Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Electronic Supplementary Information

Problem solving approach for the diastereoselective synthesis of (5′*S*)- and (5′*R*)-5′,8-cyclopurine lesions

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General information: ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in a Varian Mercury 400 NMR at 400 MHz for ^{1}H, at 101 MHz for ^{13}C and 162 MHz for ^{31}P, respectively. Chemical shifts (δ) for ^{1}H and ^{13}C are given in ppm relative to residual signals of the solvents ($CDCl_3$ at 7.26 ppm for ^{1}H NMR and 77.05 ppm for ^{13}C NMR, d_6-DMSO at 2.54 ppm for ^{1}H NMR and 40.45 ppm for ^{13}C NMR) while chemical shifts for ^{31}P are given relative to the signal of 85% H_3PO_4 in D_2O as external standard. Coupling constants are given in Hz and the multiplicity is given with the abbreviations s for singlet; d for doublet; t for triplet; q for quartet; m for multiplet; bs for broad signal. Mass (MS) and mass/mass (MS/MS) spectrum were obtained by Esquire 3000 plus ESI (Bruker Daltonics), also connected with an Agilent 1100 series HPLC. For HPLC-UV analysis a Waters 600 HPLC connected with Photodiode Array Detector 996 was used.

General methods: Purification of products was accomplished using forced-flow chromatography (FFC), chromatography under nitrogen pressure conditions, on silica gel (60-200 mesh) while for thin layer chromatography (TLC) analysis was carried out using, Merck pre-coated TLC plates (silica gel 60 F_{254}, 0.25 mm) were observed at UV light and also sprayed with acidic solution of ceric ammonium molybdate or basic solutions of potassium permangante ($KMnO_4$) for visualization.

Reverse stationary phase (RP) chromatographic purification of the diastereomers was performed on Silica gel 100 C_{18}-reversed phase fully end-capped (Fluka) and thin layer chromatography was performed on Merck pre-coated RP-18 aluminum TLC plates (RP-C18 silica gel F_{254}, 0.25 mm), using UV light as the visualizing agent. Before using, both RP-silica and TLC were activated with acetonitrile or methanol HPLC grade.

General methods for synthesis and purification of oligonucleotides (ODNs): ODNs were prepared by automated synthesis using the DMT- and β-(cyanoethyl)phosphoramidite method, on CPG supports (500 Å), with an Expedite 8900 DNA synthesizer (*Applied Biosystems*) at the 1 μmol scale. The coupling time was elongated to 13 min. for each coupling. The phosphoramidites of 5′,8-cyclonucleosides were found to be insoluble in acetonitrile, consequently, they were dissolved in dry dichloromethane at a concentration of 0.074M.

Following to their synthesis, the DMTr-on ODNs were cleaved from the solid support and deprotected by the method of two syringes using AMA reagent [NH_4OH (30%)/CH_3NH_2 (40%) 1:1] for 10′ at room temperature. The AMA solution containing the cleaved ODN was placed in a sealed vial and heated for 15′ at 55°C. The solvent was then removed in a Speedvac.

The crude 5′-DMT-on oligomers were purified and detritylated on-column by RP-HPLC (Grace Vydac C18 column, 5μm, 50x22 mm).

The ODNs were further purified by SAX HPLC (preparative DNA Pac PA-100 column, 5μm, 22x250 mm). TRIS·HCl 25 mM, pH=8 (buffer A) and TRIS·HCl 25mM, $NaClO_4$ 0.5M, pH 8.0 (buffer B) were used at a flow rate of 9 mL/min eluting with 2-30 % B in 30 min, 30% B for 10 min, then 30-45% B in 5 min monitoring at 254 nm.

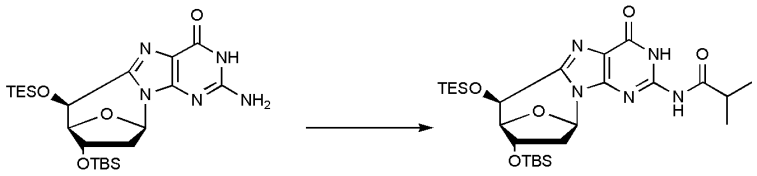
The ODNs were further purified by 15% denaturing (8 M urea) PAGE, extracted from the gel using TBE buffer (50 mM TRIS·HCl, 50 mM Boric acid, 1 mM EDTA pH 8.0) and isolated by ethanol precipitation.

The final DNA yield was estimated by UV absorption in aqueous solution measured at 254 nm on a Perkin Elmer UV/Vis Spectrometer Lambda Bio 40 following standard procedures.
Analitycal SAX HPLC chromatography, analytical PAGE and MALDI-TOF mass spectrometry were used to characterize the purified ODNs.

Starting materials: 2′-Deoxyguanosine monohydrated was purchased from Berry & Associates and used as it was. Commercial grade reagents and solvents were purchased from Link Technologies, Sigma Aldrich, Fluka, and Carlo Erba and used as received. Deionized distilled water (Mill-Q) was used for HPLC and RP chromatography.

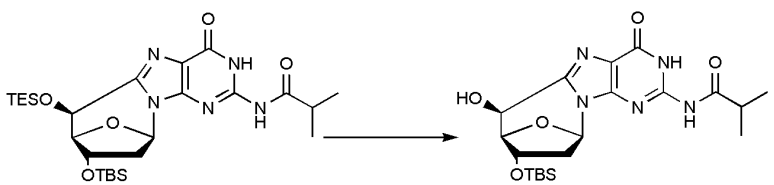
Synthetic procedures:

(5'*S*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-triethylsilyl-5',8-cyclo-2'-deoxyguanosine



(5'*S*)-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-triethylsilyl-5',8-cyclo-2'-deoxyguanosine (150 mg, 0.30 mmol) was dissolved in dry pyridine (6 mL) and stirred at 0 °C. DMAP (catalytic, 1.8 mg) was added, followed by iPrC(O)Cl (79 μL, 0.61 mmol) dropwise. The reaction mixture was stirred under Ar atmosphere for 15 minutes at 0°C and 2 hours at room temperature and was controlled with TLC (CH_2Cl_2/CH_3OH, 97:3). The reaction was quenched with 5% $NaHCO_3$ and extracted with dichloromethane (2x15 mL). Column chromatography ($CHCl_3$/MeOH 98:2) afforded 144 mg (85%) of the (5'*S*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-triethylsilyl-5',8-cyclo-2'-deoxyguanosine as white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.14 (s, 1H), 9.45 (s, 1H), 6.20 (d, *J* = 4.8 Hz, 1H), 5.11 (d, *J* = 6.1 Hz, 1H), 4.85 (dd, *J* = 7.2, 4.3 Hz, 1H), 4.50 (d, *J* = 6.1 Hz, 1H), 2.79 (dd, *J* = 8.2, 5.6 Hz, 1H), 2.43 (dd, *J* = 13.2, 7.3 Hz, 1H), 2.12 (d, *J* = 13.0 Hz, 1H), 1.26 (dd, *J* = 6.8, 4.8 Hz, 6H), 0.99 (t, *J* = 7.8 Hz, 9H), 0.89 – 0.82 (m, 9H), 0.77 (dt, *J* = 15.4, 7.8 Hz, 6H), 0.06 (s, 3H), 0.03 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 145.9, 87.2, 84.9, 69.5, 65.7, 46.3, 36.5, 25.7, 19.1, 18.9, 6.9, 4.8, -4.6, -4.8.
MS (ESI+) *m/z*: calcd for $C_{26}H_{46}N_5O_5Si_2$ $[M+H]^+$: 564.8397, found 564.8391

(5'*S*)-N^6- isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyguanosine



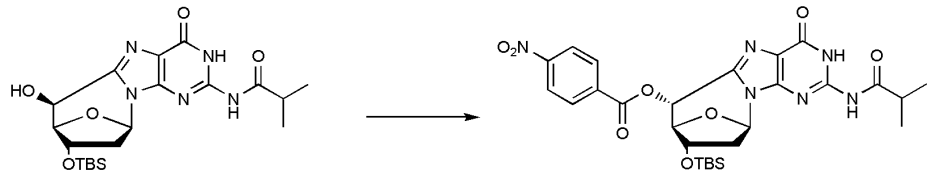
(5'*S*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-triethylsilyl-2'-deoxyguanosine (144 mg, 0.25 mmol) was taken up in THF (13 mL). The solution was cooled down to -20 °C, 0.25 mL (0.25 mmol) of 1 M solution of TBAF in THF was added, and the mixture left stirring at the same temperature. The reaction was controlled with TLC (CH_2Cl_2/CH_3OH, 95:5), and after 25 minutes, when the starting material was consumed, was quenched with saturated solution of $NaHCO_3$ and extracted with ethyl acetate (2 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was subsequently removed by rotary evaporator. The resulting residue was submitted to column chromatography on silica gel (eluent: $CHCl_3$/CH_3OH, from 99:1 to 95:5) to give 110 mg (98 %) of the (5'*S*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyguanosine as white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.15 (s, 1H), 9.38 (s, 1H), 6.29 (d, *J* = 4.6 Hz, 1H), 5.44 (s, 1H), 5.29 (d, *J* = 6.3 Hz, 1H), 4.91 (d, *J* = 2.8 Hz, 1H), 4.70 (d, *J* = 6.2 Hz, 1H), 2.89 – 2.66 (m, 1H), 2.54 (dd, *J* = 13.3, 7.3 Hz, 1H), 2.27 – 2.13 (m, 1H), 1.24 (dd, *J* = 13.2, 6.9 Hz, 6H), 0.88 (s, 9H), 0.11 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.6, 155.0, 148.4, 147.5, 145.5, 86.3, 85.4, 69.2, 64.1, 46.4, 36.2, 25.8, 19.1, 17.9, 6.6, 5.9, -4.8, -4.7.

MS (ESI+) *m/z*: calcd for $C_{20}H_{32}N_5O_5Si$ $[M+H]^+$: 450.5770, found 450.5774.

