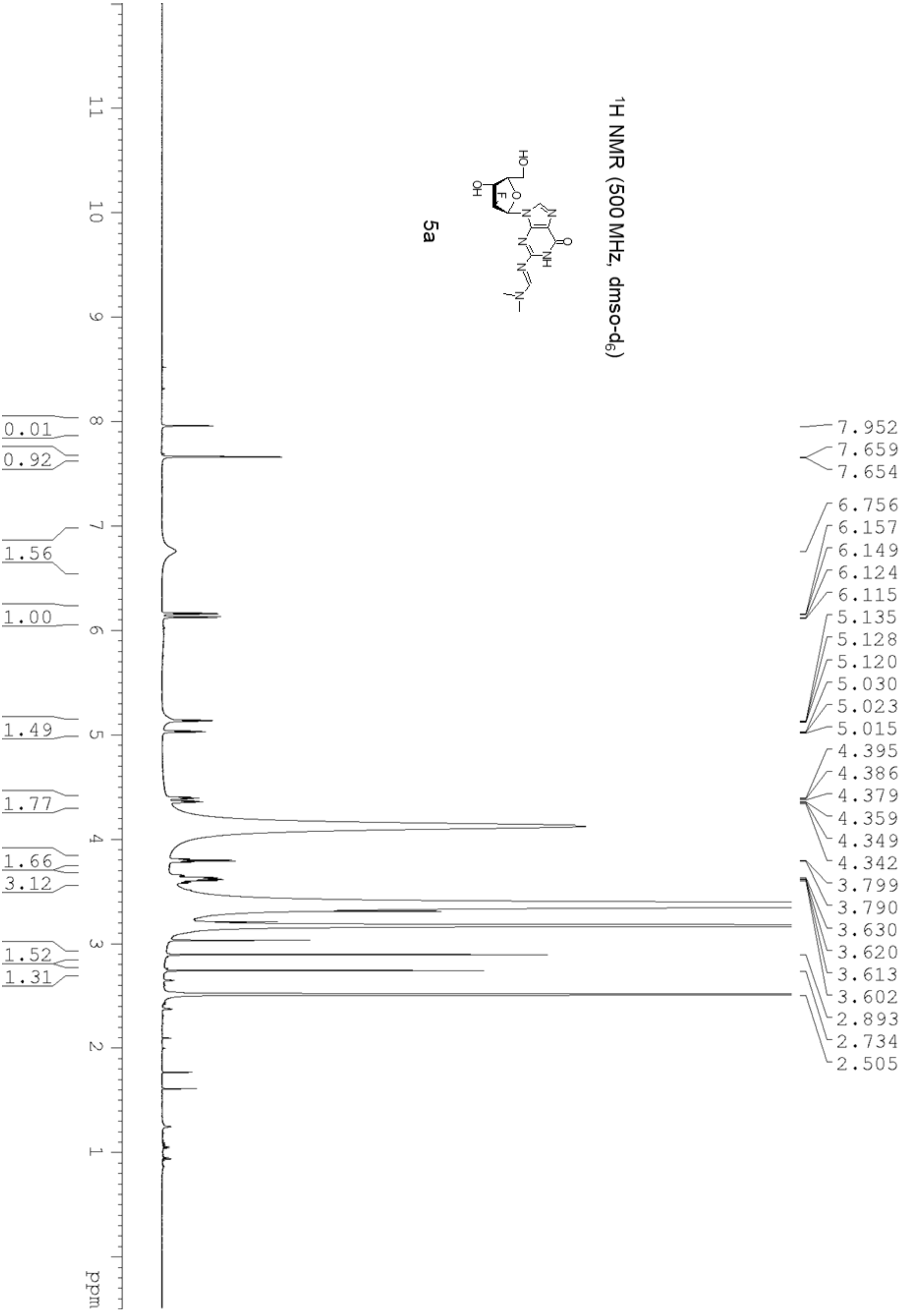

^{13}C NMR (125 MHz, dmso-d_6)

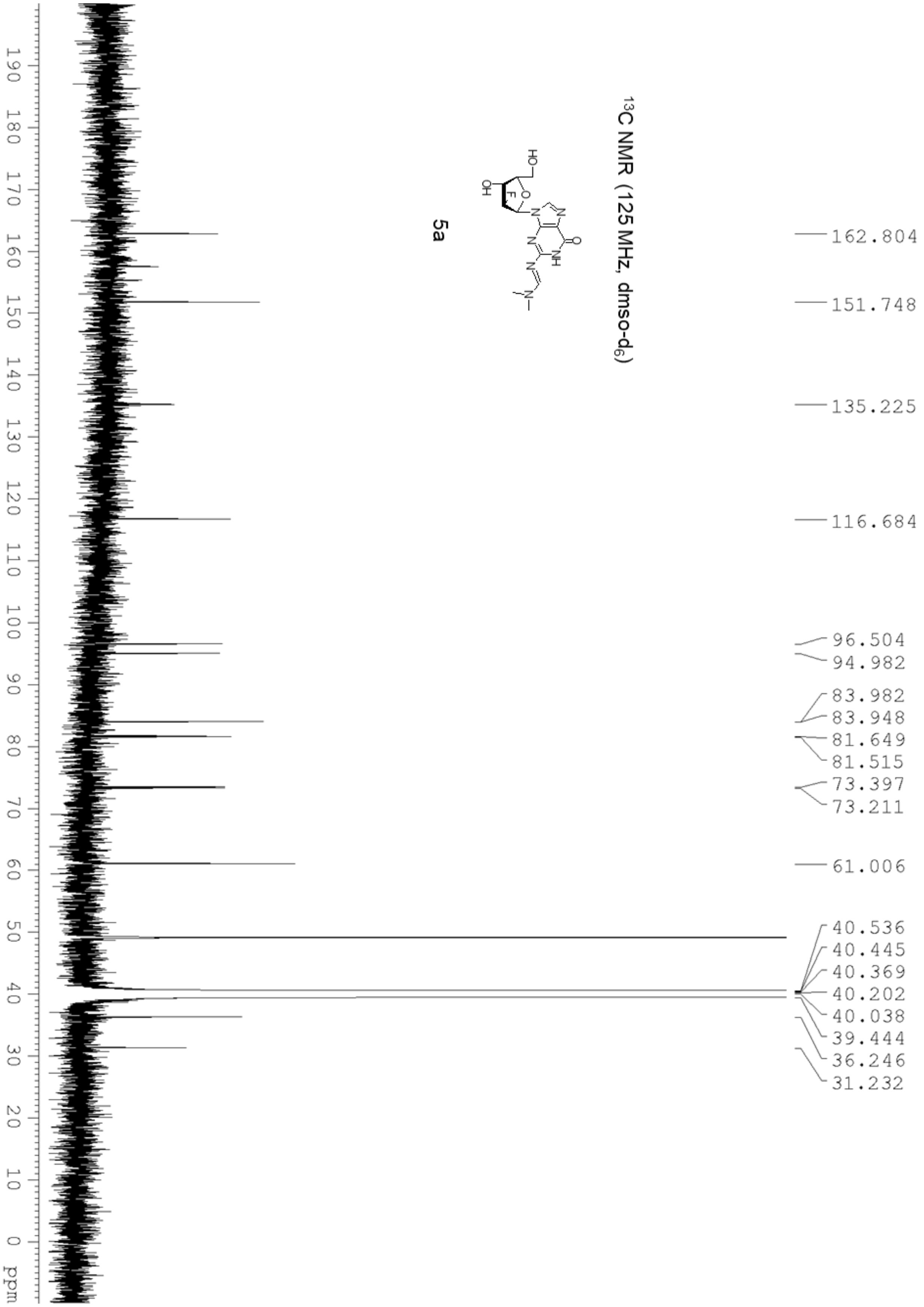
4d

^{19}F NMR (376 MHz, dmso-d_6)

4d



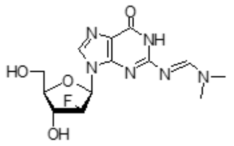

^{1}H NMR (500 MHz, dmso-d$_6$)
5a
HO
OH
F
O
N
NH
N
N
N
N
O
7.952
7.659
7.654
6.756
6.157
6.149
6.124
6.115
5.135
5.128
5.120
5.030
5.023
5.015
4.395
4.386
4.379
4.359
4.349
4.342
3.799
3.790
3.630
3.620
3.613
3.602
2.893
2.734
2.505
0.01
0.92
1.56
1.00
1.49
1.77
1.66
3.12
1.52
1.31
11
10
9
8
7
6
5
4
3
2
1
ppm



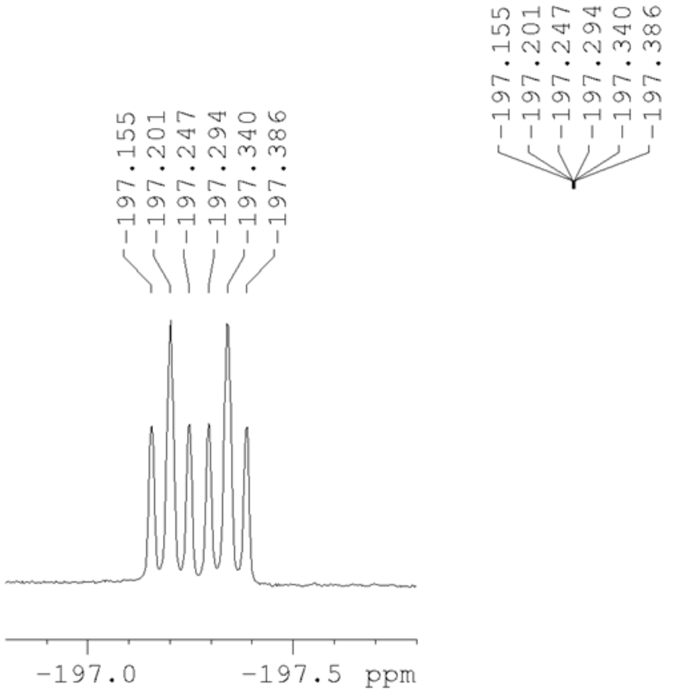
^{13}C NMR (125 MHz, dmso-d$_6$)

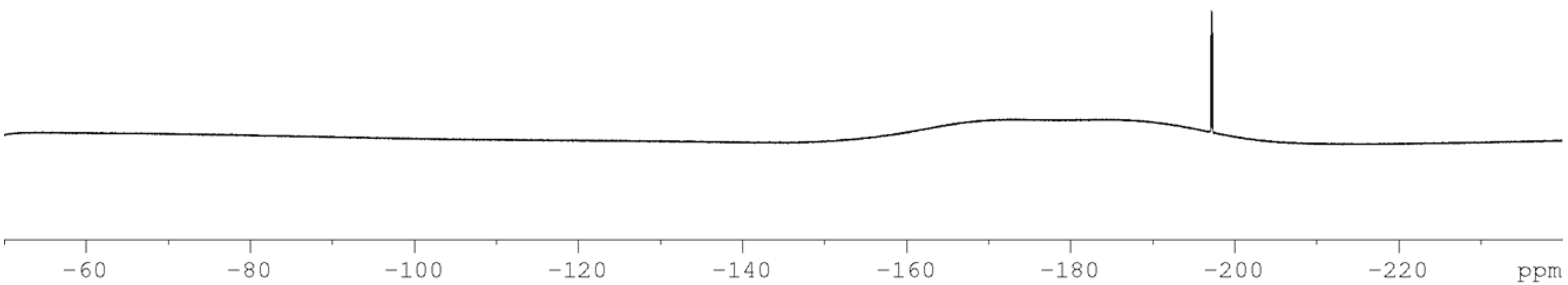
5a

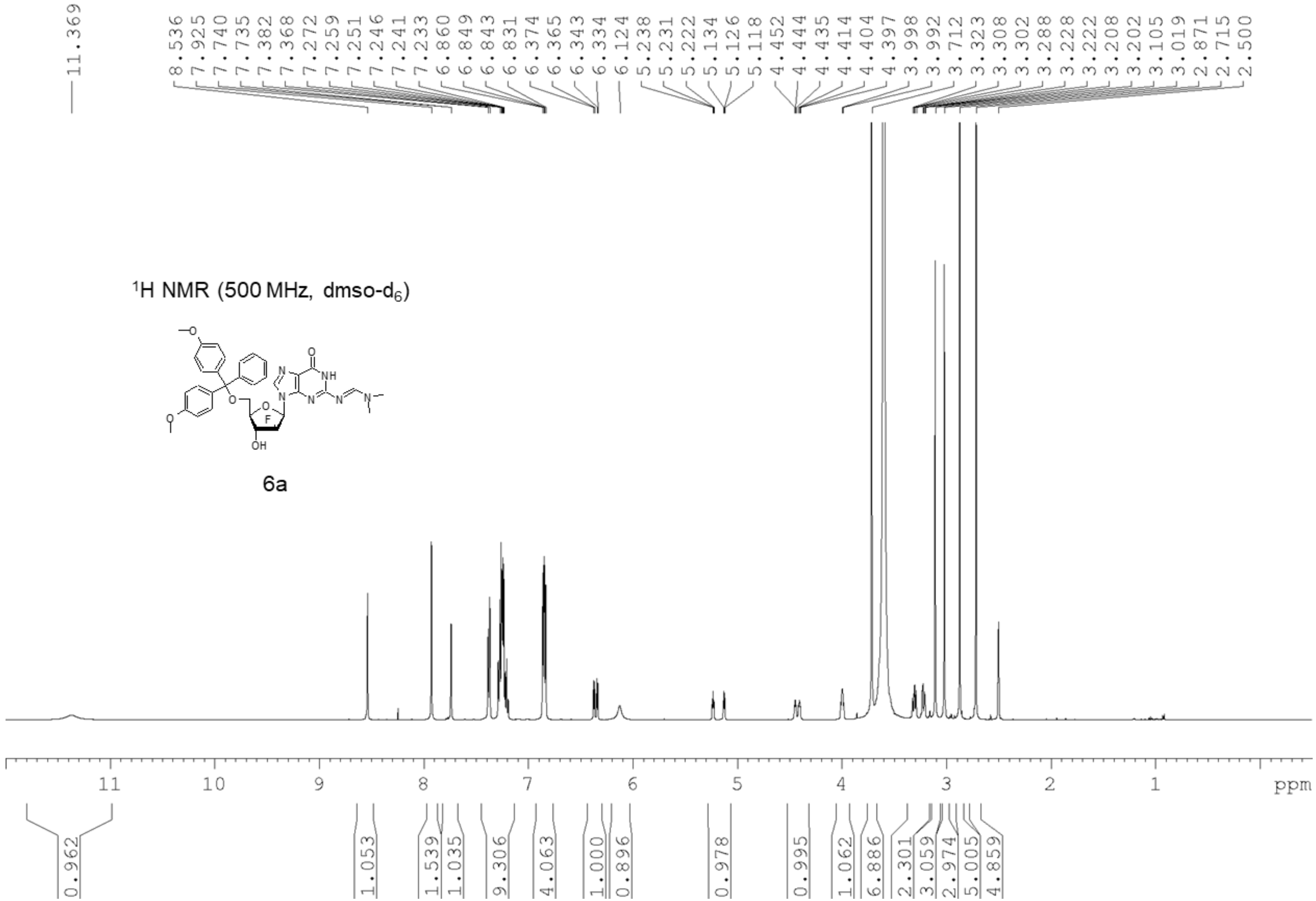
^{19}F NMR (376 MHz, dmso-d$_6$)