(5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-*p*-nitrobenzoyl-5',8-cyclo-2'-deoxyguanosine



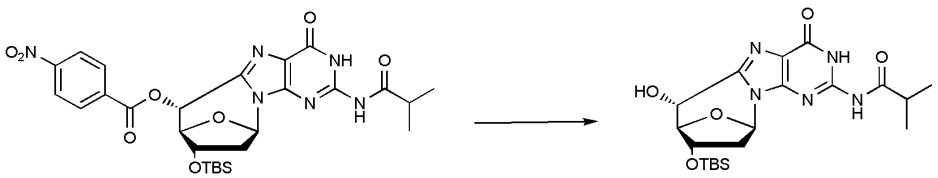
(5'*S*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyguanosine (30 mg, 0.06 mmol) was dissolved in dry THF (2 mL) and evaporated to dryness. The compound was dissolved in dry THF (2 mL) under Ar atmosphere and then PPh_3 (31.4 mg, 0.12 mmol) was added followed by DIAD (23.6 μL, 0.12 mmol) and 4-NBA (18 mg, 0.108 mmol). The mixture was left stirring overnight under Ar. The reaction was controlled with TLC (Hex/EtOAc 1:1). After the completion of the reaction, the mixture was quenched with 5% $NaHCO_3$ and extracted with EtOAc (2x10mL). Column chromatography (Hex/EtOAc 55:45) afforded 33mg (92%) of the (5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-*p*-nitrobenzoyl-5',8-cyclo-2'-deoxyguanosine as white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.06 (s, 1H), 8.65 (s, 1H), 8.22 (dd, *J* = 19.2, 7.8 Hz, 4H), 6.35 (s, 1H), 6.12 (s, 1H), 4.56 – 4.50 (m, 1H), 2.74 – 2.64 (m, 1H), 2.53 (dd, *J* = 13.4, 7.2 Hz, 1H), 2.24 (dd, *J* = 17.3, 8.4 Hz, 1H), 1.27 (t, *J* = 6.9 Hz, 6H), 0.89 (s, 9H), 0.15 (s, 3H), 0.11 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.0, 163.7, 155.2, 150.8, 148.4, 145.8, 139.0, 134.5, 131.2, 87.0, 85.3, 71.5, 68.6, 67.3, 60.5, 45.5, 36.5, 27.8, 25.7, 22.7, 22.0, 21.2, 19.0, 18.0, 14.2, -4.9, -4.8.

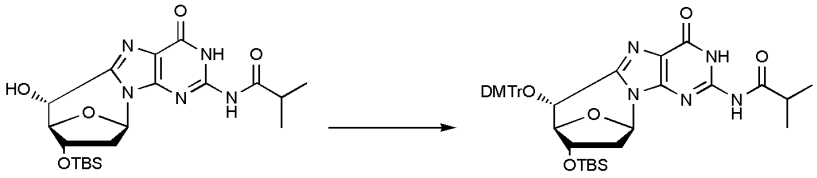
MS (ESI+) *m/z*: calcd for $C_{27}H_{35}N_6O_8Si$ $[M+H]^+$: 599.6809, found 599.6805.

(5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyguanosine



The (5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-*p*-nitrobenzoyl-5',8-cyclo-2'-deoxyguanosine (20 mg, 0.032 mmol) was dissolved in THF (0.8 mL) and a solution of LiOH 1M (2 equiv., 0.064 mmol, 64 μL) was added. The reaction reached completion after 35 minutes, as evident by TLC (CH_2Cl_2/CH_3OH, 95:5). The mixture was poured into 5 mL of $CHCl_3$ and washed with saturated NH_4Cl. Column chromatography (CH_2Cl_2/CH_3OH 95:5) afforded 14.1 mg of (5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyguanosine as white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.26 (s, 1H), 6.29 (s, 1H), 4.84 (s, 1H), 4.68 (s, 1H), 4.36 (s, 1H), 3.05 (s, 1H), 2.50 – 2.40 (m, 1H), 2.18 (d, *J* = 12.9 Hz, 1H), 1.30 (dd, *J* = 6.7, 3.9 Hz, 6H), 0.89 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 148.8, 145.3, 144.3, 89.8, 85.1, 71.3, 65.1, 45.4, 36.2, 32.0, 29.6, 29.4, 29.3, 25.8, 22.8, 19.5, 18.7, 18.0, 14.2, -4.8, -4.5.
MS (ESI+) *m/z*: calcd for $C_{20}H_{32}N_5O_5Si$ $[M+H]^+$: 450.5770, found 450.5768.

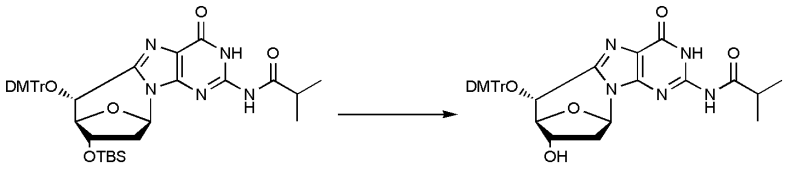
(5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine



(5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine (130 mg, 0.289 mmol) was taken up in dry pyridine (3 mL). The resulting mixture was evaporated to dryness under vacuum (the apparatus was pre-dried and prefilled with argon). The operation was repeated twice. The resulting residue was dissolved in dry pyridine (4 mL) and DMTr-Cl (130.3 mg, 0.37 mmol, 1.3 equiv.) were added. The reaction mixture was heated at 70 °C. The reaction was monitored by TLC (CH_2Cl_2/CH_3OH, 97:3) and after 30 minutes was cooled down at 4 °C and another 1.3 equiv. of DMTr-Cl was added. The same procedure was repeated once more (130.3 mg, 0.37 mmol, 1.3 equiv.). After the addition of all 4 equivalents of DMTr-Cl, the mixture was left stirring at 70°C for 5h. Then the mixture was cooled down at 4 °C and quenched with MeOH (0.5 mL). The mixture was stirred at 4 °C for 10 minutes and then CH_2Cl_2 (20 mL) was added followed by extracted with 5% $NaHCO_3$ (2x5 mL). The organic layer was dried over anhydrous Na_2SO_4, the solvents removed by rotary evaporator and the resulting residue was submitted to column chromatography on silica gel (gradient CH_2Cl_2/TEA/MeOH, from 99:1:0 to 96:1:3) to afford 184 mg (85 %) of the (5'*R*)-N^6-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine as light yellowish foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.57 (m, 3H), 7.57 – 7.50 (m, 3H), 6.86 (d, *J* = 8.0 Hz, 4H), 6.37 (d, *J* = 4.4 Hz, 1H), 4.70 (s, 1H), 3.80 (d, *J* = 15.5 Hz, 6H), 3.54 (s, 1H), 2.62 (dd, *J* = 13.7,

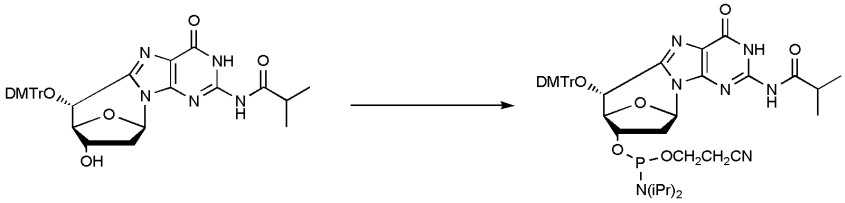
6.9 Hz, 1H), 2.20 – 2.12 (m, 1H), 1.92 (dt, *J* = 12.9, 4.5 Hz, 1H), 1.24 (dd, *J* = 6.9, 2.6 Hz, 6H), 0.76 (s, 9H), -0.10 (s, 3H), -0.14 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.4, 158.8, 155.5, 149.8, 149.6, 147.7, 145.6, 145.4, 145.2, 143.0, 136.1, 130.7, 128.6, 128.0, 127.1, 121.1, 113.4, 88.6, 87.5, 85.0, 71.0, 68.0, 55.2, 46.3, 45.9, 36.6, 29.7, 25.6, 19.2, 18.9, 17.7, 11.6, -5.0, -4.8.
MS (ESI+) *m/z*: calcd for $C_{41}H_{50}N_5O_7Si$ $[M+H]^+$: 752.9442, found 752.9438.

(5'*R*)-*N*^{6}-isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine



(5'*R*)-*N*^{6}-isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine (180 mg, 0.239 mmol) was dissolved in THF (5 mL). A solution of TBAF (550 µL, 0.549 mmol) in THF (1 M) was then added, and the mixture was stirred at room temperature. The reaction was controlled with TLC (CH_2Cl_2/CH_3OH, 95:5), showing after 15 minutes the consumption of the starting material. The solvent was removed by rotary evaporator. The resulting residue was s chromatographed on silica gel (CH_2Cl_2/CH_3OH/TEA, 98:2:1) to give 140 mg (92%) of the (5'*R*)-*N*^{6}-isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine as white foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.50 (m, 4H), 7.34 – 7.22 (m, 2H), 7.21 (t, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 8.2 Hz, 3H), 6.23 (d, *J* = 4.8 Hz, 1H), 4.67 (s, 1H), 3.89 (dd, *J* = 6.2, 2.9 Hz, 1H), 3.77 (d, *J* = 1.6 Hz, 4H), 3.17 (s, 1H), 2.66 – 2.56 (m, 1H), 2.20 (dd, *J* = 13.5, 7.3 Hz, 1H), 2.01 – 1.89 (m, 1H), 1.23 (dd, *J* = 6.9, 1.6 Hz, 3H), 1.14 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 159.0, 155.7, 147.6, 145.7, 145.0, 142.6, 136.0, 130.9, 130.7, 128.8, 128.1, 127.3, 120.9, 113.5, 84.4, 70.9, 68.2, 46.1, 44.6, 36.5, 29.7, 19.1, 10.6.
MS (ESI+) *m/z*: calcd for $C_{35}H_{36}N_5O_7$ $[M+H]^+$: 638.6832, found 638.6841.

(5'*R*)-*N*^{6}-isobutyryl-5',8-cyclo-2'-deoxyguanosine phosphoramidite derivative



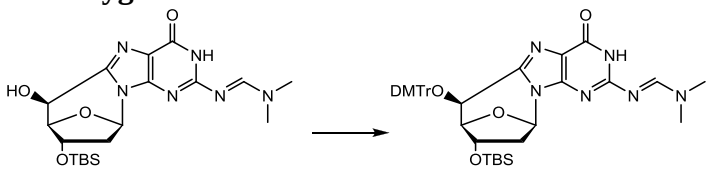
(5'*R*)-*N*^{6}-isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine (140 mg, 0.22 mmol) was taken up in dry dichloromethane (6 mL) and stirred at room temperature under Ar atmosphere. Then, dry DIEA (114 µL, 0.66 mmol) was added, followed by dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (147.1 µL, 0.66 mmol). The reaction was monitored by TLC (AcOEt/Pet/TEA, 70:30:1) and after 2 h was cooled down at 4 °C and extra dry DIEA (114 µL, 0.66 mmol) was added followed by methanol (200 µL). After 10 minutes the solvents were removed by rotary evaporator (the apparatus was pre-dried and prefilled with argon)

and the resulting residue was chromatographed on silica gel (AcOEt/CH_2Cl_2/Pet/TEA, 20:2:10:0.3) to afford 151.2 mg (82 %) of the (5'*R*)- N^6-isobutyryl-5',8-cyclo-2'-deoxyguanosine phosphoramidite derivative as white foam. Both silica gel and solvents were flushed with argon before used and the fractions were collected in 20 mL vials which were continuously flashed with Ar during collection and subsequently closed and kept under argon atmosphere.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.71 – 7.47 (m, 5H), 7.33 – 7.16 (m, 3H), 6.95 – 6.80 (m, 3H), 6.28 – 6.10 (m, 1H), 4.85 – 4.75 (m, 1H), 4.68 (dd, *J* = 8.6, 1.1 Hz, 1H), 4.24 – 4.07 (m, 2H), 3.85 – 3.75 (m, 5H), 3.71 – 3.57 (m, 2H), 3.54 – 3.40 (m, 2H), 2.82 (dd, *J* = 13.8, 7.1 Hz, 1H), 2.77 – 2.72 (m, 1H), 2.71 – 2.59 (m, 1H), 2.49 (t, *J* = 6.4 Hz, 1H), 2.04 (d, *J* = 3.1 Hz, 1H), 1.48 (d, *J* = 6.5 Hz, 3H), 1.29 – 1.21 (m, 14H), 1.14 (dt, *J* = 13.6, 6.8 Hz, 4H), 1.05 (d, *J* = 6.8 Hz, 1H), 0.99 (d, *J* = 6.8 Hz, 1H).

^{31}P NMR (162 MHz, $CDCl_3$) 149.67, 148.84 (two diastereomers).

(5'*S*)-N^6-dimethylformyl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine



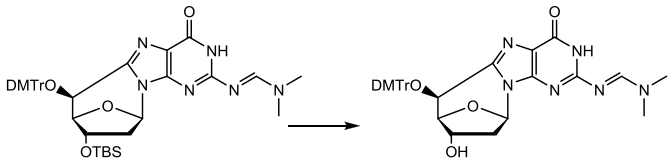
(5'*S*)-N^6-dimethylformyl-3'-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine (304 mg, 0.7 mmol) was taken up in dry pyridine (3 mL). The resulting mixture was evaporated to dryness under vacuum (the apparatus was pre-dried and prefilled with argon). The operation was repeated twice. The resulting residue was dissolved in dry pyridine (10 mL) and DMTr-Cl (949 mg, 2.8 mmol) was added in 2 portions (within 1 hour). The reaction mixture was heated at 70 °C. The reaction was monitored by TLC (CH_2Cl_2/CH_3OH, 97:3) and after 20h was cooled down at 4 °C and quenched with MeOH (0.5 mL). The mixture was stirred at 4 °C for 10 minutes and then CH_2Cl_2 (100 mL) was added followed by extracted with 5% $NaHCO_3$ (2x50 mL). The organic layer was dried over anhydrous Na_2SO_4, the solvents removed by rotary evaporator and the resulting residue was submitted to column chromatography on silica gel (gradient CH_2Cl_2/TEA/MeOH, from 99:1:0 to 96:1:3) to afford 444 mg (86 %) of the (5'*S*)-N^6-dimethylformyl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine as light yellowish foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.55 (s, 1H), 7.79 (dd, *J* = 12.0, 8.1 Hz, 4H), 7.62 (d, *J* = 8.9 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.20 (s, 1H), 6.86 (t, *J* = 8.6 Hz, 4H), 6.05 (d, *J* = 4.4 Hz, 1H), 5.11 (d, *J* = 5.3 Hz, 1H), 4.74 (dd, *J* = 7.2, 4.7 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.17 (s, 3H), 3.09 (s, 3H), 2.91 (d, *J* = 5.2 Hz, 1H), 2.40 (dd, *J* = 12.8, 7.2 Hz, 1H), 1.93 (dt, *J* = 12.6, 4.7 Hz, 1H), 0.79 (s, 9H), -0.07 (s, 3H), -0.08 (s, 3H);

^{13}C NMR (101 MHz, $CDCl_3$) δ = 158.8, 158.7, 157.9, 157.6, 156.7, 148.6, 148.3, 145.3, 144.6, 136.4, 136.2, 130.9, 130.6, 128.6, 127.9, 127, 120.1, 113.3, 113.3, 106.6, 88.3, 85.1, 84.7, 70.0, 68.5, 55.2, 47.1, 41.3, 39.2, 35.2, 25.8, 17.7, -4.5, -4.8;

MS (ESI+) *m/z*: calcd for $C_{40}H_{49}N_6O_6Si$ $[M+H]^+$: 737.9328, found 737.9351.

(5'*S*)-N^6-dimethylformyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine

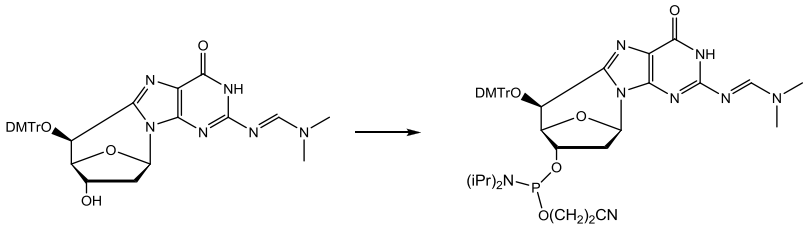


(5'*S*)-N^6-dimethylformyl-3'-*O*-*tert*-butyldimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine (369 mg, 0.5 mmol) was dissolved in THF (10 mL). A solution of TBAF (1150 µL, 1.15 mmol) in THF (1 M) was then added, and the mixture was stirred at room temperature. The reaction was controlled with TLC (CH_2Cl_2/CH_3OH, 95:5), showing after 3 hours the consumption of the starting material. The solvent was removed by rotary evaporator. The resulting residue was s chromatographed on silica gel (AcOEt/hexane/TEA, 50:10:0.6) to give 283 mg (91%) of the (5'*S*)-N^6-dimethylformyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine as white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (bs, 1H), 8.55 (s, 1H), 7.90 – 7.76 (m, 4H), 7.73 – 7.63 (m, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.25 – 7.17 (m, 1H), 6.97 – 6.84 (m, 4H), 6.08 (d, *J* = 4.7 Hz, 1H), 5.21 (d, *J* = 5.9 Hz, 1H), 4.72 (dd, *J* = 7.5, 4.2 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.17 (s, 3H), 3.09 (d, *J* = 0.5 Hz, 3H), 2.70 (d, *J* = 5.9 Hz, 1H), 2.48 (dd, *J* = 13.5, 7.6 Hz, 1H), 2.04 – 1.93 (m, 1H);

^{13}C NMR (101 MHz, $CDCl_3$) 159, 158.9, 158, 157.9, 156.7, 148.1, 145.4, 143.3, 136.8, 136.2, 130.6, 130.3, 128.4, 128.1, 127.2, 120, 113.6, 113.5, 87.72, 85.52, 84.22, 68.8, 67.5, 55.3, 55.3, 44.7, 41.4, 35.2, 29.7; MS (ESI+) *m/z*: calcd for $C_{34}H_{34}N_6O_6Na^+$ $[M + Na]^+$; 645.2438, found 645.2439.

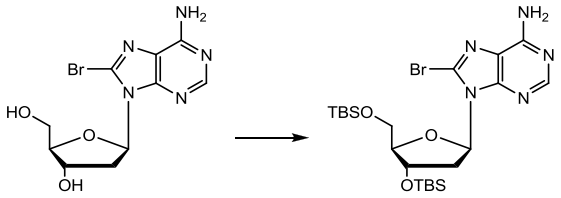
(5'*S*)- N^6-dimethylformyl-5',8-cyclo-2'-deoxyguanosine phosphoramidite derivative



(5'*S*)-N^6-dimethylformyl-5'-*O*-(4,4'-dimethoxytrityl)-5',8-cyclo-2'-deoxyguanosine (280 mg, 0.45 mmol) was taken up in dry dichloromethane (12 mL) and stirred at room temperature under Ar atmosphere. Then, dry DIEA (235 µL, 1.35 mmol) was added, followed by dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (301 µL, 1.35 mmol). The reaction was monitored by TLC (AcOEt/Hexane/TEA, 50:10:0.6) and after 1 h was cooled down at 4 °C and extra dry DIEA (235 µL, 1.35 mmol) was added followed by methanol (300 µL). After 10 minutes solvents were removed by rotary evaporator (the apparatus was pre-dried and prefilled with argon) and the resulting residue was chromatographed on silica gel (AcOEt /Hexane/TEA, 30:10:0.4) to afford 304 mg (82 %) of the (5'*S*)-5',8-cyclo-2'-deoxyguanosine phosphoramidite derivative as white foam. Both silica gel and solvents were flushed with argon before used and the fractions were collected in 24 mL vials which were continuously flashed with Ar during collection and subsequently closed and kept under argon atmosphere.

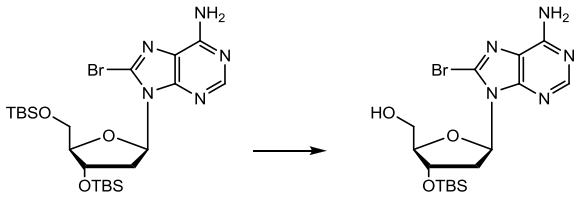
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (bs, 1H), 8.55 (d, *J* = 4.0 Hz, 1H), 7.90 – 7.72 (m, 4H), 7.63 (dd, *J* = 11.6, 4.9 Hz, 2H), 7.37 – 7.24 (m, 2H), 7.20 (dd, *J* = 9.4, 5.0 Hz, 1H), 6.85 (ddd, *J* = 9.8, 4.9, 2.3 Hz, 4H), 6.06 (dd, *J* = 9.1, 4.5 Hz, 1H), 5.14 (dd, *J* = 6.7, 5.5 Hz, 1H), 4.80 (dd, *J* = 18.5, 10.9 Hz, 1H), 3.81 – 3.74 (m, 6H), 3.64 – 3.55 (m, 2H), 3.50 (ddd, *J* = 13.7, 8.5, 4.6 Hz, 2H), 3.16 (d, *J* = 1.7 Hz, 3H), 3.08 (d, *J* = 2.8 Hz, 3H), 2.79 – 2.70 (m, 1H), 2.59 – 2.47 (m, 2H), 1.15 – 1.02 (m, 12H);
^{31}P NMR (162 MHz, $CDCl_3$) 150.1, 149.5 (two diastereomers).