5a

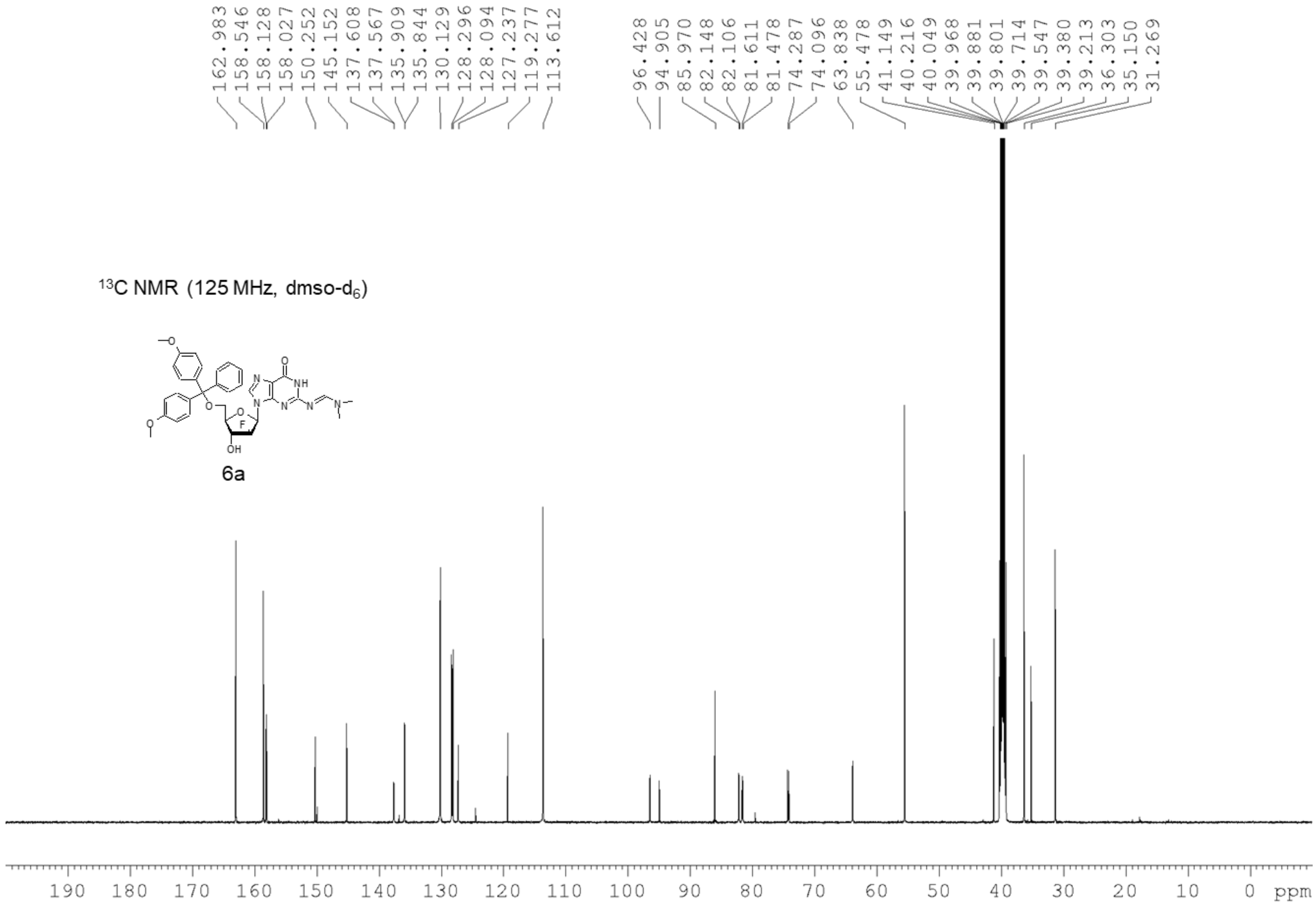






^{1}H NMR (500 MHz, dmso-d_6)

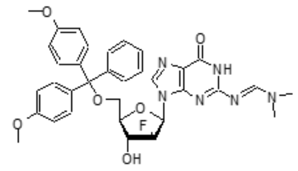
6a



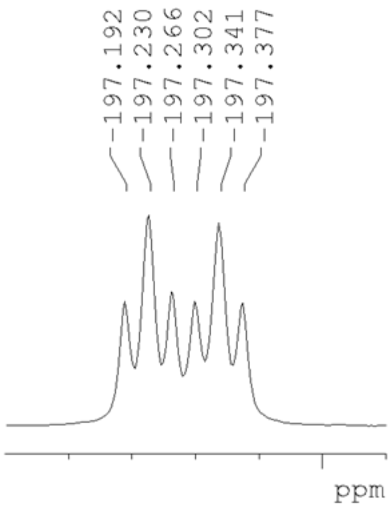
^{13}C NMR (125 MHz, dmso-d_6)

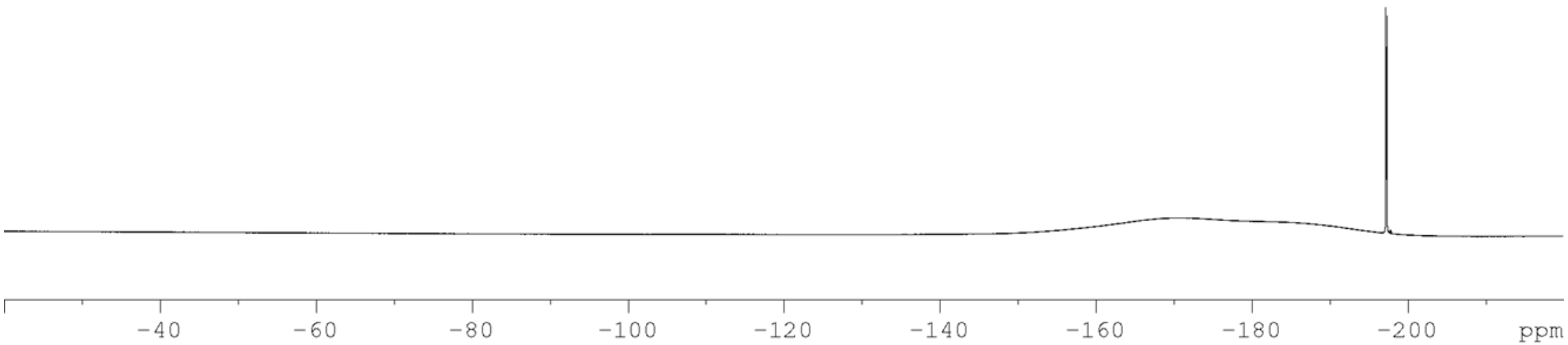
6a

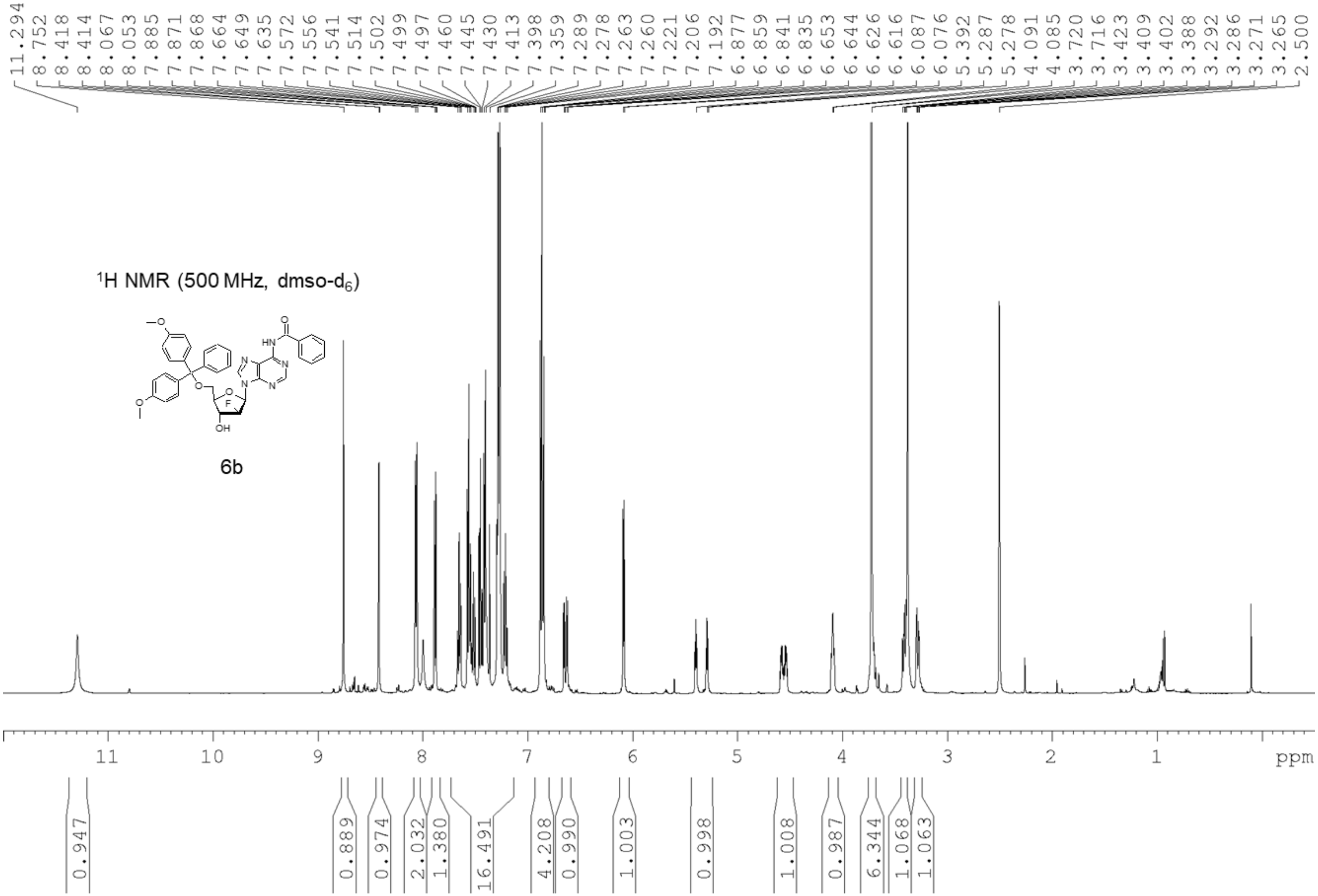
^{19}F NMR (470 MHz, dmso-d$_6$)

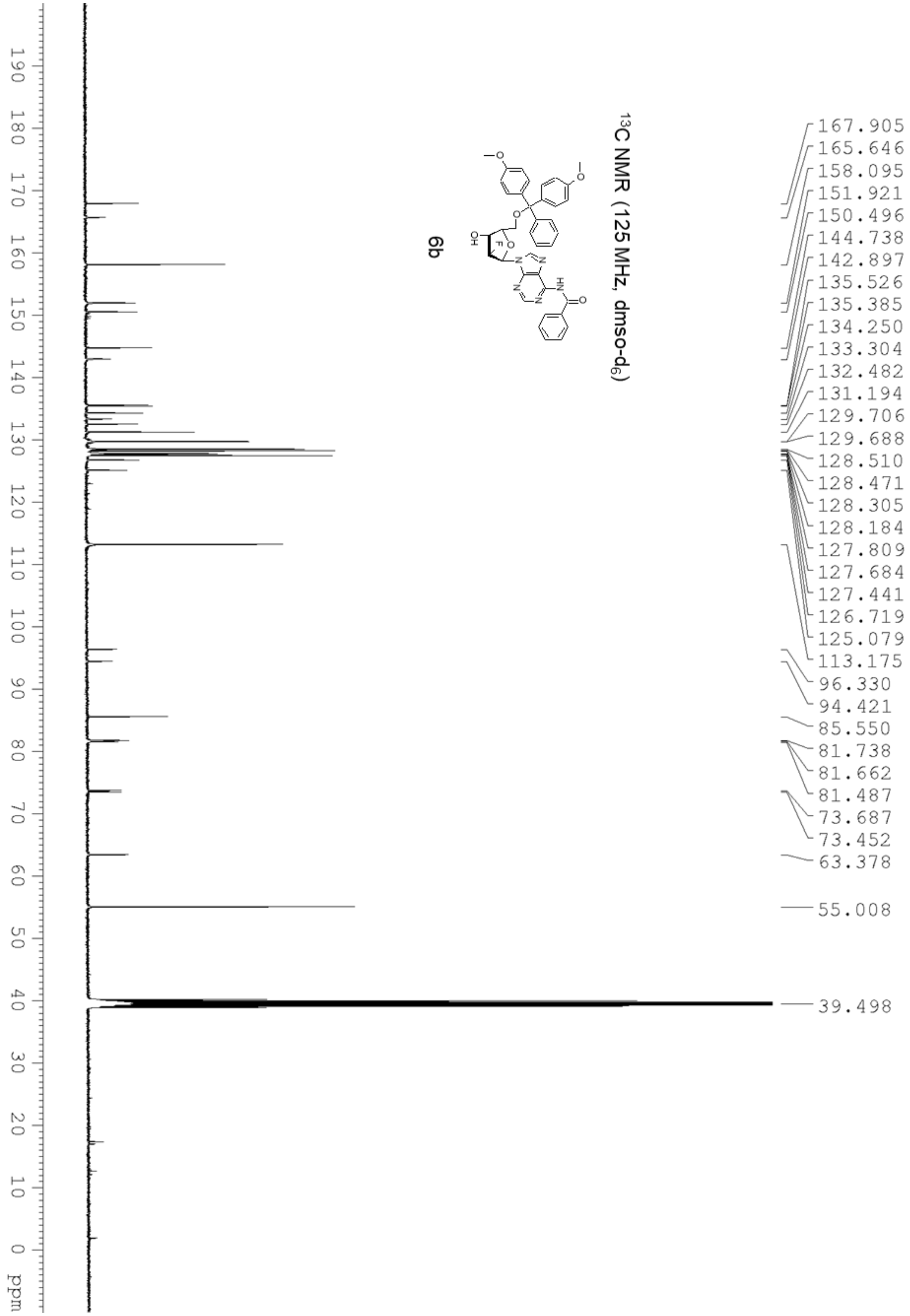


6a





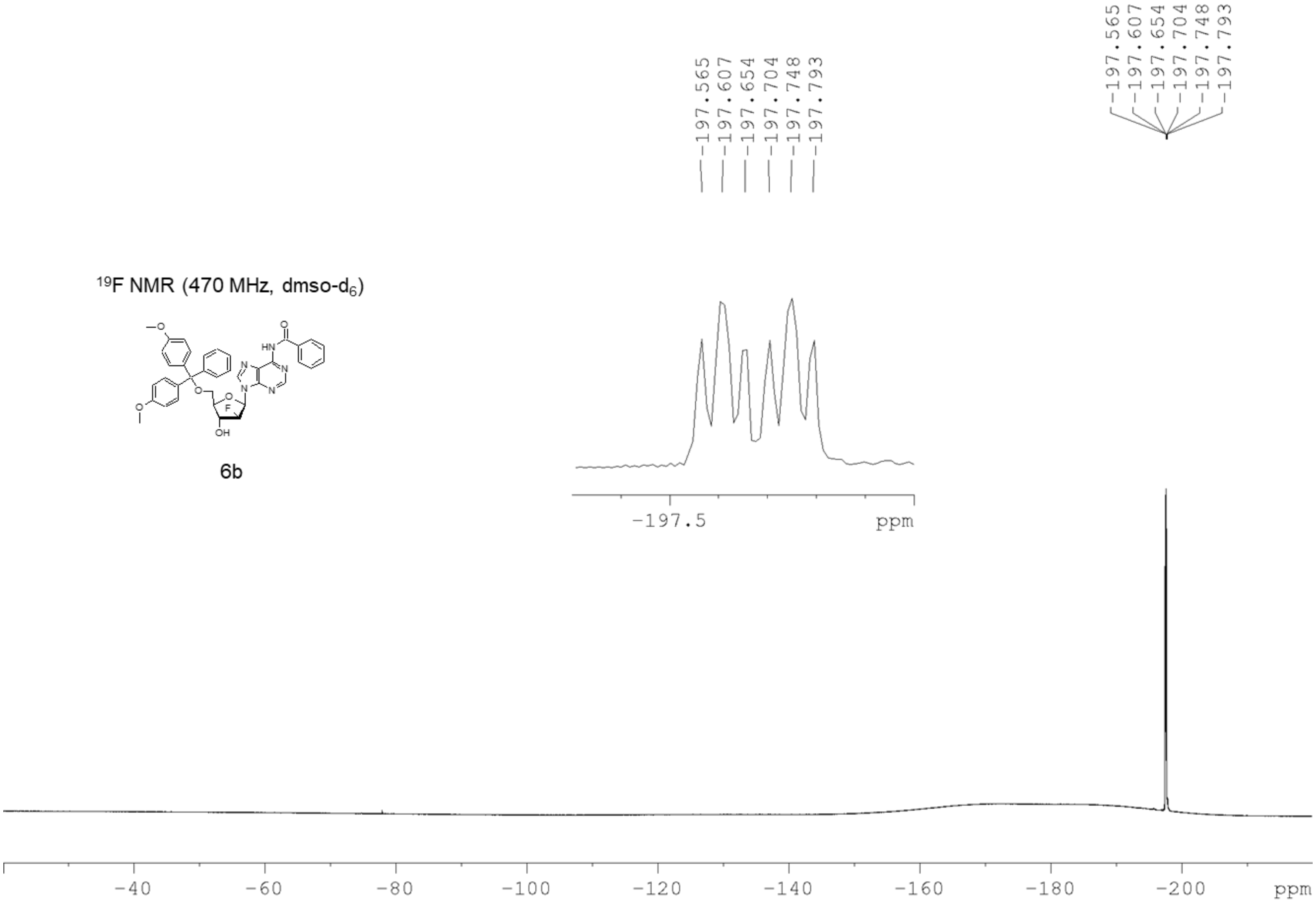

¹H NMR (500 MHz, dmso-d₆)
HN
O
N
F
OH
6b
11.294
8.752
8.418
8.414
8.067
8.053
7.885
7.871
7.868
7.664
7.649
7.635
7.572
7.556
7.541
7.514
7.502
7.499
7.497
7.460
7.445
7.430
7.413
7.398
7.359
7.289
7.278
7.263
7.260
7.221
7.206
7.192
6.877
6.859
6.841
6.835
6.653
6.644
6.626
6.616
6.087
6.076
5.392
5.287
5.278
4.091
4.085
3.720
3.716
3.423
3.409
3.402
3.388
3.292
3.286
3.271
3.265
2.500
11
10
9
8
7
6
5
4
3
2
1
ppm
0.947
0.889
0.974
2.032
1.380
16.491
4.208
0.990
1.003
0.998
1.008
0.987
6.344
1.068
1.063



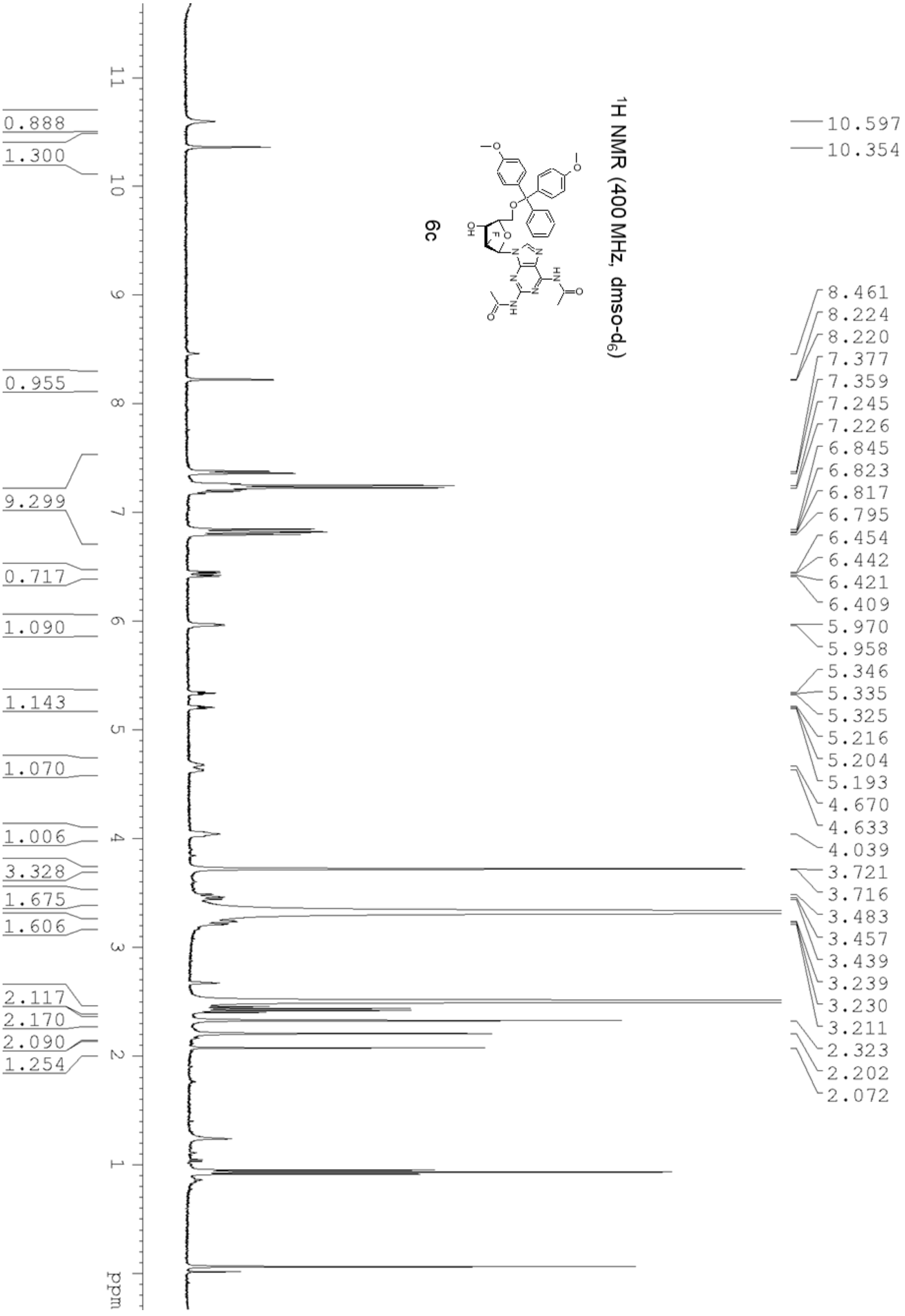
^{13}C NMR (125 MHz, dmso-d_6)

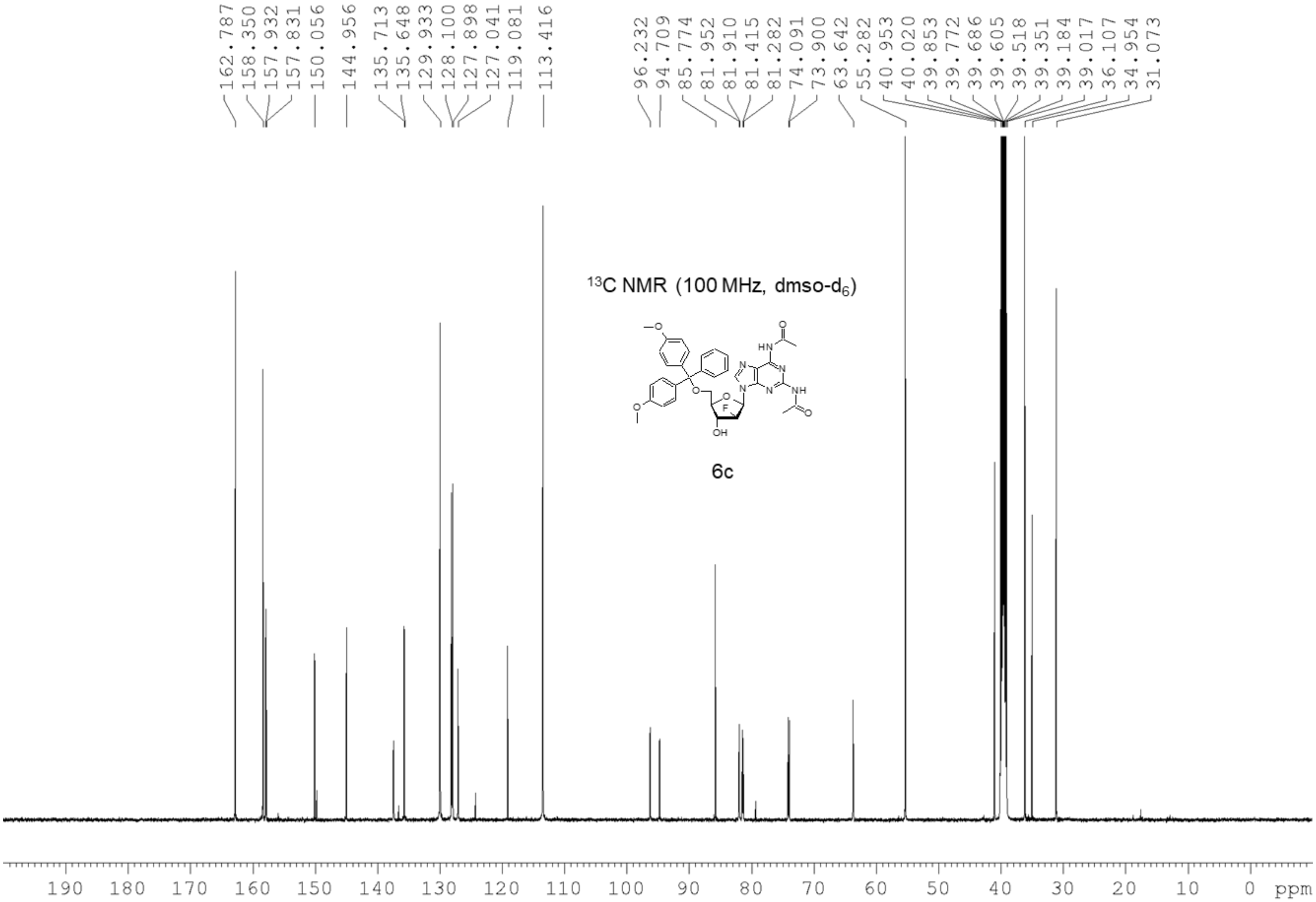
6b

^{19}F NMR (470 MHz, dmso-d$_6$)



6b

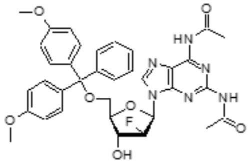

¹H NMR (400 MHz, dmso-d₆)
6c
F
OH
O
N
HN
NH
10.597
10.354
8.461
8.224
8.220
7.377
7.359
7.245
7.226
6.845
6.823
6.817
6.795
6.454
6.442
6.421
6.409
5.970
5.958
5.346
5.335
5.325
5.216
5.204
5.193
4.670
4.633
4.039
3.721
3.716
3.483
3.457
3.439
3.239
3.230
3.211
2.323
2.202
2.072
0.888
1.300
0.955
9.299
0.717
1.090
1.143
1.070
1.006
3.328
1.675
1.606
2.117
2.170
2.090
1.254
11
10
9
8
7
6
5
4
3
2
1
ppm



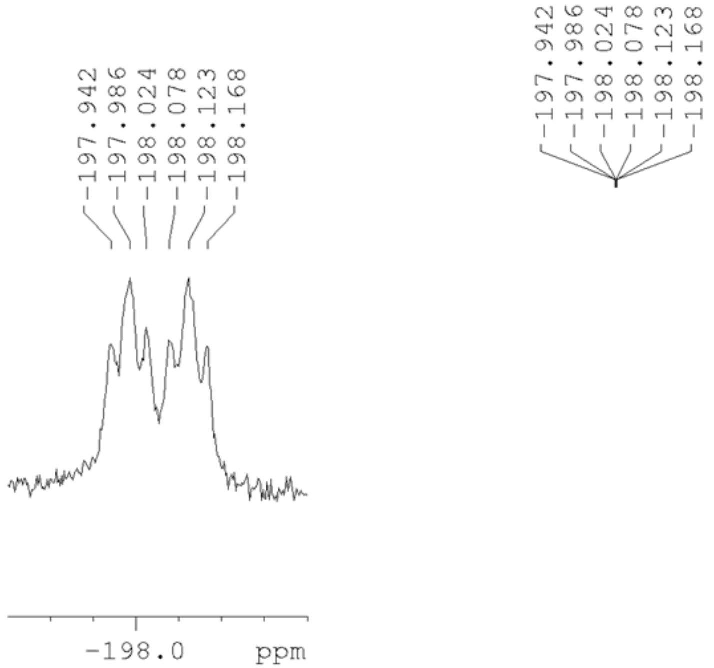
^{13}C NMR (100 MHz, dmso-d$_6$)

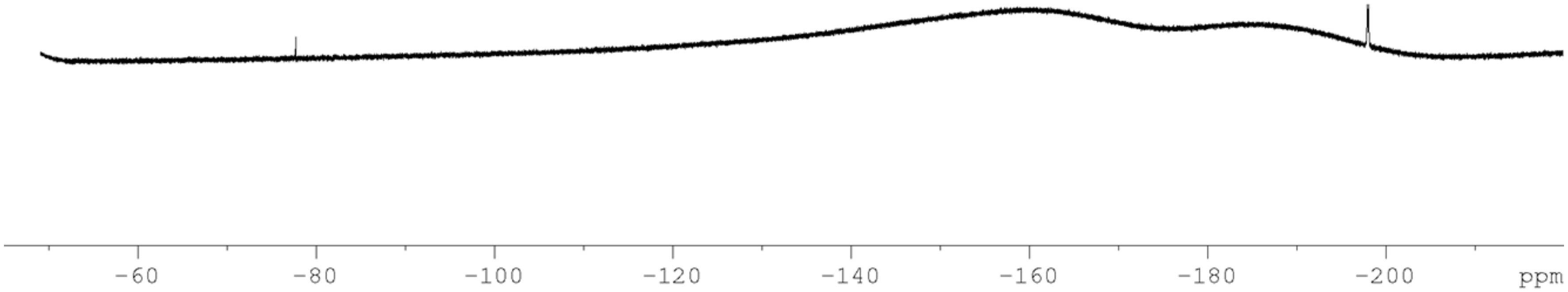
6c

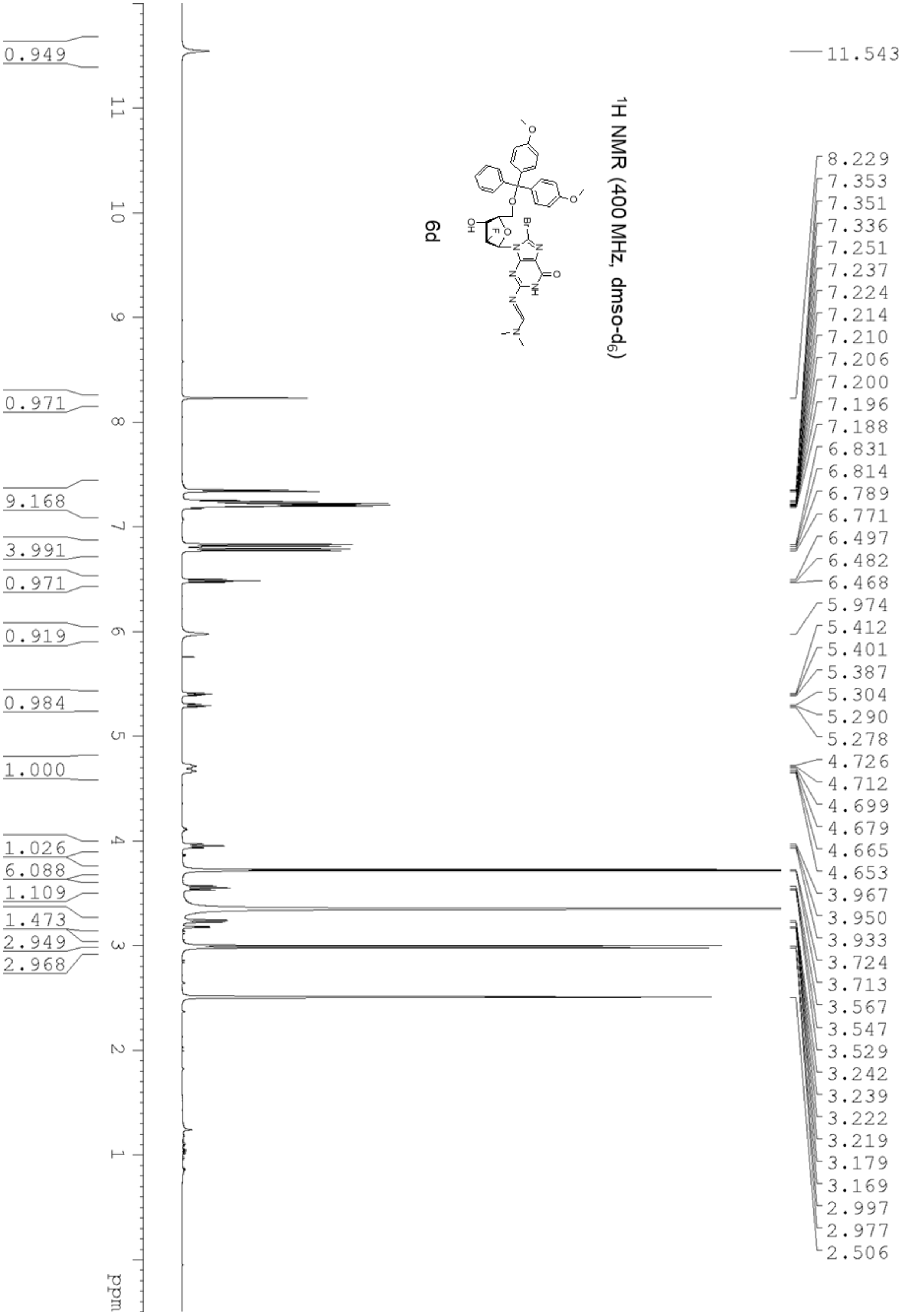
^{19}F NMR (376 MHz, dmso-d_6)