Synthesis of 8-Bromo-O3',O5'-bis(*tert*-butyldimethylsilyl)-2'-deoxyadenosine.



In dry DMF (45 mL) 8-bromo-2'-deoxyadenosine (6.8 g, 20.6 mmol), imidazole (3.5 g, 51.5 mmol) and (dimethylamino)pyridine (103 mg, 0.84 mmol) were dissolved. Next *tert*-butyldimethylsilyl chloride (TBS-Cl) (7.76 g, 51.5 mmol) was added to the solution and the mixture stirred at r.t. After 1.5 h the reaction was quenched with 10% aqueous $NaHCO_3$, 200 mL of dichloromethane added and the mixture transferred in a separatory funnel. The organic layer was washed with 10% aqueous $NaHCO_3$ (2 x 200 mL) and water (2 x 200 mL) and dried over dry Na_2SO_4. The solvent was evaporated in a rotary evaporator and the residue submitted to column chromatography on silica using AcOEt/Hexanes (1:1 → 2:1) mixture as eluent affording 11.48 g (95% yield) of light brownish solid product.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 6.33 (t, *J* = 6.7 Hz, 1H), 5.77 (s, 2H), 4.85 (ddd, *J* = 6.1, 3.9 Hz, 1H), 3.97 – 3.84 (m, 2H), 3.71 – 3.57 (m, 2H), 2.22 (ddd, *J* = 13.1, 7.0, 4.2 Hz, 1H), 0.92 (s, 9H), 0.81 (s, 9H), 0.13 (s, 6H), -0.02 (s, 3H), -0.06 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.3, 152.6, 151.0, 128.2, 120.5, 87.8, 86.3, 72.36, 62.7, 36.8, 25.9, 18.4, 18.1, 4.6, -4.7, -5.4, -5.5
MS (ESI+) *m/z*: calcd for $C_{22}H_{41}BrN_5O_3Si_2$ $[M+H]^+$: 559.660, found 559.641.

Synthesis of 8-Bromo-O3'-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine.



In a solution of THF (48 mL) containing 8-bromo-O3',O5'-bis(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (1 g, 1.79 mmol) at 0 °C, 12 mL of trofluoroacetic acid/water 1:1 mixture were added and the mixture was allowed to stir at 0 °C. The reaction optimization was made based on HPLC-UV analysis by taking sample every 10 min followed by quenching and direct injection to the HPLC. After 1 hour and 10 min the reaction was neutralized by the addition of saturated

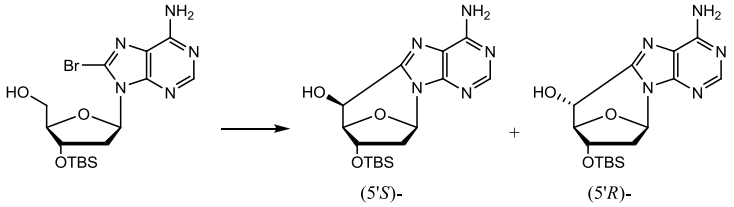
solution of Na_2CO_3. In the resulting mixture ethyl acetate (200 mL) was added and the organic layer was washed with water (2 x 200) and dried over Na_2SO_4. The solvent was removed in a rotary evaporator and the residue was purified on silica gel column by using AcOEt/Hexenes (2:1 → 1:0) as eluents. The 390 mg [49% yield (65% taking in to account the recovery of the unreacted started material)] of product were obtained as white solid:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 6.48 (d, *J* = 1.6 Hz, 1H), 6.43 (dd, *J* = 9.7, 5.2 Hz, 1H), 5.67 (s, 2H), 4.71 (d, *J* = 4.6 Hz, 1H), 4.14 (s, 1H), 3.95 (d, *J* = 12.9 Hz, 1H), 3.79 – 3.68 (m, 1H), 3.00 (ddd, *J* = 12.9, 9.7, 5.1 Hz, 1H), 2.21 – 2.11 (m, 1H), 0.93 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 152.3, 145.0, 126.8, 120.9, 90.5, 88.7, 74.0, 63.3, 40.7, 25.8, 18.0, -4.7, -4.7.

MS (ESI+) *m/z*: calcd for $C_{16}H_{27}BrN_5O_3Si$ $[M+H]^+$: 445.3990, found 445.3972.

Synthesis of (5'*R*)- and (5'*S*)-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine



A solution acetonitrile/water 1:1 (100 mL) of 5 mM 8-bromo-O3'-*tert*-butyldimethylsilyl-2'-deoxyadenosine (222 mg, 0.5 mmol) was prepared and degassed with argon for 15 min in a photoreactor. Next the solution irradiated with UV light (290 nm, medium pressure Hg lamp, 125W) for 80 minutes and subsequently quenched with saturated $NaHCO_3$. The solvents removed in a rotary evaporator and the residue dissolved in AcOEt (200 mL), washed with water (2 x 200 mL) and dried over Na_2SO_4. The mixture submitted to column chromatography on silica. First AcOEt/Hexanes 10:1 was used until the remained started material was eluted. Then a mixture of CH_2Cl_2/MeOH/EtOH 95:2.5:2.5 was used and gradually changed to CH_2Cl_2/MeOH/EtOH 90:5:5. The two products obtained pure as white solid (5′*R*, 53 mg, 0.145 mmol, 29% (or 39% considering the starting material recovered); 5′*S*, mg, 22 mg 0.06 mmol, 12% (16% considering the starting material recovered)).

(5'*R*)-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 6.80 (s, 2H), 6.49 (d, *J* = 4.6 Hz, 1H), 4.73 (s, 1H), 4.71 (s, 1H), 4.20 (dd, *J* = 6.8, 4.5 Hz, 1H), 2.38 (dd, *J* = 13.3, 7.3 Hz, 1H), 2.20 (dt, *J* = 13.2, 4.6 Hz, 1H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2, 153.8, 146.4, 145.0, 117.7, 89.5, 84.9, 71.5, 65.7, 45.4, 25.7, 18.1, -4.8, -4.9.

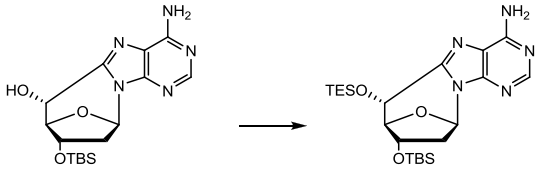
MS (ESI+) *m/z*: calcd for $C_{16}H_{26}N_5O_3Si$ $[M+H]^+$: 364.4886, found 364.4870.

(5'*S*)-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine

^{1}H NMR (400 MHz, DMSO) δ 8.30 (s, 1H), 7.79 (s, 2H), 6.60 (s, 1H), 6.47 (d, *J* = 4.8 Hz, 1H), 5.22 (d, *J* = 6.1 Hz, 1H), 4.94 (dd, *J* = 7.4, 4.2 Hz, 1H), 4.63 (d, *J* = 6.3 Hz, 1H), 2.62 (s, 1H), 2.57 (dd, *J* = 13.3, 7.4 Hz, 1H), 2.24 (dt, *J* = 13.1, 4.6 Hz, 1H), 0.93 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H).

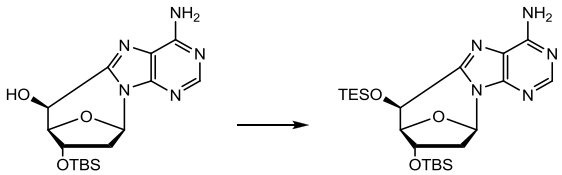
^{13}C NMR (101 MHz, *d6*-DMSO) δ 158.1, 154.0, 153.9, 151.5, 123.3, 91.5, 89.6, 83.6, 83.3, 83.0, 74.0, 69.2, 30.6, 22.6, 0.1, 0.1.
MS (ESI+) *m/z*: calcd for $C_{16}H_{26}N_5O_3Si$ $[M+H]^+$: 364.4886, found 364.4899.

Synthesis of (5'*R*)-3'-O-tert-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine



(5'*R*)-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine (284 mg, 0.78 mmol) was dissolved in dry dichloromethane (20 mL) along with imidazole (79.7 mg, 1.17 mmol). Triethylsilyl chloride (196 μL, 1.17 mmol) was then added dropwise and the mixture was stirred at ambient temperature under Ar atmosphere for 30 min until the TLC showed the consumption of the starting material. Next the mixture was quenched with saturated $NaHCO_3$ solution, extracted with dichloromethane, washed with 5% $NaHCO_3$ solution (2 x 50 mL) and the organic layer dried over Na_2SO_4. After the solvent was removed by a rotary evaporator, the crude reaction mixture chromatographed on silica gel (CH_2Cl_2/MeOH/TEA 96.5:3:0.5) to give 243 mg (90%) of product as light-brown foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 6.42 (d, *J* = 4.7 Hz, 1H), 5.21 (d, *J* = 6.1 Hz, 1H), 5.05 – 4.68 (m, 3H), 4.60 (d, *J* = 6.1 Hz, 1H), 2.55 (dd, *J* = 13.7, 7.2 Hz, 1H), 2.26 (d, *J* = 13.7 Hz, 1H), 1.05 (t, *J* = 7.8 Hz, 9H), 0.88 (s, 9H), 0.86 – 0.71 (m, 6H), 0.08 (s, 3H), 0.06 (d, *J* = 5.6 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 155.6, 152.7, 147.1, 145.1, 119.0, 90.1, 85.0, 71.4, 66.7, 45.4, 29.6, 25.6, 17. 9, 6.6, 4.8, -4.9, -5.0.
MS (ESI+) *m/z*: calcd for $C_{22}H_{40}N_5O_3Si_2$ $[M+H]^+$: 478.7500, found 478.7446.

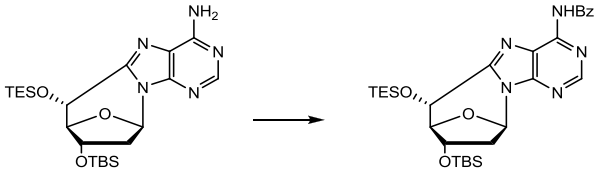
Synthesis of (5'*S*)-3'-O-(*tert*-butyldimethylsilyl)-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine



(5'*S*)-3'-O-tert-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine (280 mg, 0.80 mmol) dissolved in 40 mL of dry dimethylformamide/dichloromethane 1:4 mixture along with imidazole (163.4 mg, 2.40 mmol). Then triethylsilyl chloride (402 μL, 2.4 mmol) was added dropwise and the reaction controlled with TLC. The starting material was consumed after 30 min and the same procedure described for the (5'*R*)-3'-O-tert-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine was followed. After purification, 249.2 mg (90%) of (5'*S*)-3'-O-tert-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine were obtained as a white foam.