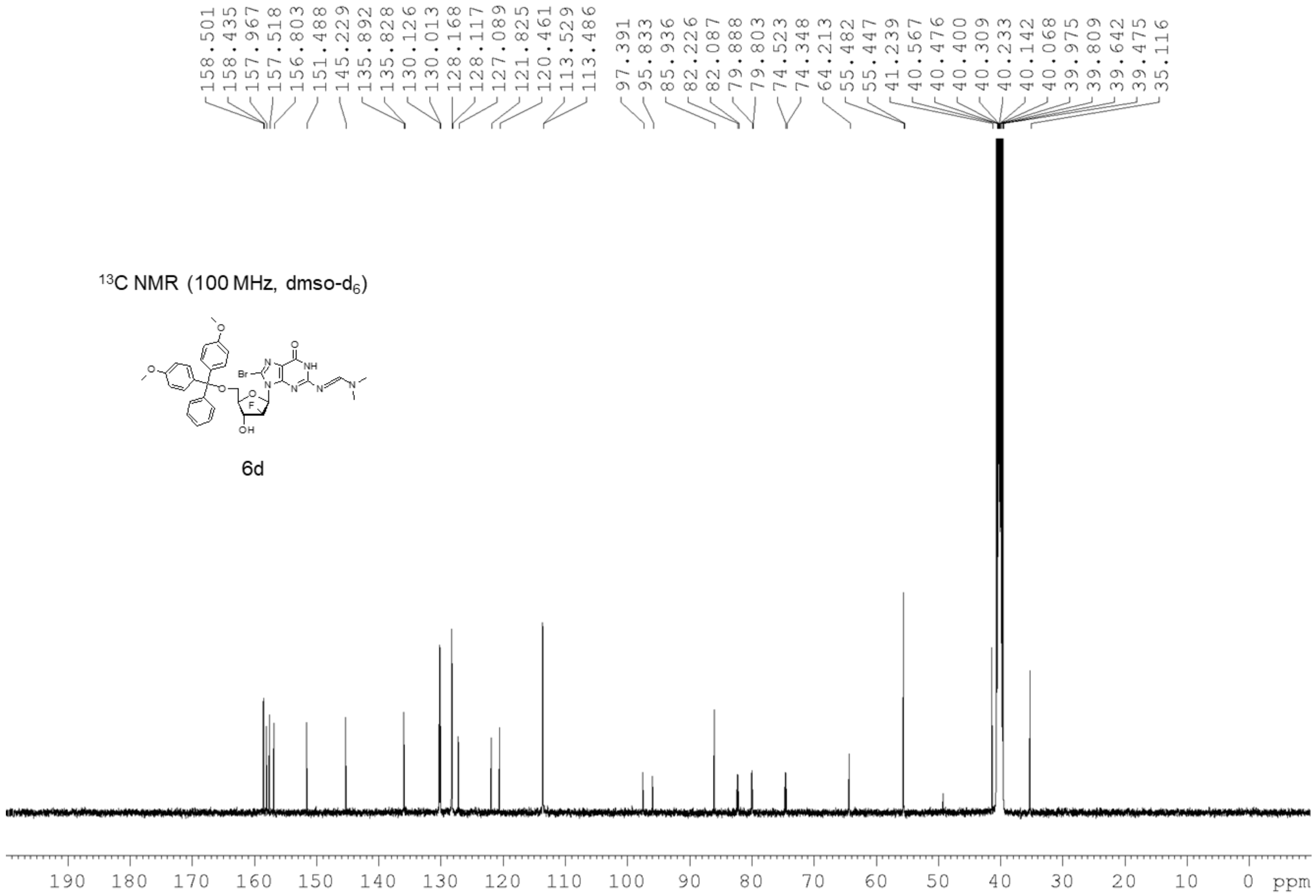


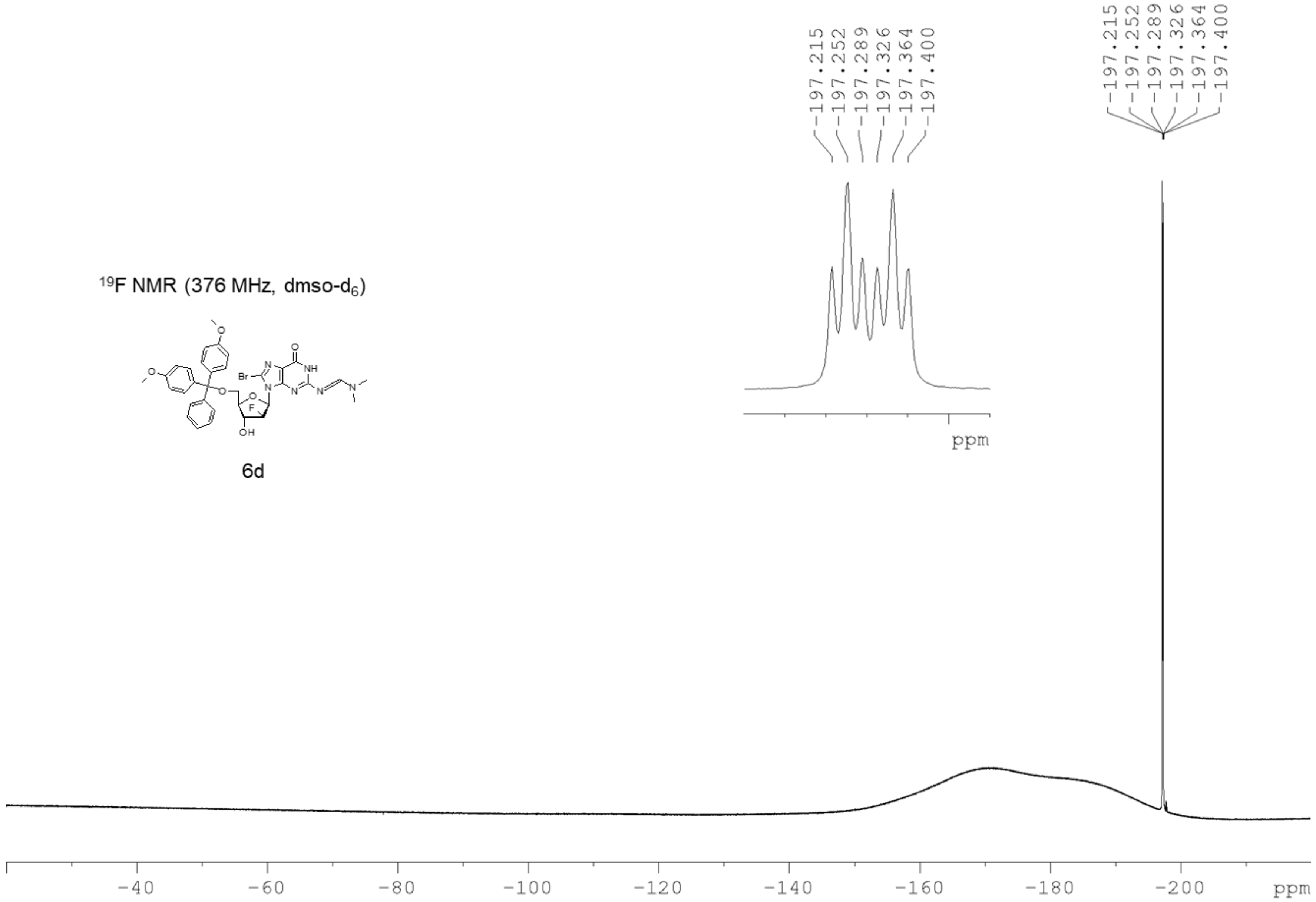
6c





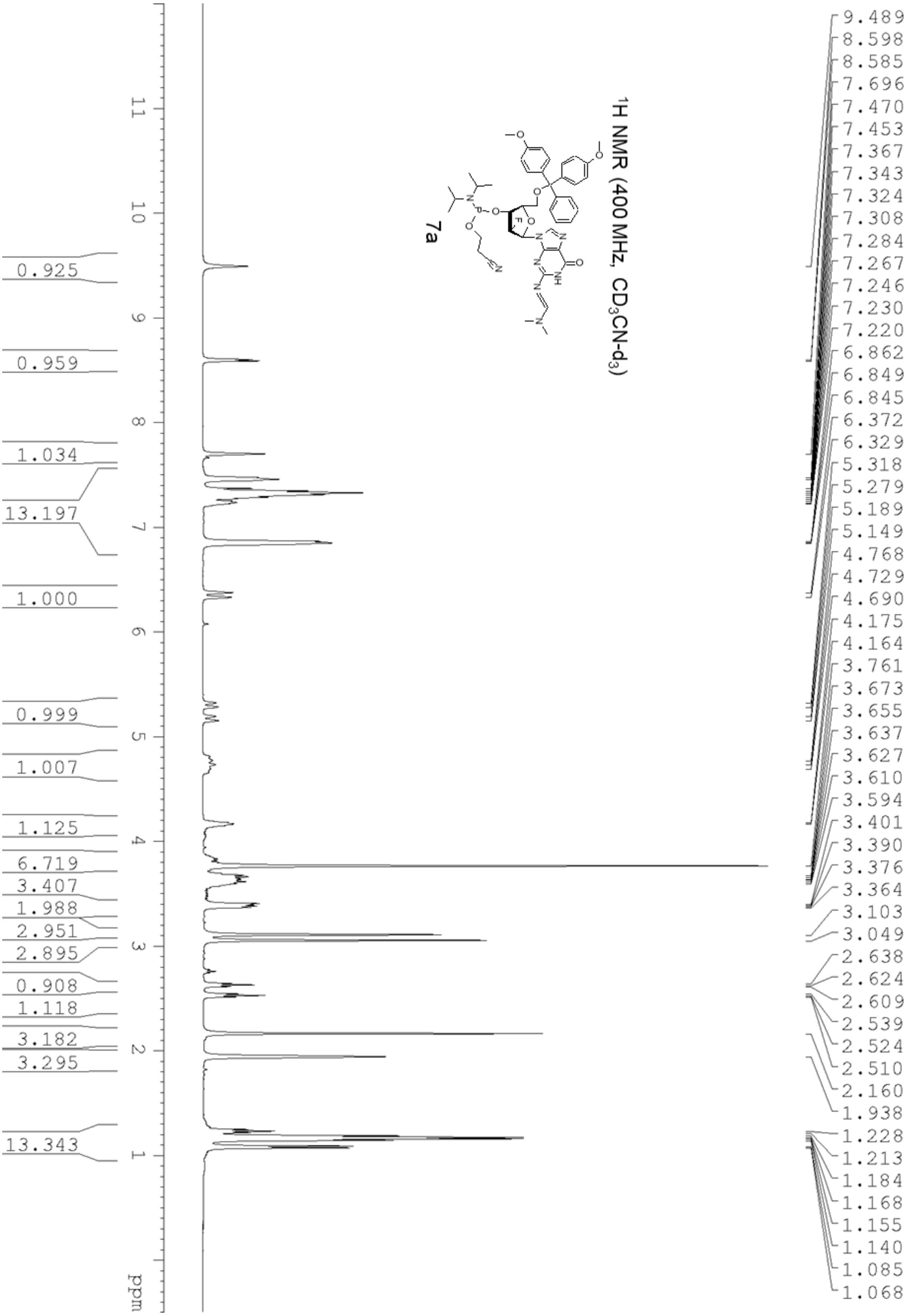

¹H NMR (400 MHz, dmso-d₆)
6d
Br
N
O
NH
OH
F
11.543
8.229
7.353
7.351
7.336
7.251
7.237
7.224
7.214
7.210
7.206
7.200
7.196
7.188
6.831
6.814
6.789
6.771
6.497
6.482
6.468
5.974
5.412
5.401
5.387
5.304
5.290
5.278
4.726
4.712
4.699
4.679
4.665
4.653
3.967
3.950
3.933
3.724
3.713
3.567
3.547
3.529
3.242
3.239
3.222
3.219
3.179
3.169
2.997
2.977
2.506
0.949
0.971
9.168
3.991
0.971
0.919
0.984
1.000
1.026
6.088
1.109
1.473
2.949
2.968
11
10
9
8
7
6
5
4
3
2
1
ppm


158.501
158.435
157.967
157.518
156.803
151.488
145.229
135.892
135.828
130.126
130.013
128.168
128.117
127.089
121.825
120.461
113.529
113.486
97.391
95.833
85.936
82.226
82.087
79.888
79.803
74.523
74.348
64.213
55.482
55.447
41.239
40.567
40.476
40.400
40.309
40.233
40.142
40.068
39.975
39.809
39.642
39.475
35.116
^{13}C NMR (100 MHz, dmso-d$_6$)
Br
NH
O
N
F
OH
6d
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm



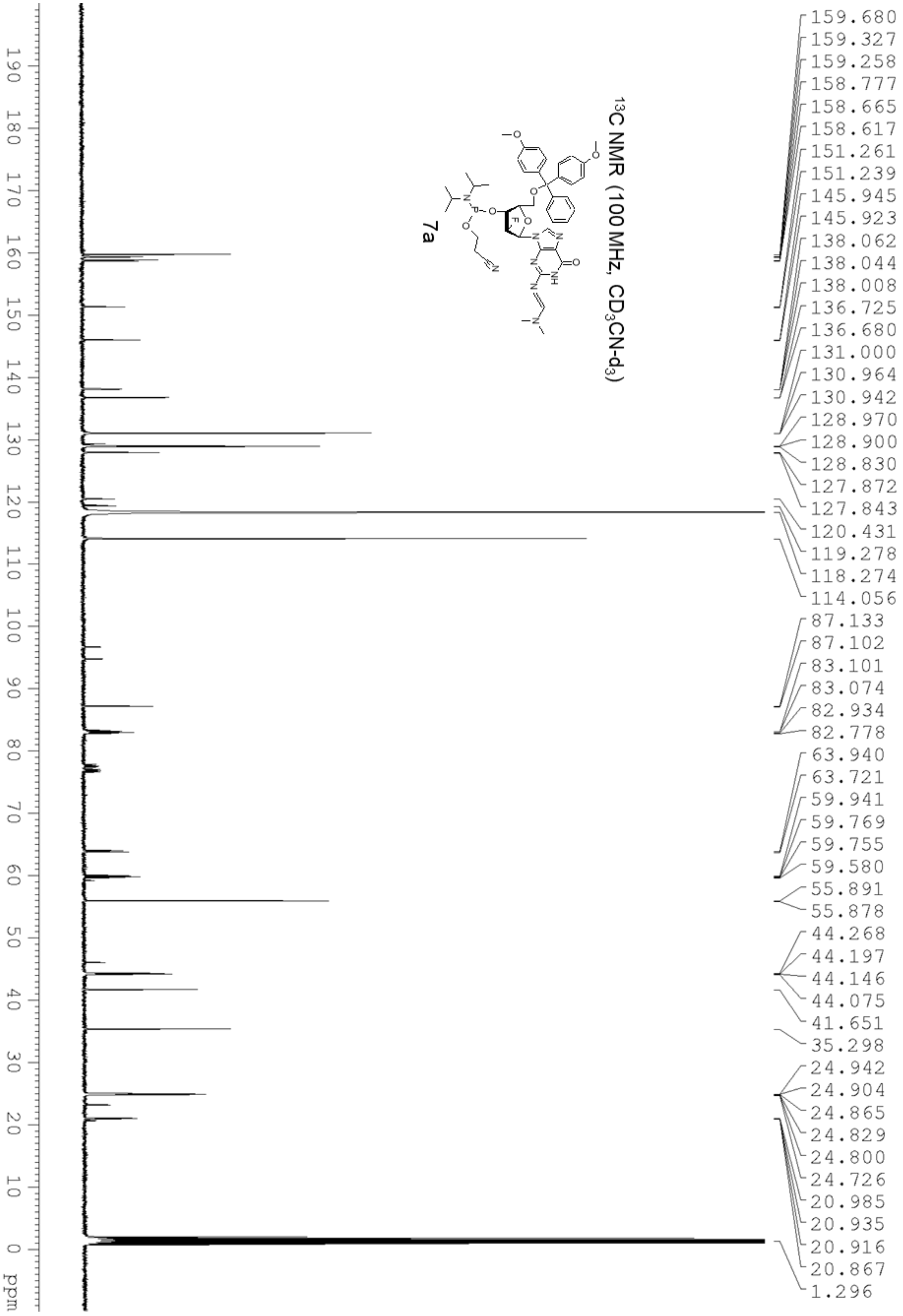
¹⁹F NMR (376 MHz, dmso-d_6)

6d



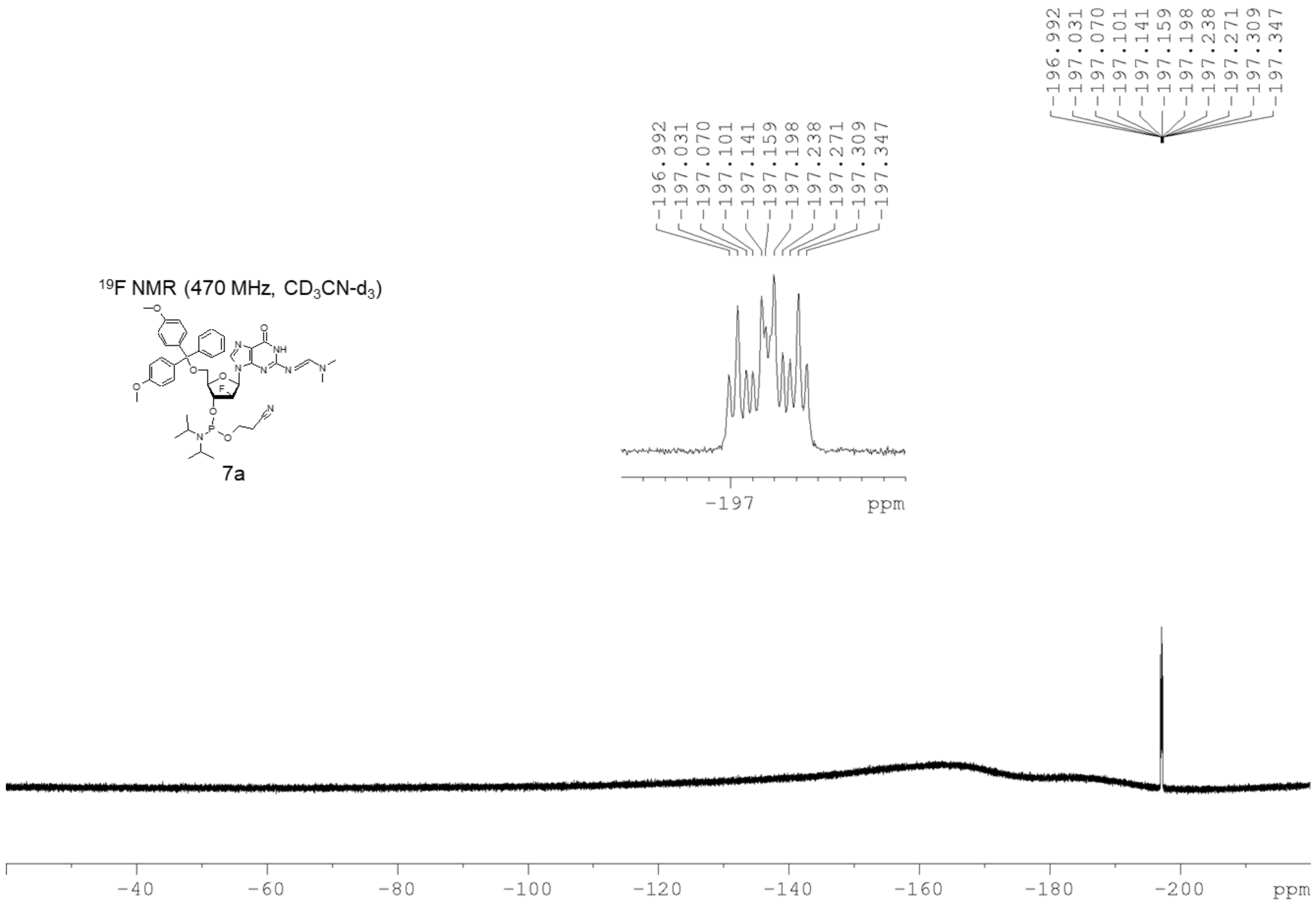
^{1}H NMR (400 MHz, CD_3CN-d_3)

7a



^{13}C NMR (100 MHz, CD$_3$CN-d$_3$)

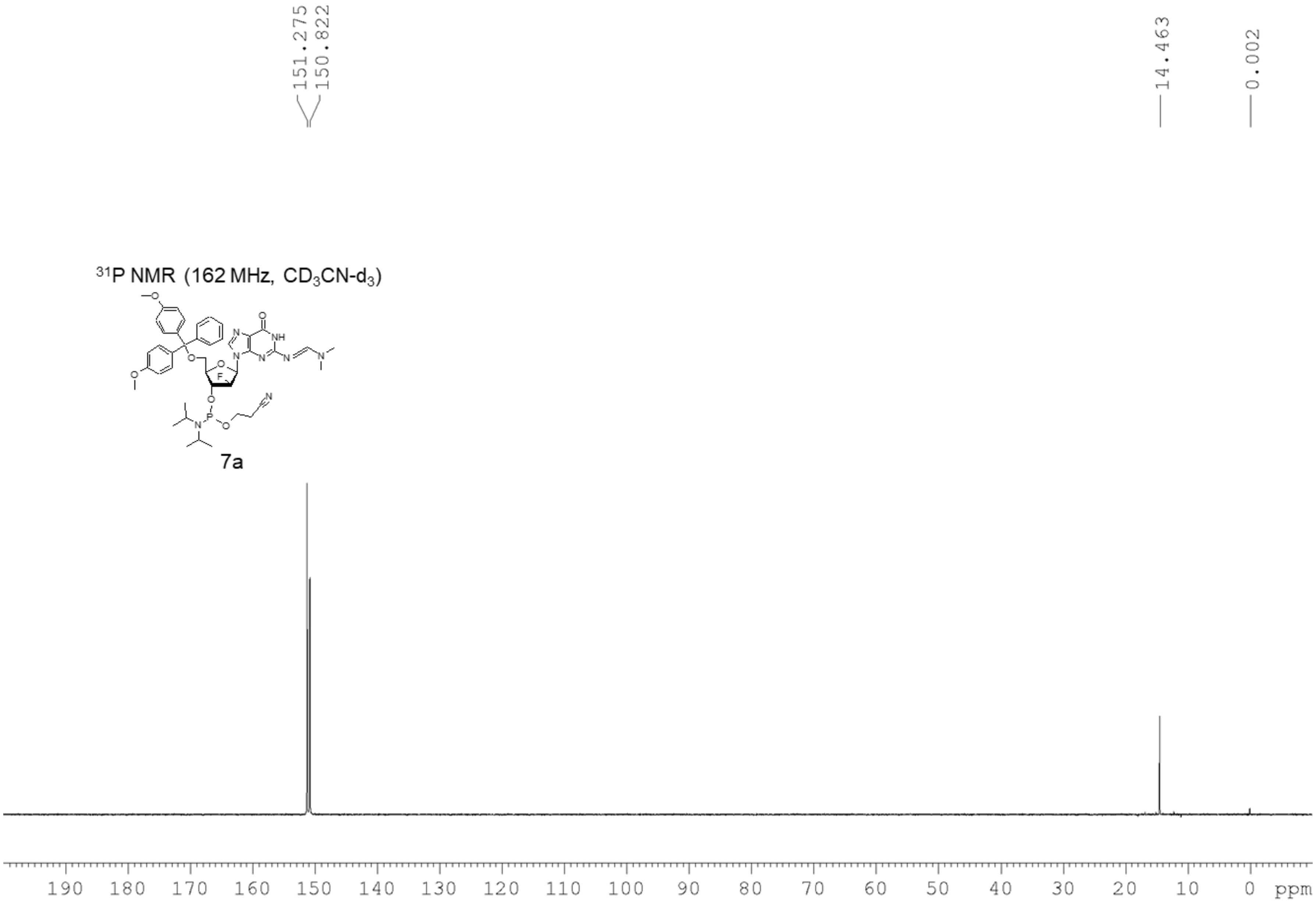
7a



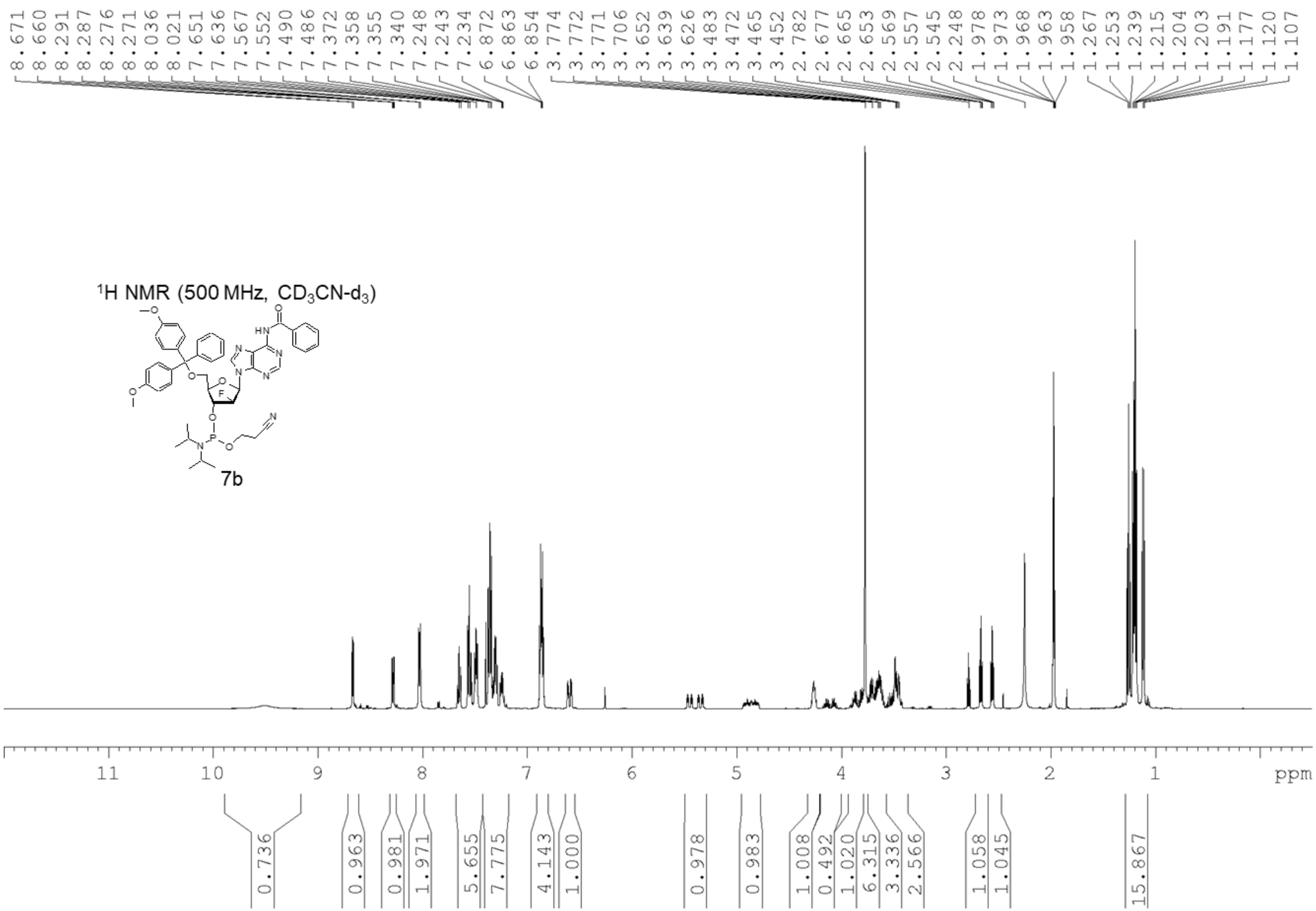
^{19}F NMR (470 MHz, CD_3CN-d_3)

7a

^{31}P NMR (162 MHz, CD_3CN-d_3)

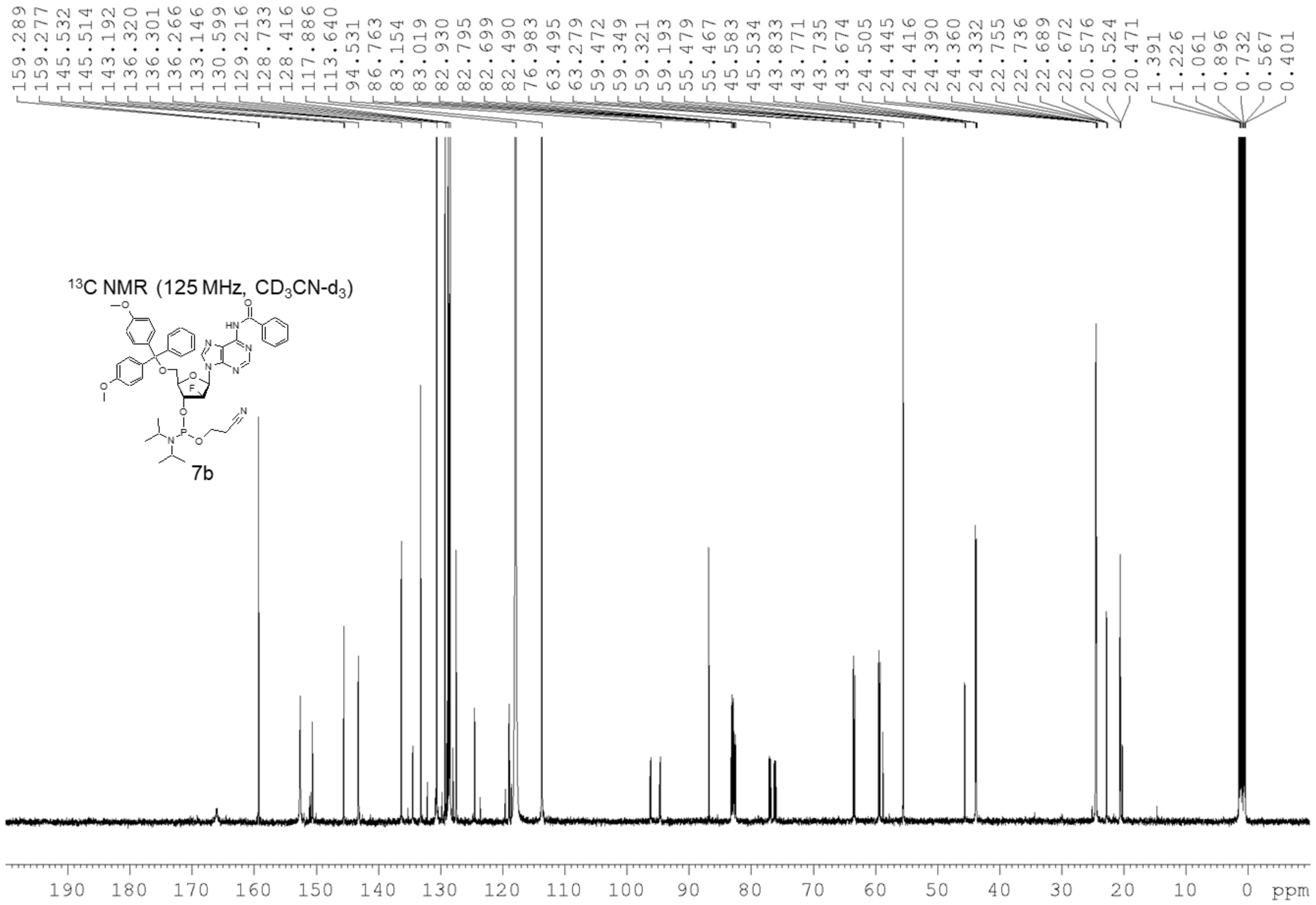


7a



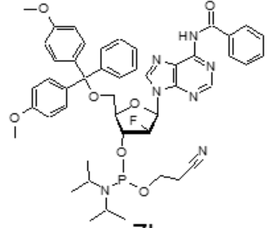
^{1}H NMR (500 MHz, CD_3CN-d_3)