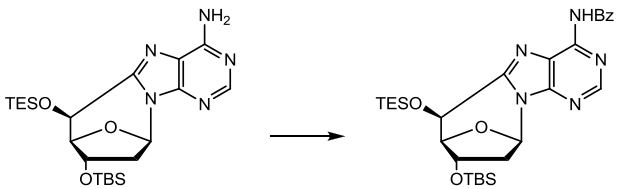
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 6.40 (d, *J* = 4.8 Hz, 1H), 5.66 (s, 2H), 5.20 (d, *J* = 6.1 Hz, 1H), 4.84 (dd, *J* = 7.3, 4.3 Hz, 1H), 4.57 (d, *J* = 6.1 Hz, 1H), 2.52 (dd, *J* = 13.2, 7.4 Hz, 1H), 2.20 (dt, *J* = 13.2, 4.6 Hz, 1H), 1.06 (t, *J* = 7.9 Hz, 6H), 0.88 (s, 9H), 0.81 (ddd, *J* = 20.9, 10.4, 4.7 Hz, 6H), 0.07 (s, 3H), 0.04 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8, 152.3, 148.1, 147.6, 119.5, 87.1, 85.2, 69.6, 66.2, 46.4, 25.7, 17.9, 6.9, 4.86, -4.6, -4.8.
MS (ESI+) *m/z*: calcd for $C_{22}H_{40}N_5O_3Si_2$ $[M+H]^+$: 478.7500, found 478.7443.

Synthesis of (5'*R*)-N6-benzoyl-O-3'-O-(*tert*-butyldimethylsilyl)-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine



(5'*R*)-3'-O-tert-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine (380 mg, 1.10 mmol) dissolved in dry pyridine (15 mL) and triethylamine (223 μL, 1.6 mmol) was added. The mixtrure was cooled down to 0 ^{o}C and while stirring under argon atmosphere benzoyl chloride (268 μL, 2.31 mmol) was added dropwise. The mixture was stirred for 3 h upon the TLC showed the consumption of the starting material. The reaction was then quenched with 1 mL of 2M NH_4OH solution and the solvents removed in rotary evaporator. Next the crude mixture redissolved in 15 mL of THF, 6 mL of 2M NH_4OH solution were added and the mixture stirred for 1 extra hour at room temperature. The reaction was then quenched with 1 M HCl solution, the mixture extracted with dichloromethane, washed with water and chromatographed on silica (AcOEt/hexanes/TEA 66:33:1) to give 441 mg (69%) of product as yellowish foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.63 (s, 1H), 7.98 – 7.88 (m, 2H), 7.53 (dd, *J* = 10.9, 3.9 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 6.54 (d, *J* = 4.6 Hz, 1H), 4.76 (s, 1H), 4.58 (s, 1H), 4.26 (dd, *J* = 7.0, 4.4 Hz, 1H), 2.43 (dd, *J* = 13.3, 7.2 Hz, 1H), 2.20 (dt, *J* = 13.3, 4.5 Hz, 1H), 0.95 (t, *J* = 7.8 Hz, 9H), 0.85 (s, 9H), 0.79 – 0.63 (m, 6H), 0.05 (s, 3H), 0.02 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 164.4, 152.4, 149.3, 148.9, 147.8, 133.8, 132.5, 128.7, 127.6, 122.2, 90.1, 85.1, 71.3, 66.6, 45.3, 25.5, 17.8, 6.6, 4.7, -4.9, -5.1.
MS (ESI+) *m/z*: calcd for $C_{29}H_{46}N_5O_3Si_2$ $[M+H]^+$: 568.8732, found 568.8700.

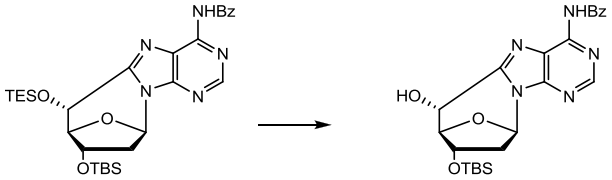
Synthesis of (5'*S*)-N6-benzoyl-O3'-(*tert*-butyldimethylsilyl)-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine



The procedure described for the (5'*R*)-N6-benzoyl-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine was followed also for the (5'*S*)- diastereoisomer. Starting from (5'*S*)-3'-O-tert-

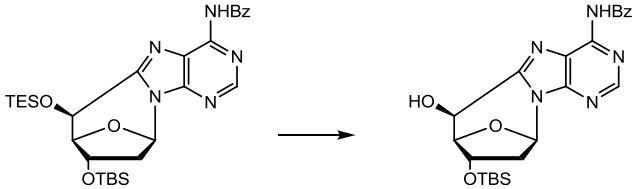
butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine (480 mg, 1.38 mmol) after purification, 561 mg (70%) of (5'*S*)-N5-benzoyl-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine were obtained as a yellowish foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.75 (s, 1H), 8.00 (dd, *J* = 8.4, 1.2 Hz, 2H), 7.65 – 7.57 (m, 1H), 7.56 – 7.48 (m, 2H), 6.49 (d, *J* = 4.8 Hz, 1H), 5.26 (d, *J* = 6.1 Hz, 1H), 4.85 (dd, *J* = 7.3, 4.3 Hz, 1H), 4.62 (d, *J* = 6.1 Hz, 1H), 2.56 (dd, *J* = 13.3, 7.3 Hz, 1H), 2.26 (dt, *J* = 13.3, 4.6 Hz, 1H), 1.09 (t, *J* = 7.9 Hz, 8H), 0.92 – 0.79 (m, 16H), 0.08 (s, 3H), 0.05 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 152.5, 150.8, 149.1, 148.9, 133.9, 132.8, 129.0, 127.6, 122.2, 87.0, 85.5, 69.5, 66.2, 46.5, 3.72, 17.9, 6.9, 4.9, -4.6, -4.8.
MS (ESI+) *m/z*: calcd for $C_{29}H_{46}N_5O_3Si_2$ $[M+H]^+$: 568.8732, found 568.8768.

Synthesis of (5'*R*)-N6-benzoyl-O-3'-O-(*tert*-butyldimethylsilyl)-5',8-cyclo-2'-deoxyadenosine



(5'*R*)-N6-benzoyl-O3'-*tert*-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine (360 mg, 0.62 mmol) dissolved in THF (10 mL) and the mixture was cooled down to 0 ^{o}C. Next 1M TBAF solution in THF (430 μL, 0.43 mmol) was added while stirring. After 10 min upon the TLC showed the consumption of the starting material the reaction mixture extracted with AcOEt (100 mL), subsequently washed with water (2 x 30 mL) and dried over Na_2SO_4. Purification on silica (CH_2Cl_2/MeOH 98:2 → 93:7) gave 275 mg (95%) of product as white foam
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.09 (d, *J* = 7.3 Hz, 2H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 2H), 6.59 (d, *J* = 4.7 Hz, 1H), 4.98 (s, 1H), 4.58 (s, 1H), 4.33 (dd, *J* = 7.0, 4.1 Hz, 1H), 2.50 (dd, *J* = 13.5, 7.2 Hz, 1H), 2.29 (dt, *J* = 13.5, 4.5 Hz, 1H), 0.88 (s, 9H), 0.04 (s, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 165.4, 152.8, 149.3, 149.0, 148.5, 133.4, 132.9, 128.8, 128.3, 128.2, 121.2, 88.8, 85.2, 71.6, 65.4, 45.4, 25.7, 25.7, 17.9, 1.0, -4.8, -4.9.
MS (ESI+) *m/z*: calcd for $C_{23}H_{32}N_5O_3Si$ $[M+H]^+$: 454.6117, found 454.6129.

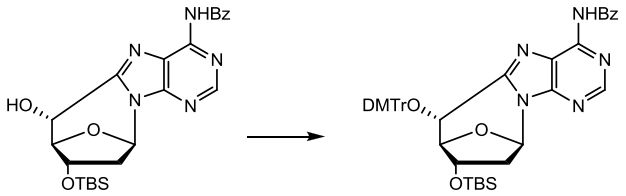
Synthesis of (5'*S*)-N6-benzoyl-O3'-(*tert*-butyldimethylsilyl)-5',8-cyclo-2'-deoxyadenosine



The procedure described for the (5'*R*)-N6-benzoyl-O-3'-O-(*tert*-butyldimethylsilyl)-5',8-cyclo-2'-deoxyadenosine was followed also for the (5'*S*)- diastereoisomer. Starting from (5'*S*) -N6-benzoyl-O3'-*tert*-butyldimethylsilyl-5'-O-triethylsilyl-5',8-cyclo-2'-deoxyadenosine (240 mg, 0.41 mmol) after purification, 183 mg (95%) of product were obtained as a white foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.00 (d, *J* = 7.3 Hz, 2H), 7.60 – 7.51 (m, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 6.87 (s, 1H), 6.40 (d, *J* = 4.7 Hz, 1H), 5.49 (d, *J* = 6.2 Hz, 1H), 4.71 (dd, *J* = 6.9,

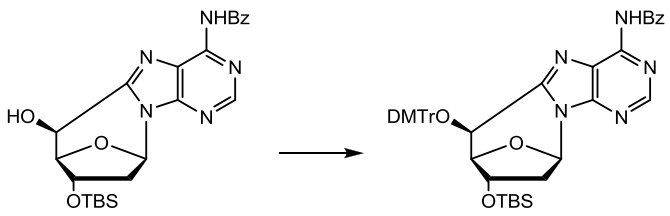
4.3 Hz, 1H), 4.15 (d, *J* = 5.4 Hz, 1H), 2.43 (dd, *J* = 13.5, 7.3 Hz, 1H), 2.17 (dt, *J* = 13.3, 4.5 Hz, 1H), 0.80 (s, 9H), -0.05 (s, 3H), -0.06 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 152.4, 152.3, 149.7, 148.5, 133.4, 132.9, 128.3, 128.0, 122.3, 86.1, 85.3, 69.1, 64.5, 46.3, 25.7, 17.8, -4.8, -5.0.
MS (ESI+) *m/z*: calcd for $C_{23}H_{32}N_5O_3Si$ $[M+H]^+$: 454.6117, found 454.6156.