7b

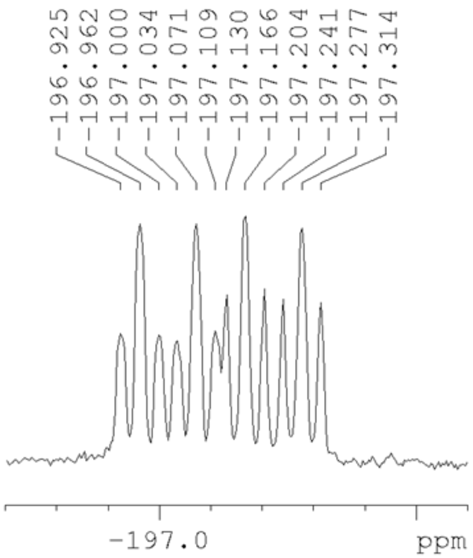


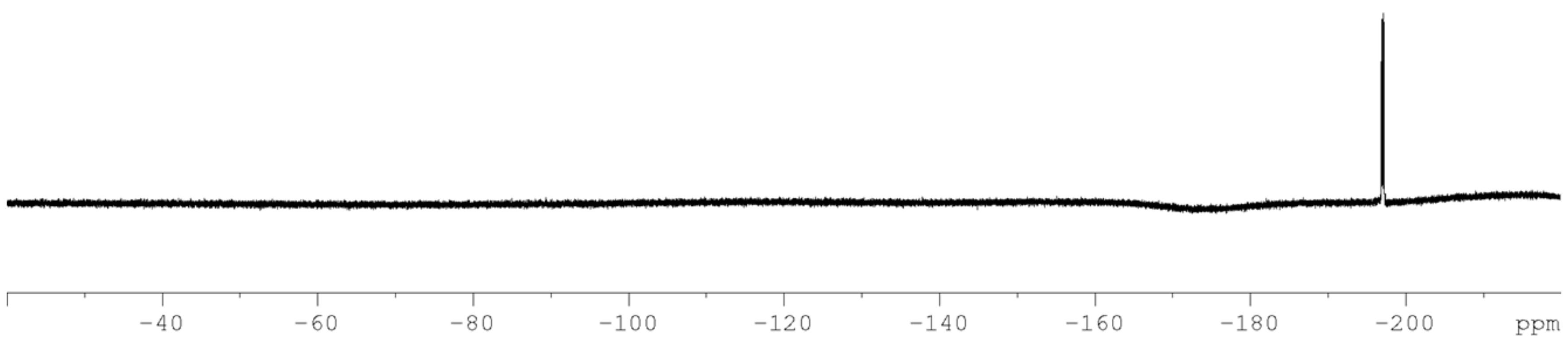
^{13}C NMR (125 MHz, CD_3CN-d_3)

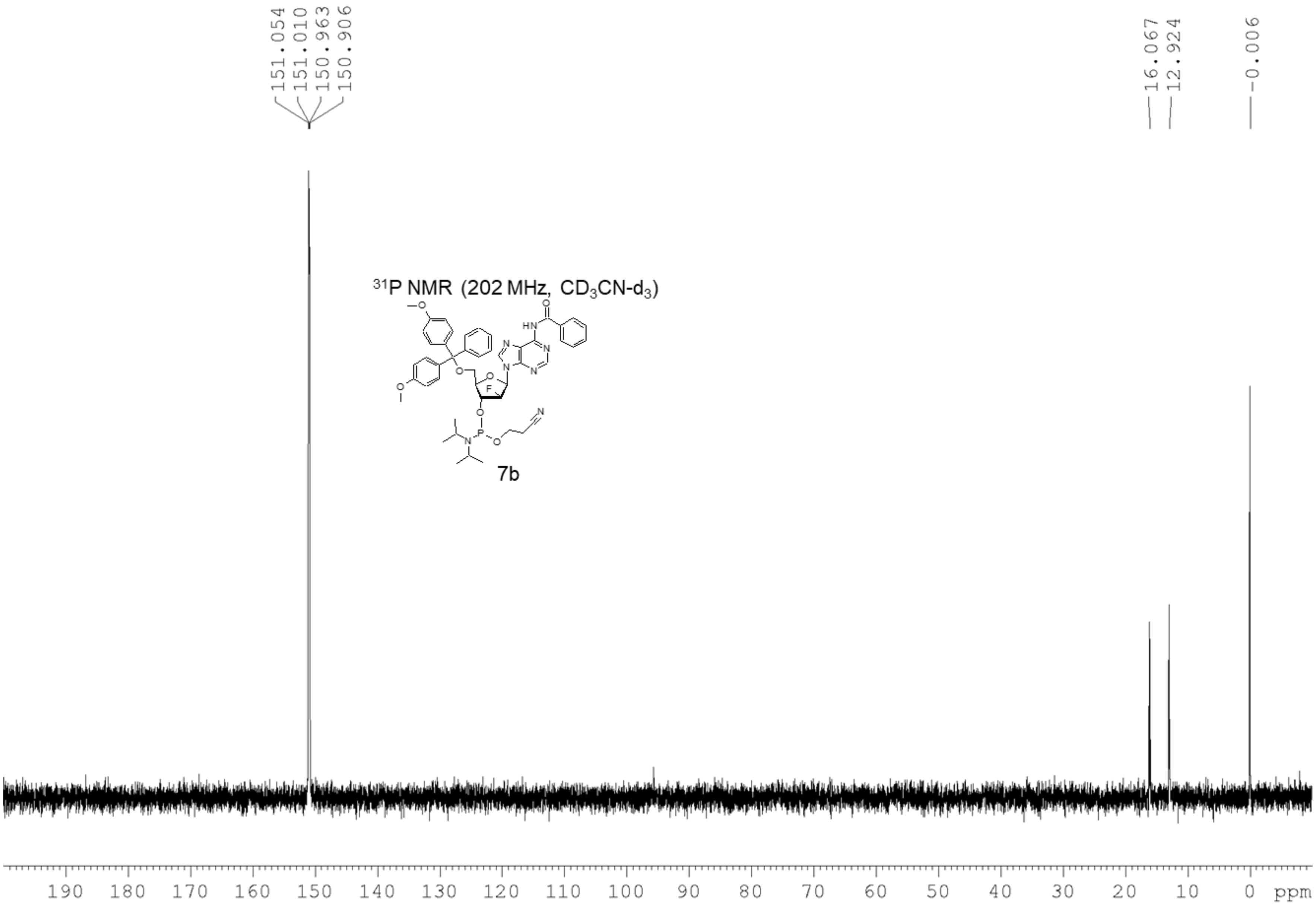
^{19}F NMR (470 MHz, CD_3CN-d_3)



7b

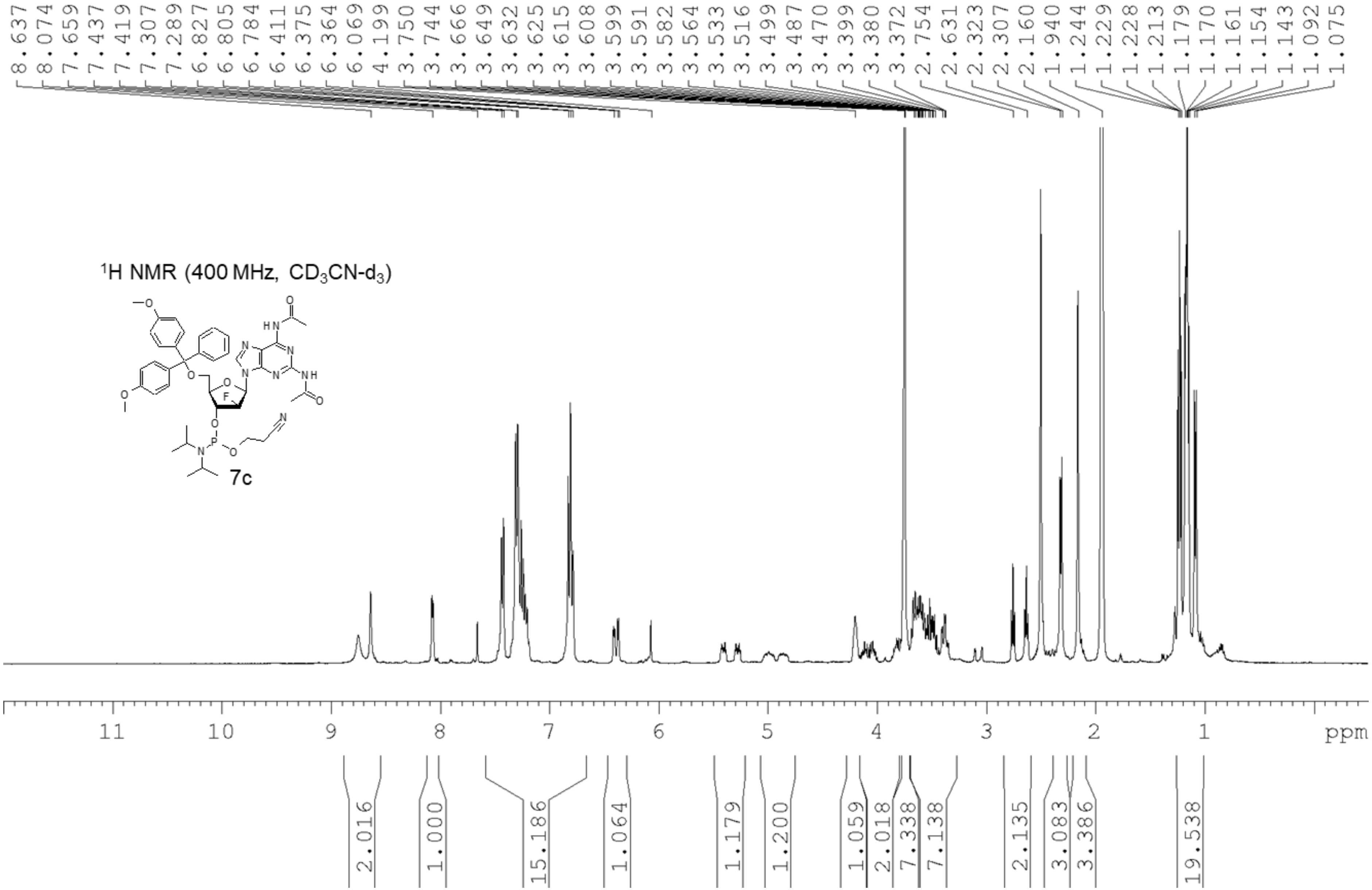






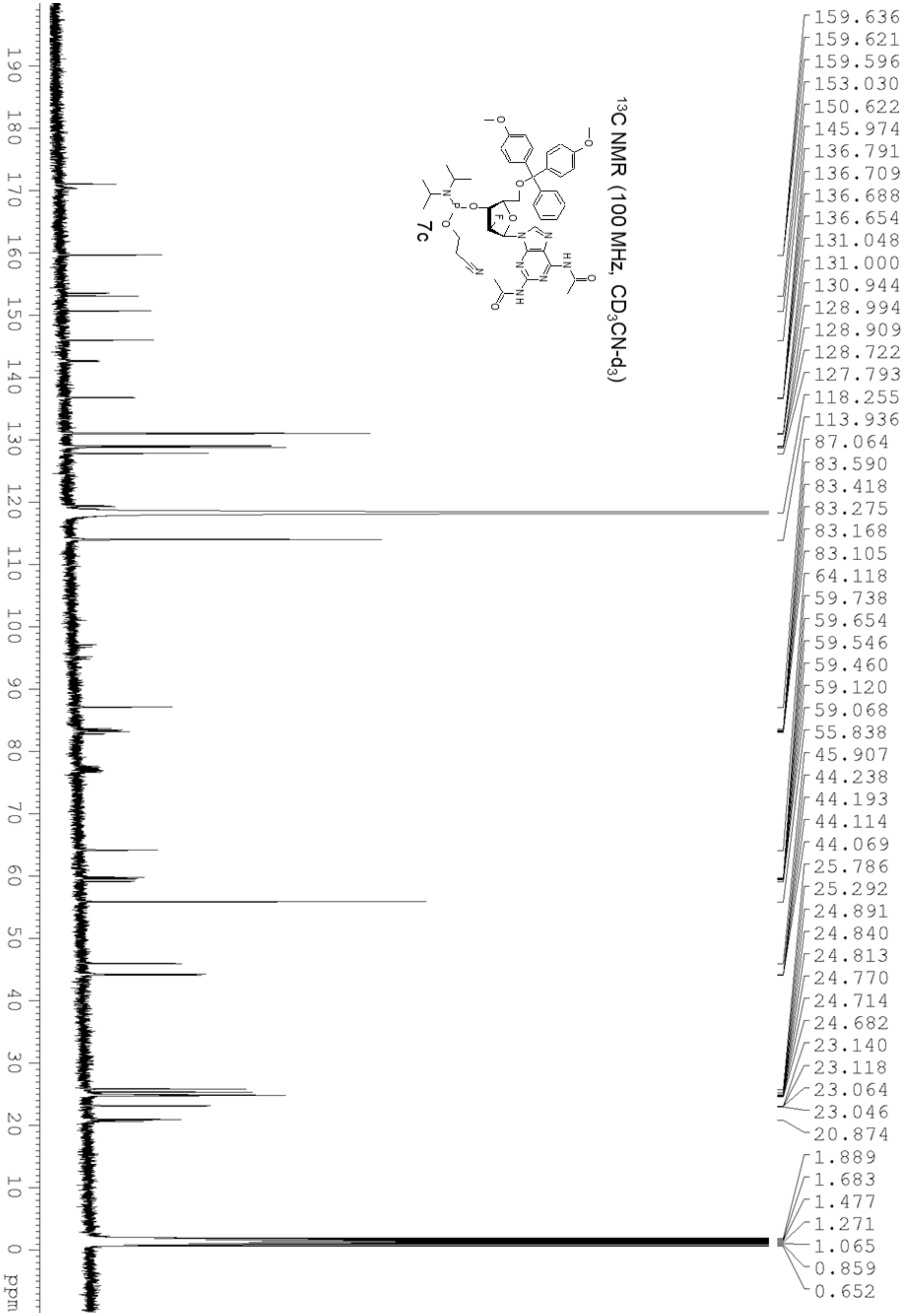
^{31}P NMR (202 MHz, CD_3CN-d_3)

7b



^{1}H NMR (400 MHz, CD_3CN-d_3)

7c

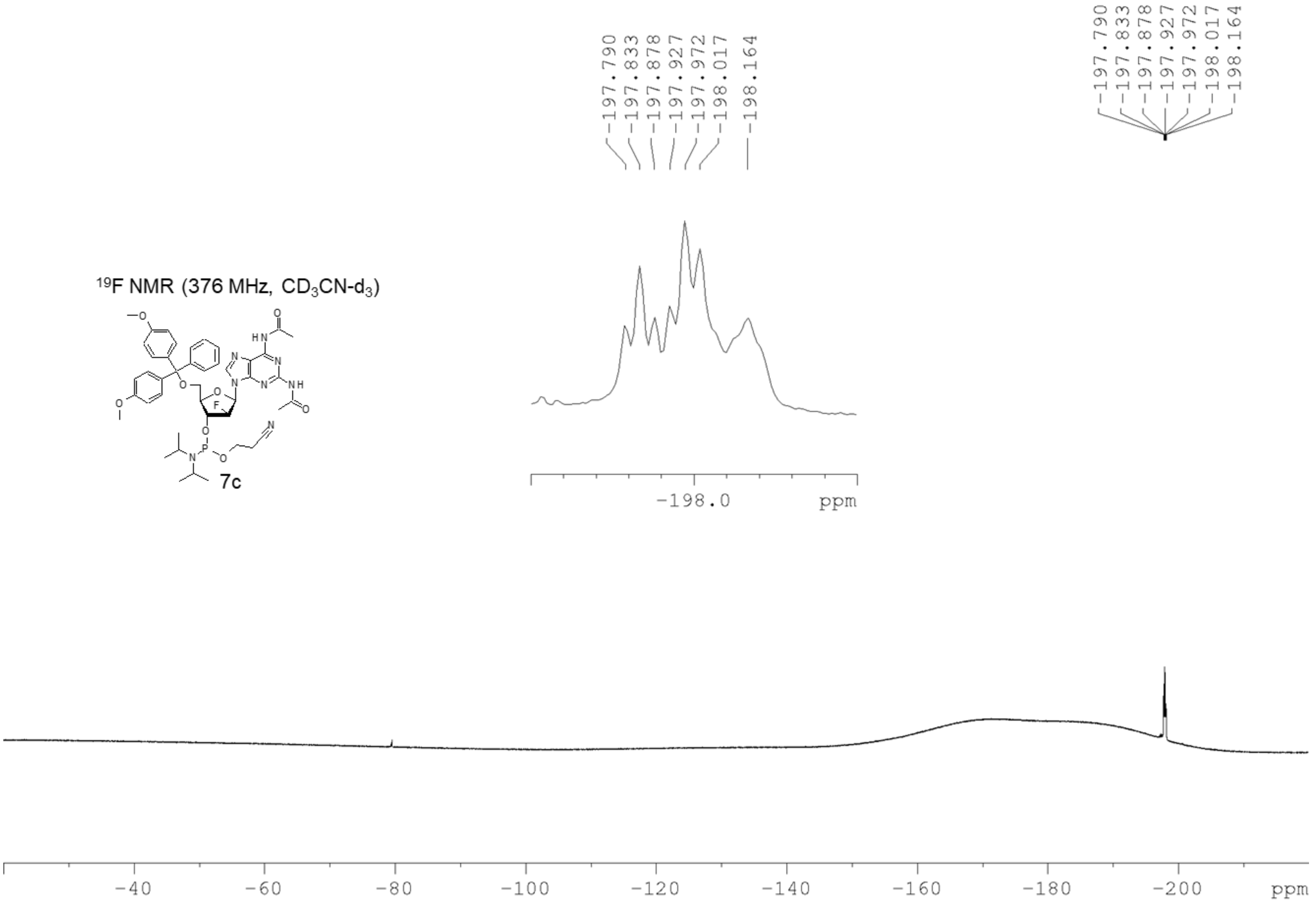


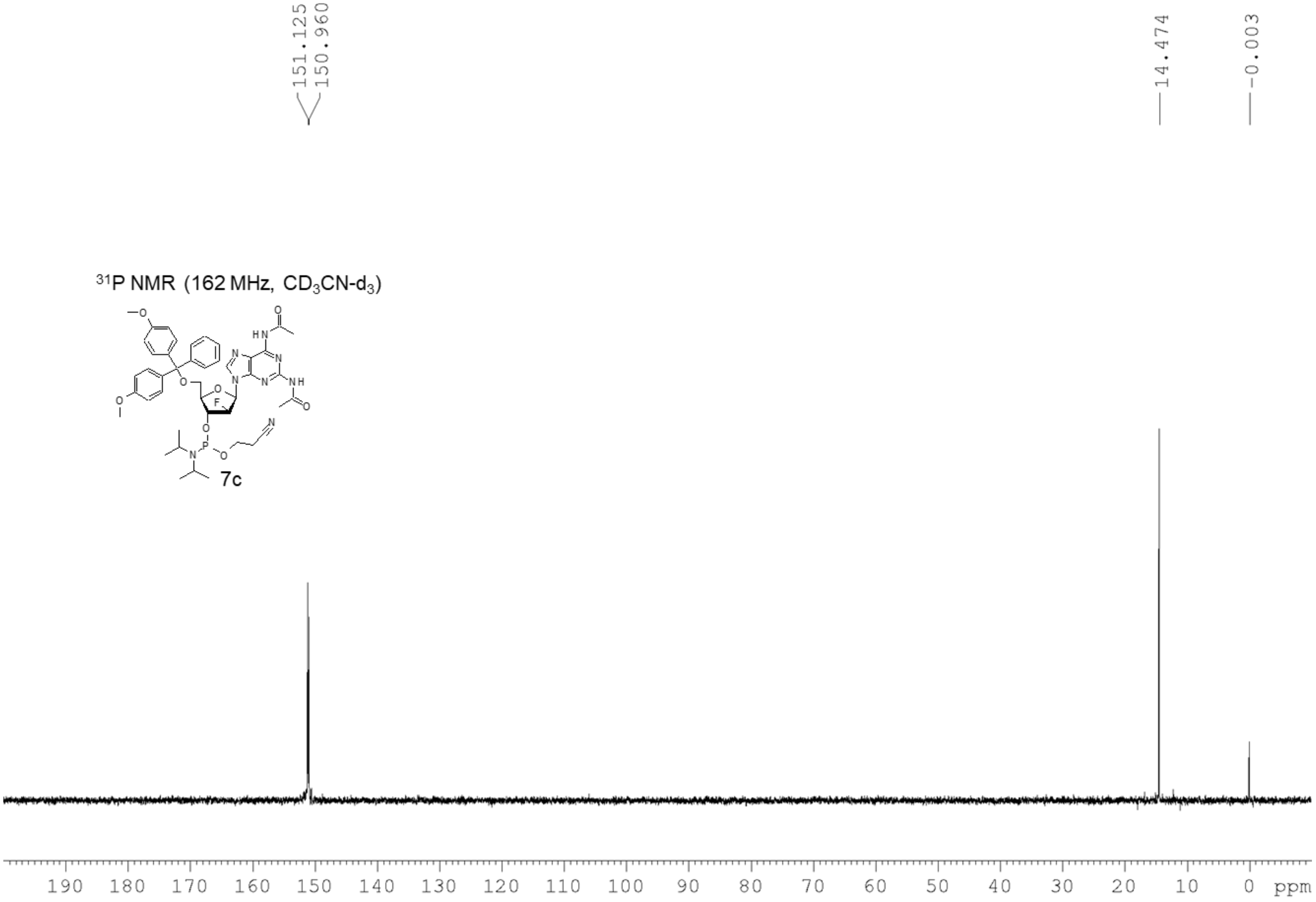
^{13}C NMR (100 MHz, CD_3CN-d$_3$)

7c

^{19}F NMR (376 MHz, CD_3CN-d_3)

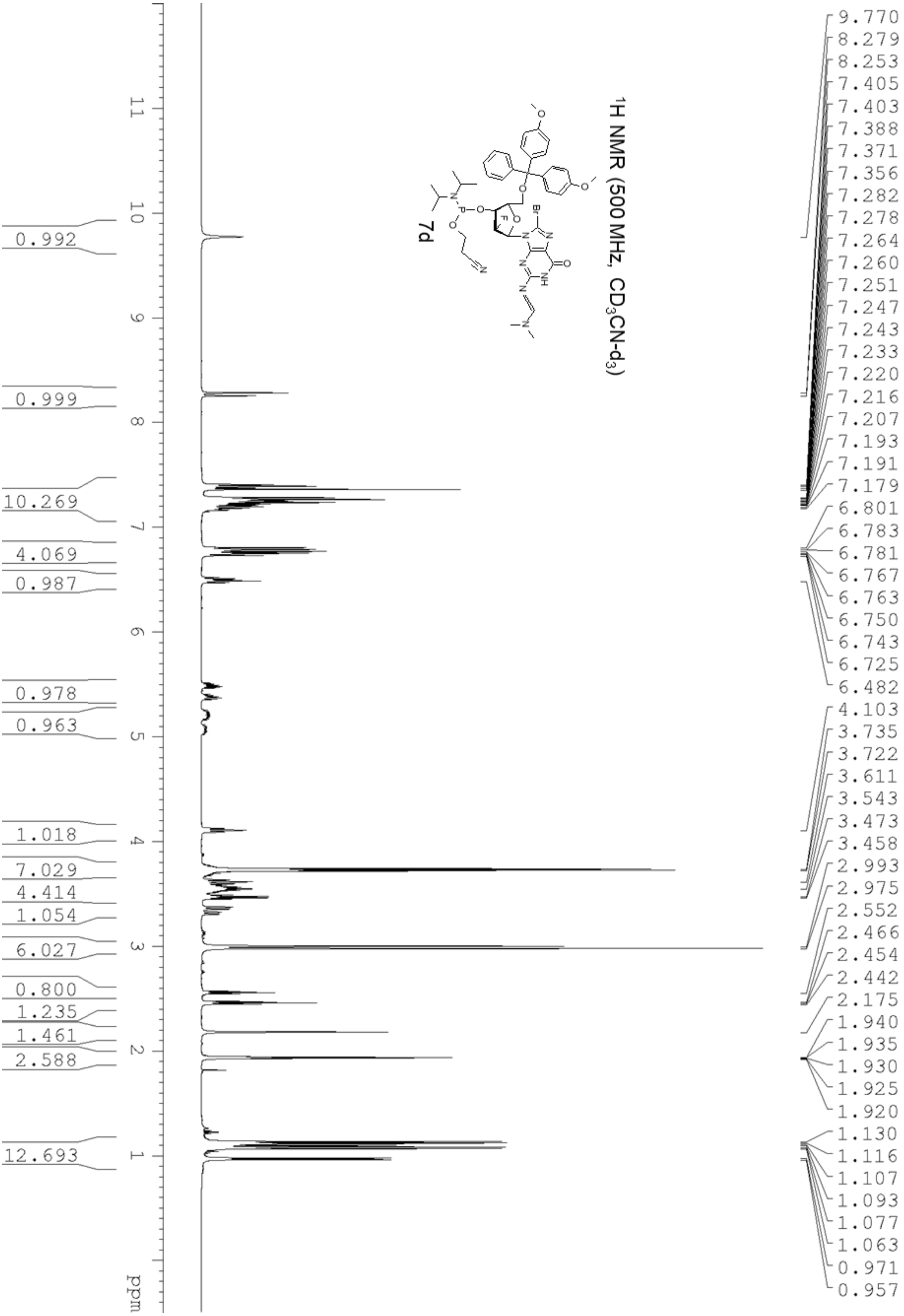
7c



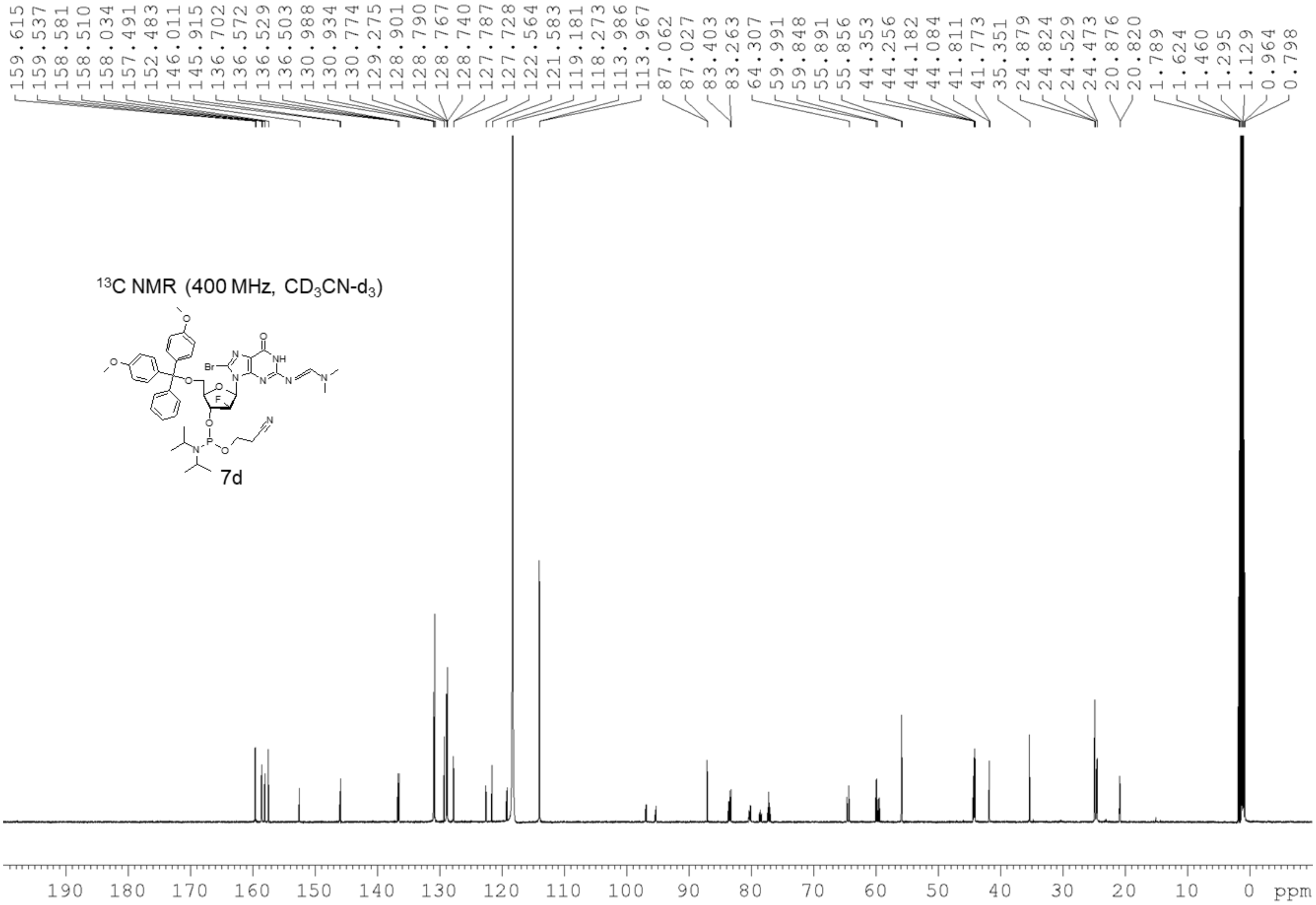


^{31}P NMR (162 MHz, CD_3CN-d_3)

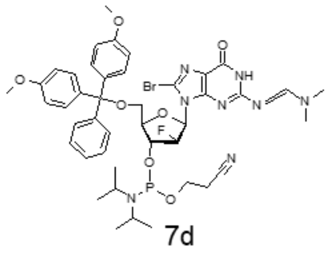
7c


^{1}H NMR (500 MHz, CD_3CN-d_3)

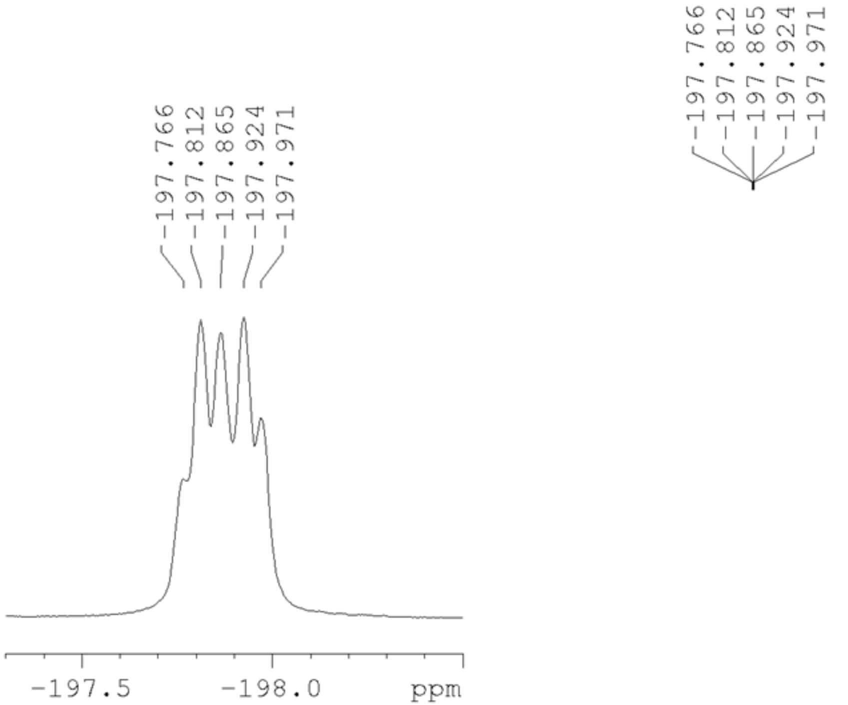
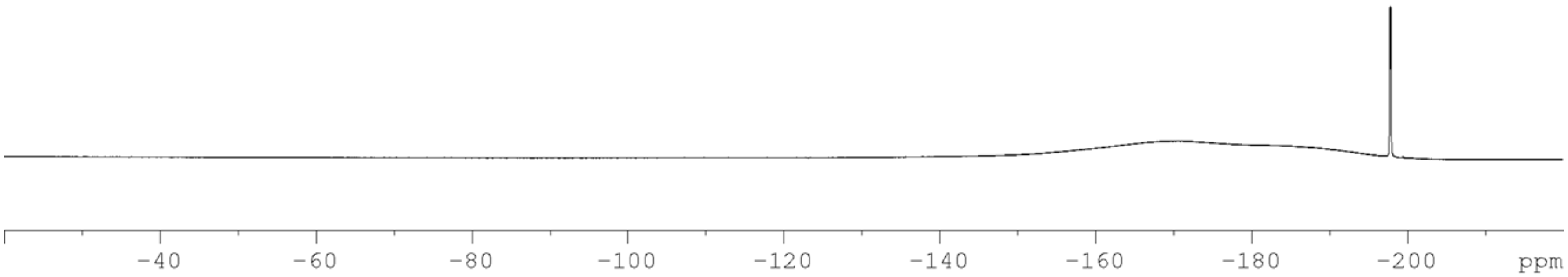
7d