Synthesis of (5'*R*)-N6-benzoyl-O-3'-(*tert*-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine



(5'*R*)-N6-benzoyl-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine (230 mg, 0.49 mmol) was put in a round bottom flask and dissolved in dry pyridine (3 mL). The resulting mixture was evaporated to dryness in rotarvapor (which was pre-dried and prefilled with argon). The operation was repeated twice. The resulting residue was dissolved for third time in dry pyridine (10 mL) and kept under argon atmosphere. Next triethylamine (68.7 µL, 0.49 mmol) added followed by DMTr-Cl (498 mg, 1.47 mmol) addition in 2 portions (within 1 hour). The reaction mixture was heated at 70 °C and monitored by TLC. After 18h it was cooled down at 5 °C and methanol (0.5 mL) added. The mixture was then extracted with dichloromethane (50 mL), washed with $NaHCO_3$ 5% solution (2 x 10 mL) and water (2 x 10 mL), and the organic layer dried over Na_2SO_4. The solvent removed by rotary evaporator and the resulting residue was submitted to column chromatography on silica gel (CH_2Cl_2 / TEA / MeOH, 94:1:5) affording 330 mg (87 %) of the product as light yellowish foam.
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.76 (s, 1H), 8.04 (d, *J* = 8.1 Hz, 2H), 7.64 – 7.52 (m, 8H), 7.32 – 7.26 (m, 2H), 7.24 – 7.14 (m, 2H), 6.90 – 6.81 (m, 4H), 6.58 (d, *J* = 4.4 Hz, 1H), 4.75 (s, 1H), 3.87 – 3.81 (m, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.70 (s, 1H), 2.27 (dd, *J* = 13.2, 7.1 Hz, 1H), 2.06 (dt, *J* = 13.1, 4.5 Hz, 1H), 0.79 (s, 9H), -0.10 (s, 3H), -0.12 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 158.8, 158.6, 152.1, 149.4, 149.2, 147.7, 145.1, 139.5, 135.9, 133.8, 132.8, 130.8, 130.8, 129.2, 129.0, 128.7, 127.9, 127.9, 127.8, 127.2, 127.1, 122.2, 113.4, 113.3, 113.2, 68.0, 55.3, 55.2, 55.1, 45.8, 25.7, 25.6, 17.7, -4.7, -5.0.
MS (ESI+) *m/z*: calcd for $C_{44}H_{50}N_5O_5Si$ $[M+H]^+$: 756.9800, found 756.9827.

Synthesis of (5'*S*)-N6-benzoyl-O-3'-(*tert*-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine



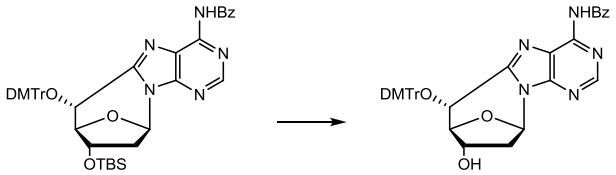
The procedure described for the (5'*R*)-N6-benzoyl-O-3'-(*tert*-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine was followed also for the (5'*S*)- diastereoisomer. Starting from (5'*S*)-N6-benzoyl-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine (360 mg, 0.77 mmol) after purification, 479 mg (74%) of (5'*S*)-N5-benzoyl-O3'-*tert*-butyldimethylsilyl-5',8-cyclo-2'-deoxyadenosine were obtained as a yellowish foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.69 (s, 1H), 8.09 – 8.02 (m, 2H), 7.82 – 7.71 (m, 4H), 7.67 – 7.50 (m, 5H), 7.36 – 7.17 (m, 3H), 6.94 – 6.81 (m, 4H), 6.34 (d, *J* = 4.5 Hz, 1H), 5.31 – 5.23 (m, 1H), 4.89 (dd, *J* = 7.2, 4.7 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.30 (d, *J* = 5.7 Hz, 1H), 2.53 (dd, *J* = 13.1, 7.3 Hz, 1H), 2.08 (dt, *J* = 13.0, 4.6 Hz, 1H), 0.84 (s, 8H), -0.01 (s, 5H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.2, 159.0, 159.0, 152.6, 150.2, 148.9, 145.0, 135.9, 135.85, 134.0, 132.8, 130.9, 130.8, 129.0, 128.6, 127.9, 127.7, 127.2, 113.4, 113.3, 88.8, 85.3, 85.2, 70.0, 68.3, 55.2, 55.2, 46.7, 25.7, 17.7, -4.4, -4.9.

MS (ESI+) *m/z*: calcd for $C_{44}H_{50}N_5O_5Si$ $[M+H]^+$: 756.9800, found 756.9869.

Synthesis of (5'*R*)-N6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine



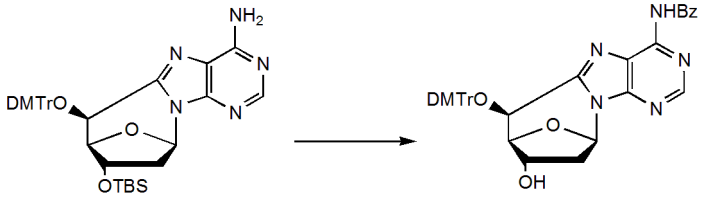
(5'*R*)-N6-benzoyl-O-3'-(*tert*-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine (320 mg, 0.42 mmol) dissolved in THF (20 mL) at room temperature. Next 1M TBAF solution in THF (550 µL, 0.55 mmol) was added while stirring. After 1 h upon the TLC showed the consumption of the starting material the reaction mixture extracted with AcOEt (100 mL), subsequently washed with $NaHCO_3$ 5% (2 x 30 mL) and dried over Na_2SO_4. Purification on silica (AcOEt/TEA/Hexanes 50:0.6:10) gave 243 mg (89%) of product as brownish foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (s, 1H), 8.70 (s, 1H), 7.97 (d, *J* = 7.5 Hz, 2H), 7.71 – 7.39 (m, 9H), 7.28 (t, *J* = 7.5 Hz, 2H), 7.21 (t, *J* = 7.2 Hz, 1H), 6.94 – 6.81 (m, 4H), 6.53 (d, *J* = 4.8 Hz, 1H), 4.68 (s, 1H), 3.87 (s, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.46 (s, 1H), 2.28 (dd, *J* = 13.7, 7.3 Hz, 1H), 2.08 (dt, *J* = 13.7, 4.5 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.5, 159.0, 158.9, 152.5, 149.3, 149.2, 147.3, 144.8, 135.8, 134.0, 132.7, 130.8, 130.7, 128.8, 128.7, 127.9, 127.7, 127.2, 122.2, 113.4, 113.3, 88.7, 87.6, 84.7, 70.8, 68.2, 55.3, 55.2, 46.0, 44.7, 10.8.

MS (ESI+) *m/z*: calcd for $C_{38}H_{36}N_5O_5$ $[M+H]^+$: 642.7185, found 642.7180.

Synthesis of (5'*S*)-N6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine



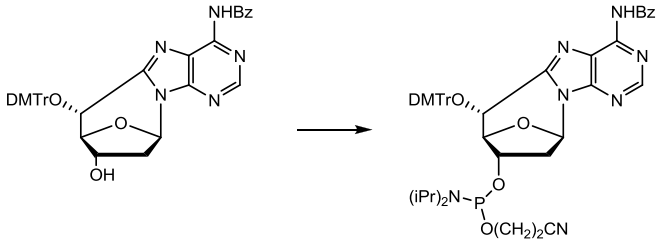
The procedure described for the (5'*R*)-N6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine was followed also for the (5'*S*)- diastereoisomer. Starting from (5'*S*)-N6-benzoyl-O-3'-(*tert*-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine (220 mg, 0.29 mmol) and by using 1M TBAF solution (750 μL, 0.75 mmol) after 3 h, 165 mg (88%) of product were obtained as a white foam after purification.

1H NMR (400 MHz, CDCl3) δ 9.14 (s, 1H), 8.71 (s, 1H), 8.10 – 8.03 (m, 2H), 7.85 – 7.74 (m, 4H), 7.70 – 7.63 (m, 2H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.54 (dd, *J* = 8.1, 6.7 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.30 – 7.22 (m, 1H), 6.95 – 6.86 (m, 4H), 6.35 (d, *J* = 4.8 Hz, 1H), 5.31 (d, *J* = 6.0 Hz, 1H), 4.84 (dd, *J* = 7.3, 4.2 Hz, 1H), 3.78 (d, *J* = 3.1 Hz, 6H), 3.76 – 3.72 (m, 1H), 2.92 (d, *J* = 6.0 Hz, 1H), 2.59 (dd, *J* = 13.7, 7.6 Hz, 1H), 2.13 (dt, *J* = 13.6, 4.6 Hz, 1H), 1.6 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.2, 159.2, 159.1, 152.6, 149.4, 149.3, 149.0, 145.0, 136.3, 135.8, 134.0, 132.8, 130.5, 129.0, 128.3, 128.2, 127.7, 127.4, 122.4, 113.6, 88.3, 85.4, 84.8, 68.9, 67.6, 55.3, 55.3, 44.9.

MS (ESI+) *m/z*: calcd for $C_{38}H_{36}N_5O_5$ $[M+H]^+$: 642.7185, found 642.7121.

(5'*R*)-5',8-cyclo-2'-deoxyadenosine phosphoramidite derivative.

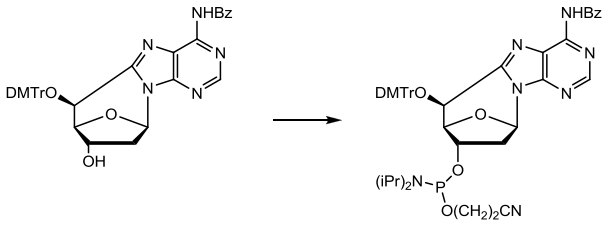


(5'*R*)-N6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine (150 mg, 0.19 mmol) was put in a round bottom flask, dissolved in dry dichloromethane (10 mL) and kept stirring at room temperature under argon atmosphere. Then, dry DIEA (99 μL, 0.57 mmol) was added, followed by dropwise addition of 2-cyanoethyl *N*,*N*diisopropylchlorophosphoramidite (135 μL, 0.57 mmol). The reaction was monitored by TLC and after 30 min was cooled down at 5 °C and extra dry DIEA (99 μL, 0.57 mmol) was added along with methanol (300 μL). After 10 minutes the solvents were removed by rotarvapor (which was pre-dried and prefilled with argon) and the resulting residue was submitted to column chromatography on silica gel (gradient AcOEt / TEA / hexanes 60:0.9:30) to afford 138 mg (85 %) of the (5'*R*)-5',8-cyclo-2'-deoxyadenosine phosphoramidite derivative as white foam. Both the silica and the solvents were flushed with argon before using and the fractions were collected in 24ml vials which were flashed with argon during the collection and subsequently closed and kept under argon atmosphere all time.