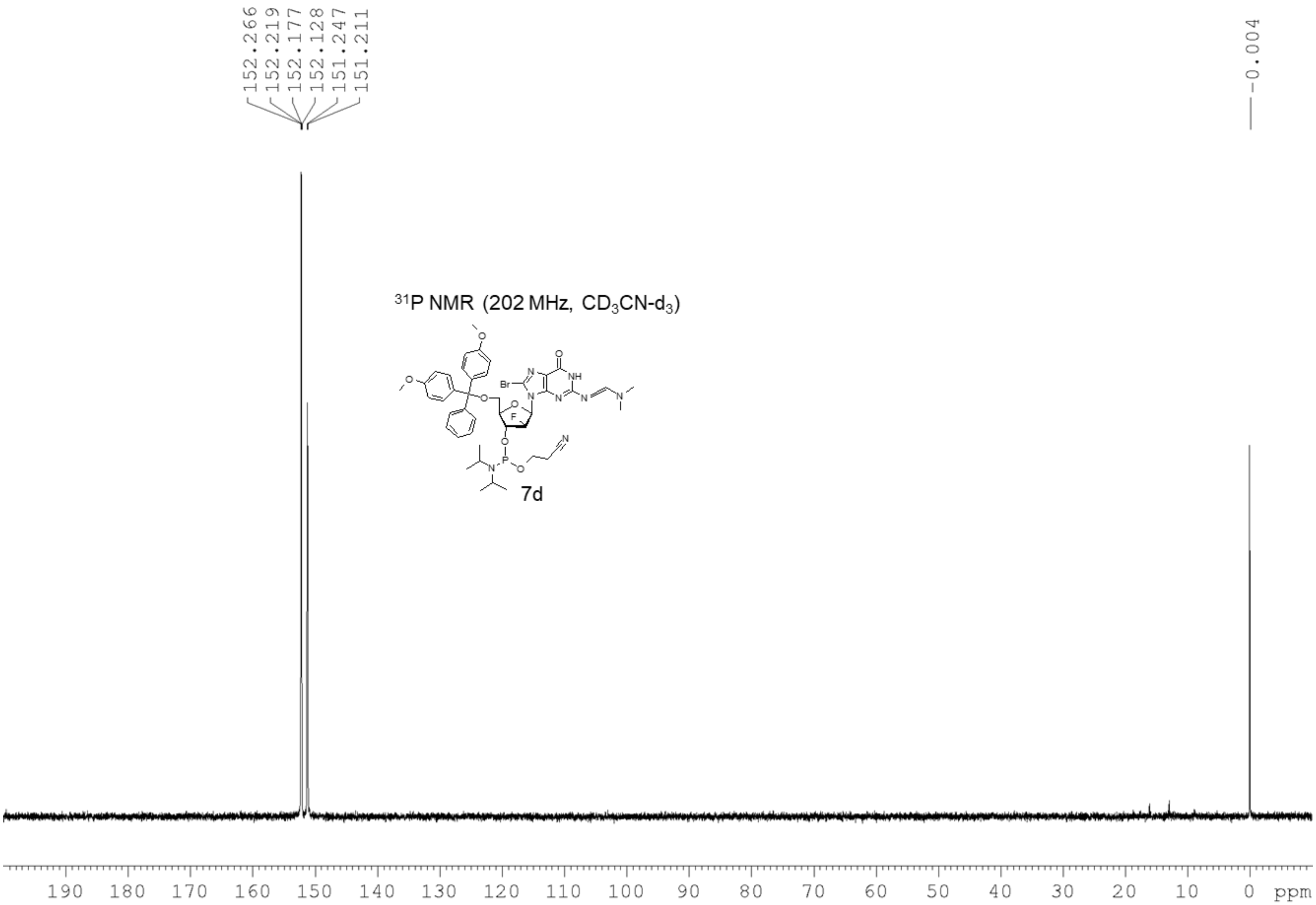
^{13}C NMR (400 MHz, CD_3CN-d_3)
7d
Br
NH
O
N
F
159.615
159.537
158.581
158.510
158.034
157.491
152.483
146.011
145.915
136.702
136.572
136.529
136.503
130.988
130.934
130.774
129.275
128.901
128.790
128.767
128.740
127.787
127.728
122.564
121.583
119.181
118.273
113.986
113.967
87.062
87.027
83.403
83.263
64.307
59.991
59.848
55.891
55.856
44.353
44.256
44.182
44.084
41.811
41.773
35.351
24.879
24.824
24.529
24.473
20.876
20.820
1.789
1.624
1.460
1.295
1.129
0.964
0.798
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

^{19}F NMR (470 MHz, CD_3CN-d_3)



7d



^{31}P NMR (202 MHz, CD_3CN-d_3)

7d

S5. Polyacrylamide gels (kinetic assay).

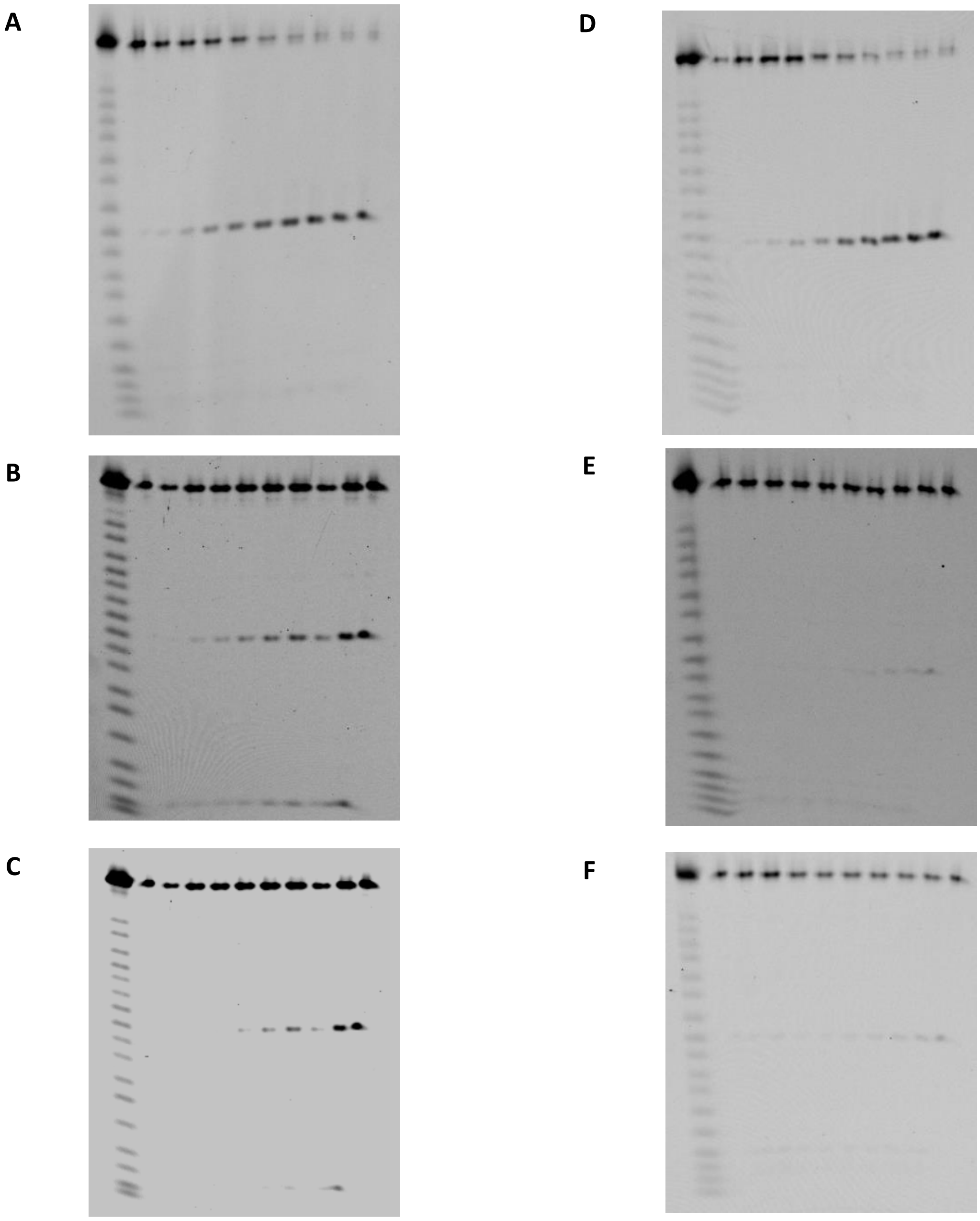
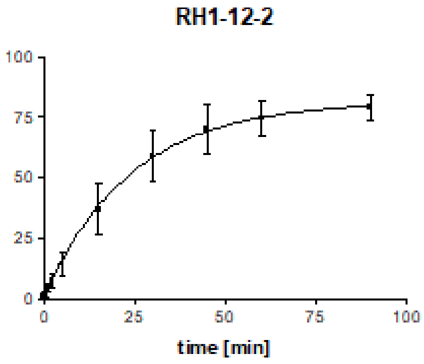
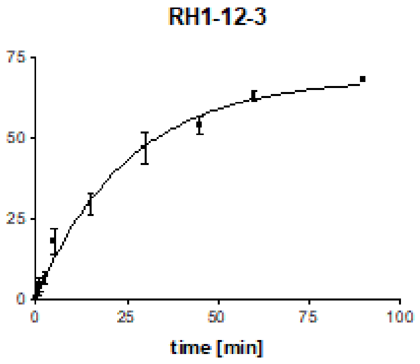
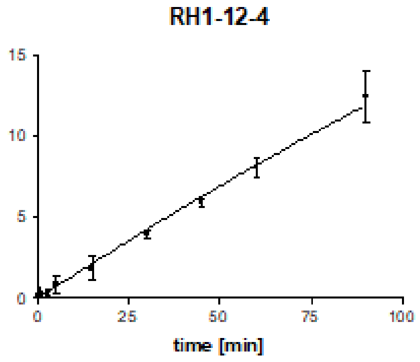


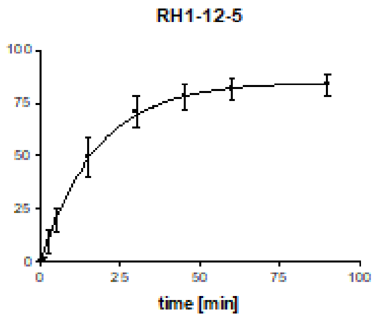
Figure S5. Examples of 12% polyacrylamide denaturing gels electrophoresis of chosen ribozymes kinetic assay A – RH1, B – RZ6A, C – RZ6C, D – RH1-12-5, E – RH1-8-5, F – RZ6-12-8 (Table 3). Far-left lane shows the Mg-ladder of FAM-labeled target RNA oligonucleotide. From left to right 0, 0.5, 1, 2.5, 5, 15, 30, 45, 60 and 90 min quenching time of respective ribozyme reaction. The observed cleavage is as expected after the NUH sequence in the FAM-labeled target oligoribonucleotide (Figure 1), giving a visible band of 10nt product.

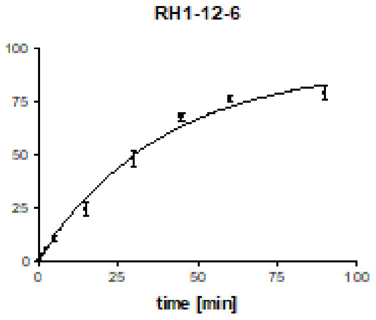
S6. Reaction rate plots.

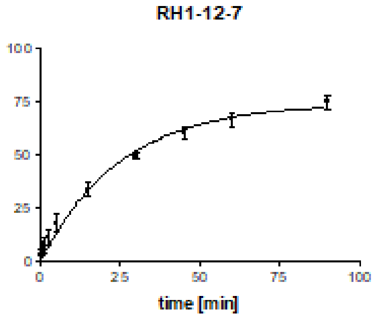


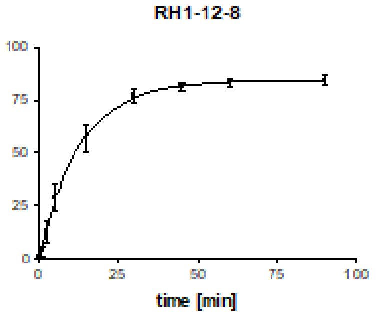


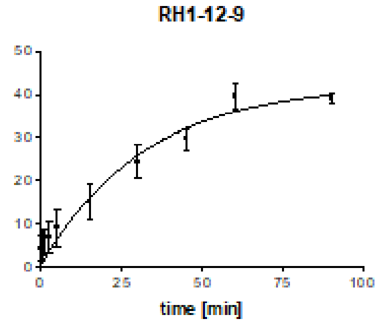












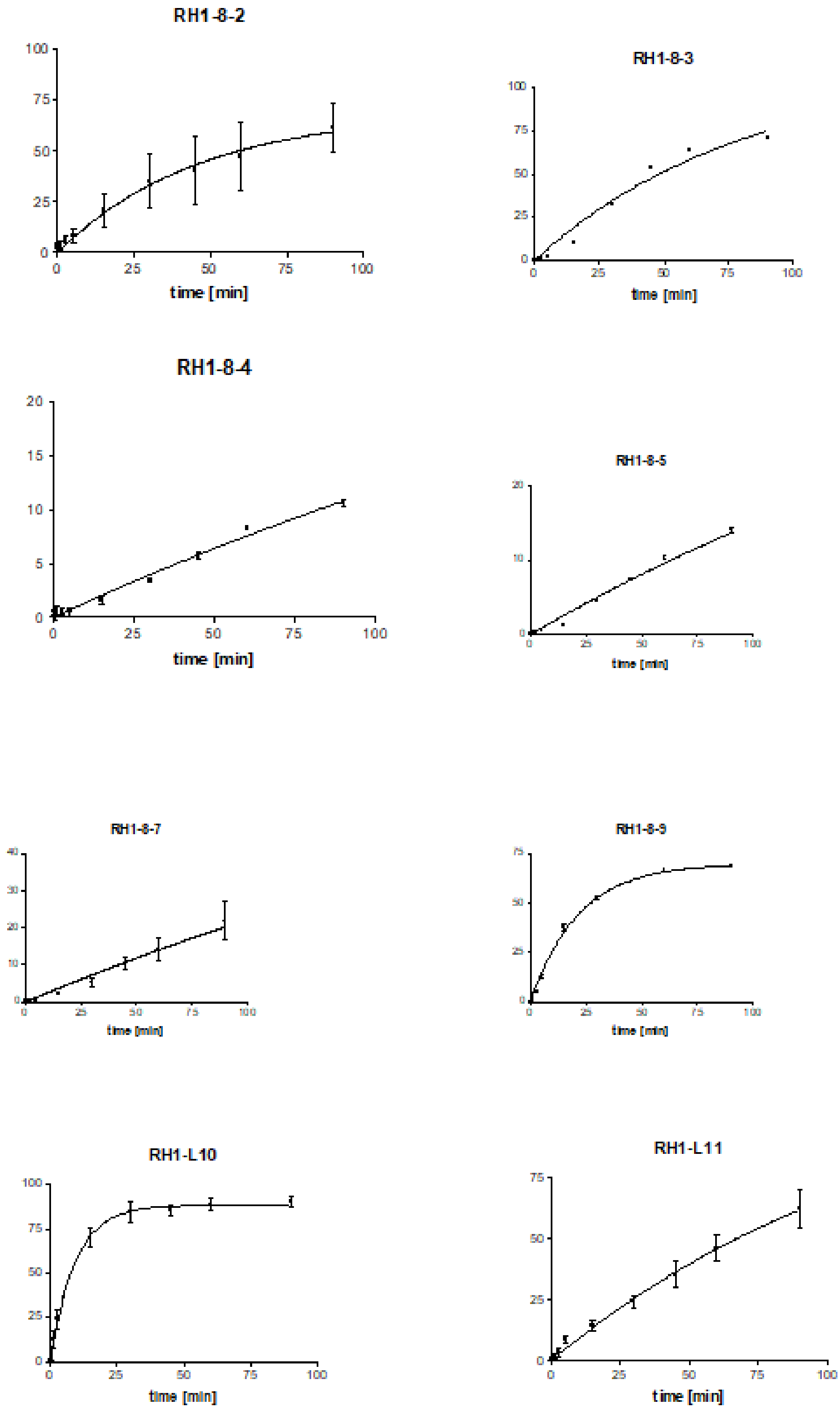


Figure S6. Kinetic curves of tested ribozymes. The bands from PAGE (Figure S5) were quantified, and k_{obs} parameters were obtained by fitting to $f = 1-e^{-kt}$ formula and collected in Table 4.

S7. Suplemental references.

1. Piasecka, J., Lenartowicz, E., Soszynska-Jozwiak, M., Szutkowska, B., Kierzek, R., and Kierzek, E. (2020). RNA secondary structure motifs of the influenza A virus as targets for siRNA-mediated RNA interference. Molecular Therapy - Nucleic Acids *19*, 627–642.