1H NMR (400 MHz, CDCl3) δ 9.00 (s, 1H), 8.77 (s, 1H), 8.02 (d, *J* = 7.3 Hz, 2H), 7.68 – 7.45 (m, 8H), 7.27 (dd, *J* = 6.8, 5.4 Hz, 2H), 7.23 – 7.16 (m, 1H), 6.91 – 6.79 (m, 4H), 6.58 (d, *J* = 1.9 Hz, 1H), 4.72 (s, 1H), 4.0 – 3.40 (m, 12H), 2. 6 – 2.3 (m, 4H), 1.18 – 1.0 (m, 12H).

^{31}P NMR (162 MHz, $CDCl_3$) δ 149.1, 148.7

(5'*S*)-5',8-cyclo-2'-deoxyadenosine phosphoramidite derivative.



The procedure described for the (5'*R*)-5',8-cyclo-2'-deoxyadenosine phosphoramidite was followed also for the (5'*S*)- diastereoisomer. Starting from (5'*S*)-N6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-5',8-cyclo-2'-deoxyadenosine (287 mg, 0.44 mmol) and after purification as described previously, 329 mg (88%) of product were obtained as a white foam. 1H NMR (400 MHz, CDCl3) δ 8.89 (s, 1H), 8.73 (s, 1H), 8.14 – 6.79 (m, 18H), 6.38 (d, 1H), 5.29 (d , 1H), 5.02 (m, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.71 – 3.49 (m, 6H), 2.63 (m, 1H), 2.45 – 2.23 (m, 4H), 1.15 (dd, *J* = 16.9, 6.8 Hz, 12H).
^{31}P NMR (162 MHz, $CDCl_3$) δ 149.1, 148.4.

ODNs characterization:

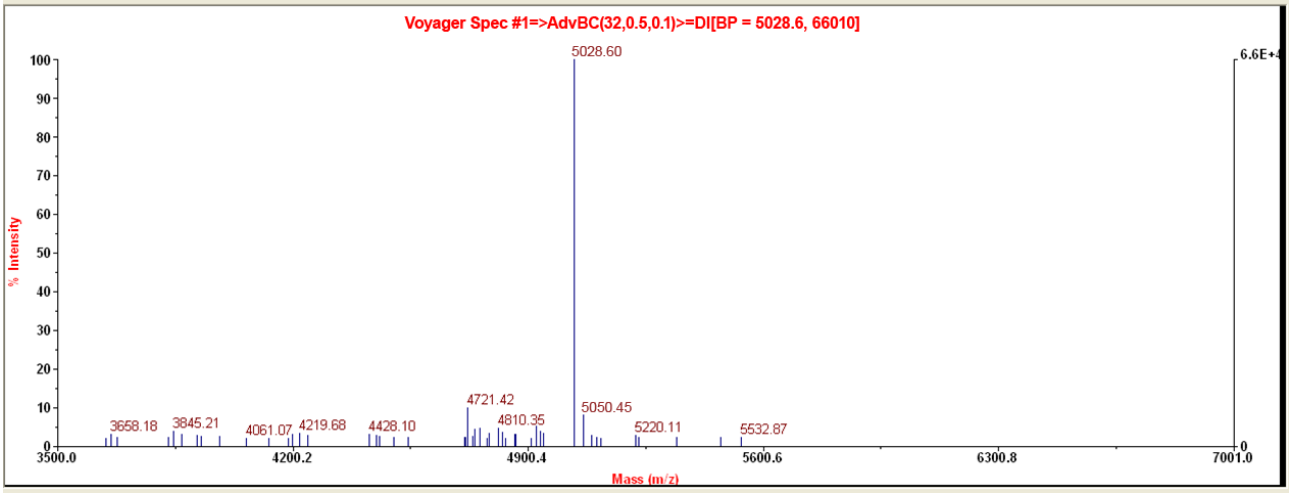
Strands	Sequence (5'-3')[a]	Mass calcd (Da)	Mass[b] found (Da)
ODN-1	CCA CCA ACG CTA CCA CC	5028,30	5028.60
ODN-2	CCA CCA ACX CTA CCA CC	5026,30	5027.88
ODN-3	CCA CCA ACX CTA CCA CC	5026,30	5027.12
ODN-4	CCA CCA ACA CTA CCA CC	5034,30	5035.76
ODN-5	CCA CCA ACX CTA CCA CC	5032,30	5033.10
ODN-6	CCA CCA ACX CTA CCA CC	5032,30	5032.80

Table S1. Sequences and molecular masses of the synthesized ODNs

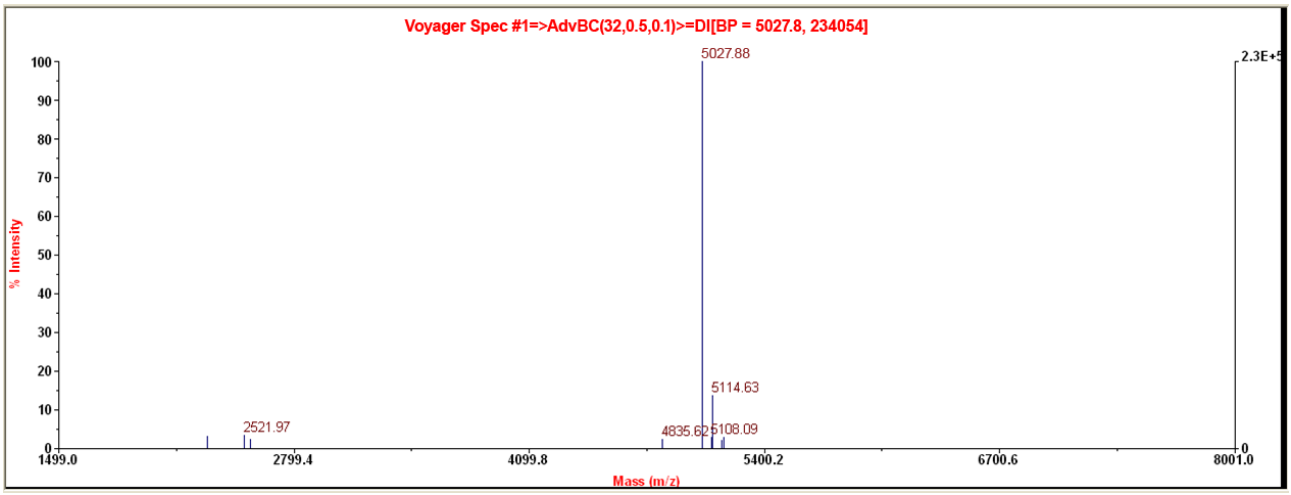
[a] X is (5′*S*)-cdG for ODN-2, (5′*R*)-cdG for ODN-3, (5′*S*)-cdA for ODN-5 and (5′*R*)-cdA for ODN-6.
[b]All the oligonucleotide masses were obtained by MALDI-TOF in negative mode. The mass was found corresponding to $[M-H^+]^-$ for oligonucleotides ODN1-ODN3; and to $[M-2H^+{+}Na^+]^-$ for oligonucleotides ODN4-ODN6.

Maldi-TOF spectra:

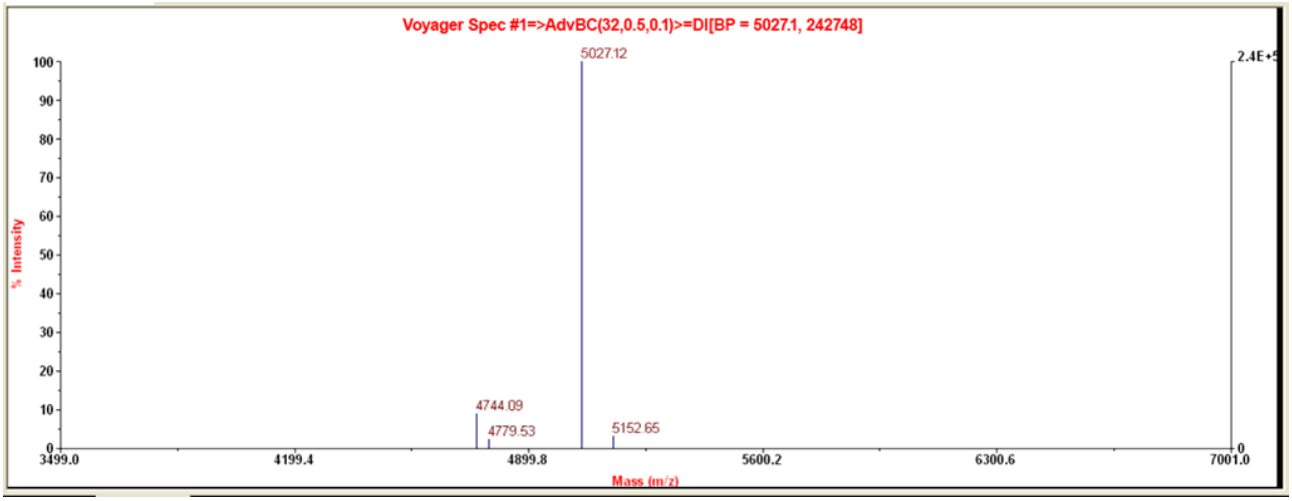
ODN1



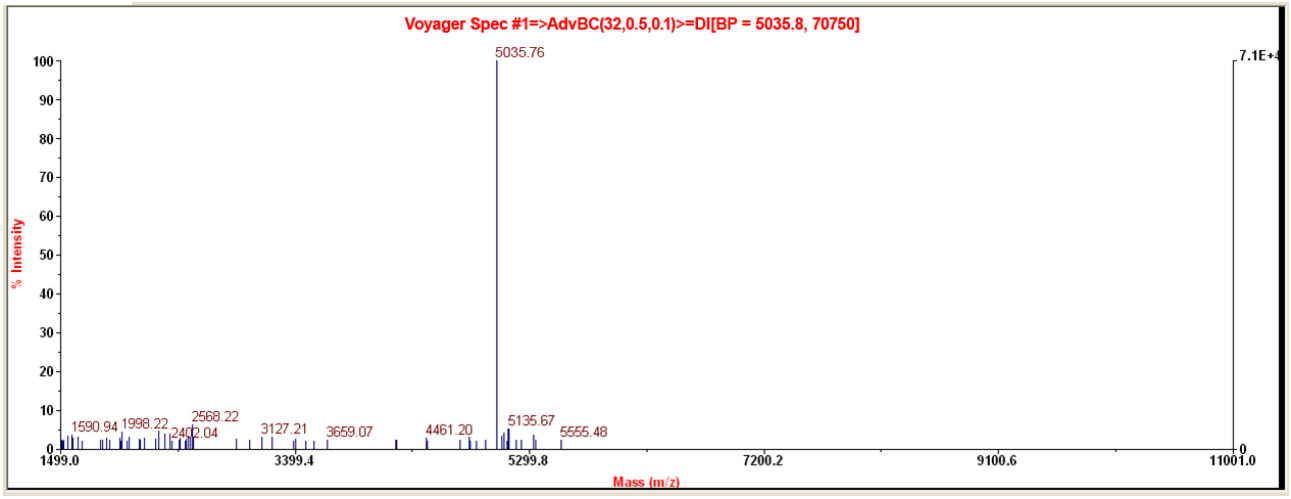
ODN2



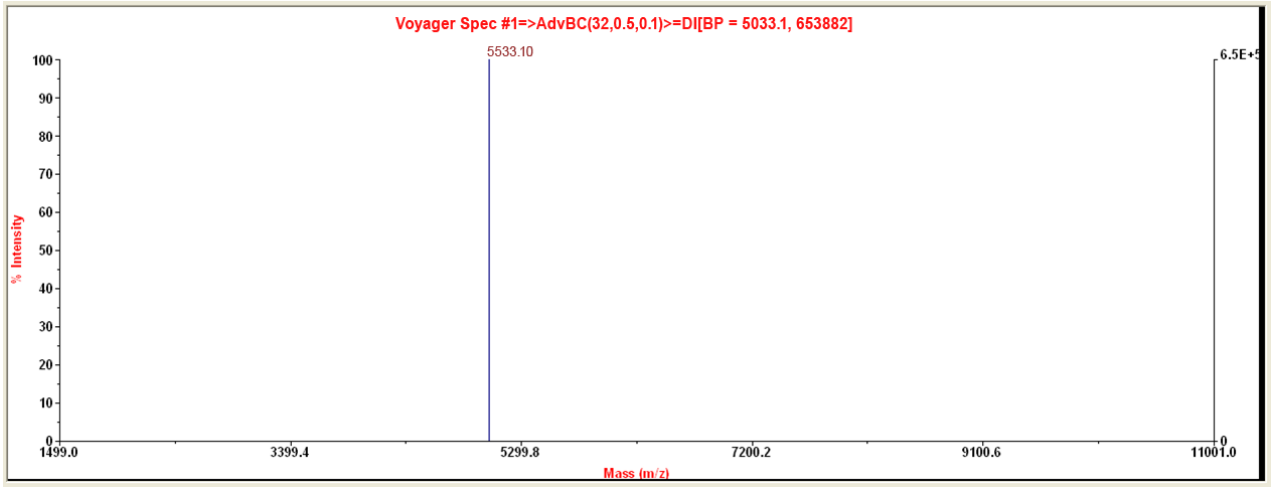
ODN3



ODN4



ODN5



ODN6

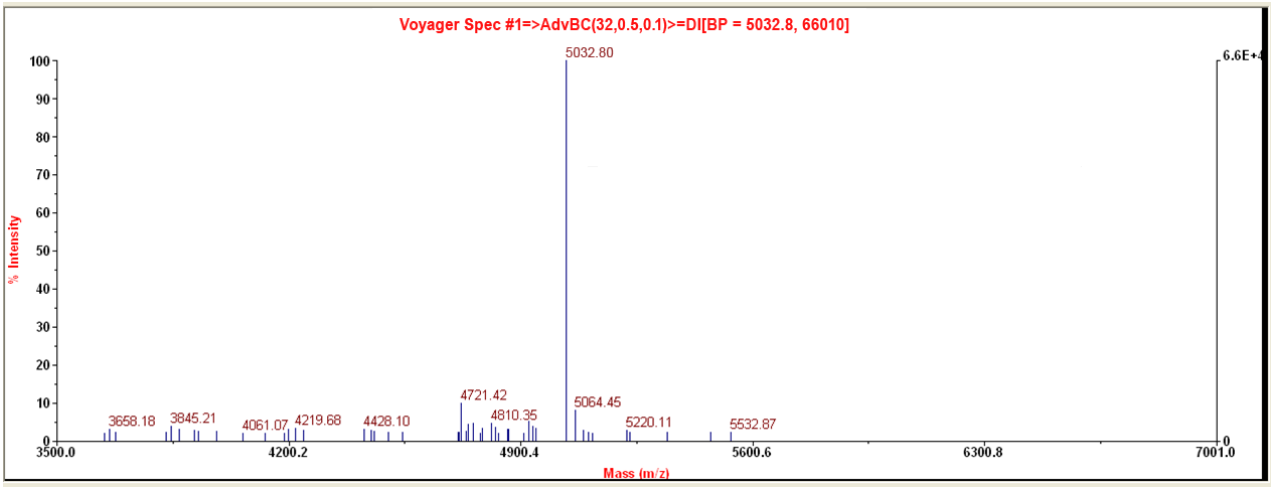


Figure S1. MALDI-TOF analysis was performed using a Voyager DE Pro (Applied Biosystems, Foster City, CA) equipped with a pulsed N2 laser operating at 337 nm. Whole oligonucleotides negative ion spectra were acquired in linear mode over a m/z range from 3500 to 7000 using a 20000-V accelerating voltage, a 17000-V grid voltage, and a delay extraction time of 200 ns. The spectrum for each spot was obtained by averaging the result of 100 laser shots. External mass calibration was performed using peptides standard mixture (mass range: 1000-6000). The analyses were performed by spotting on the target plate 1 µL of the sample mixed with an equal volume of the matrix solution, 50 mg/mL 3-hydroxypicolinic acid/ 50 mg/mL diammonium citrate (9/1) (v/v) in water.

SAX-HPLC chromatograms:

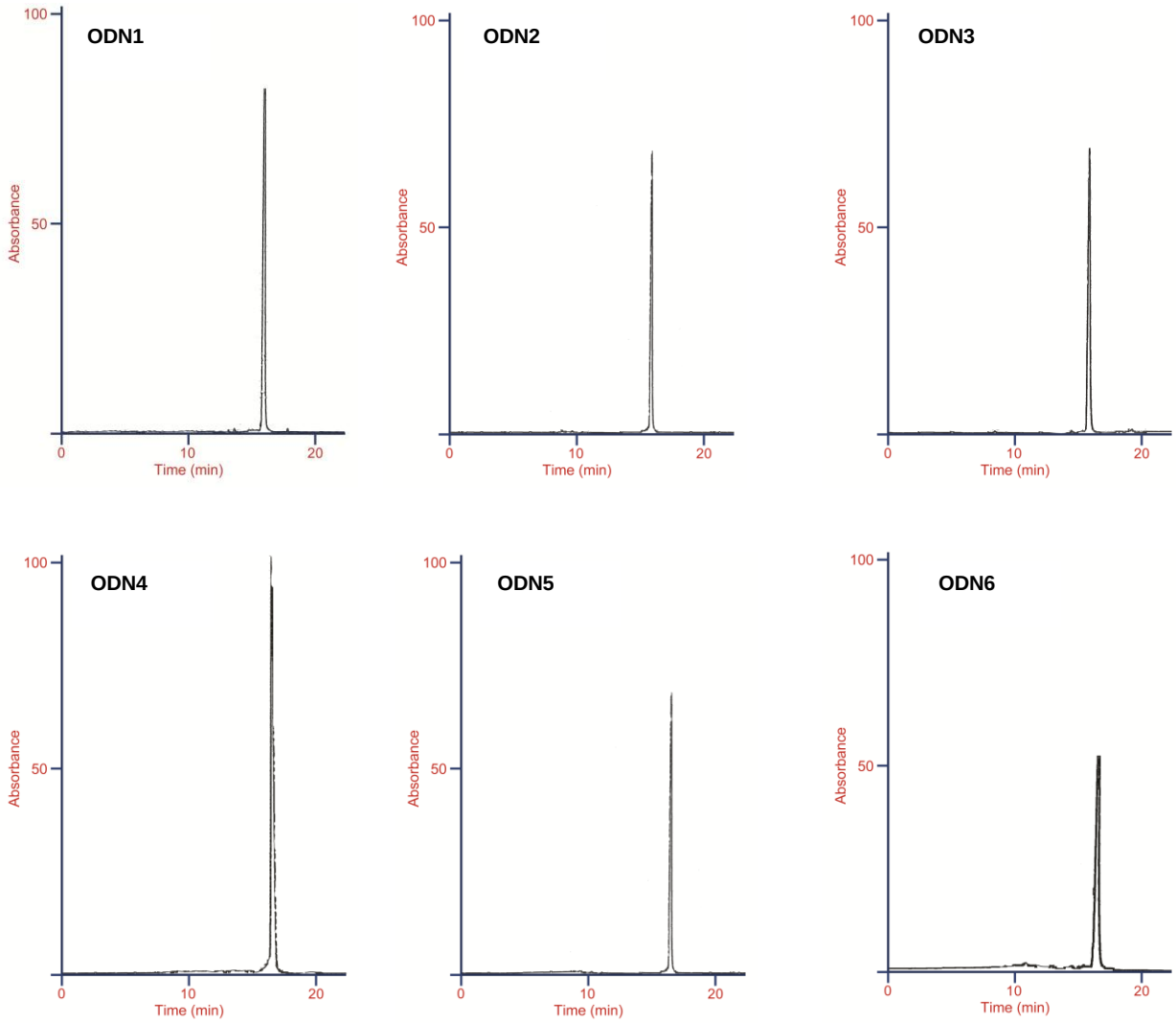


Figure S2: Analitycal SAX HPLC chromatograms of purified ODNs. Column and conditions: SAX DNAPac PA-100 column, 5µm, 4x250 mm; mobile phase A: TRIS·HCl 25 mM, pH 8.0, mobile phase B: TRIS·HCl 25mM, $NaClO_4$ 0.5M, pH 8.0. Gradient: 2-30 % B in A in 30 min. Flow rate was 1 mL/min.

Analytical Page:

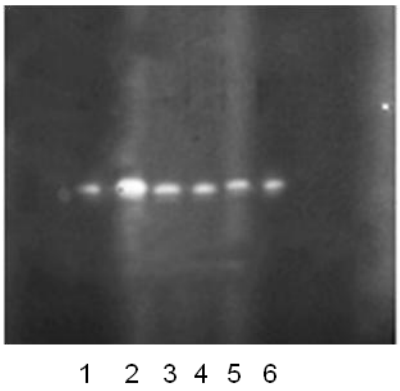
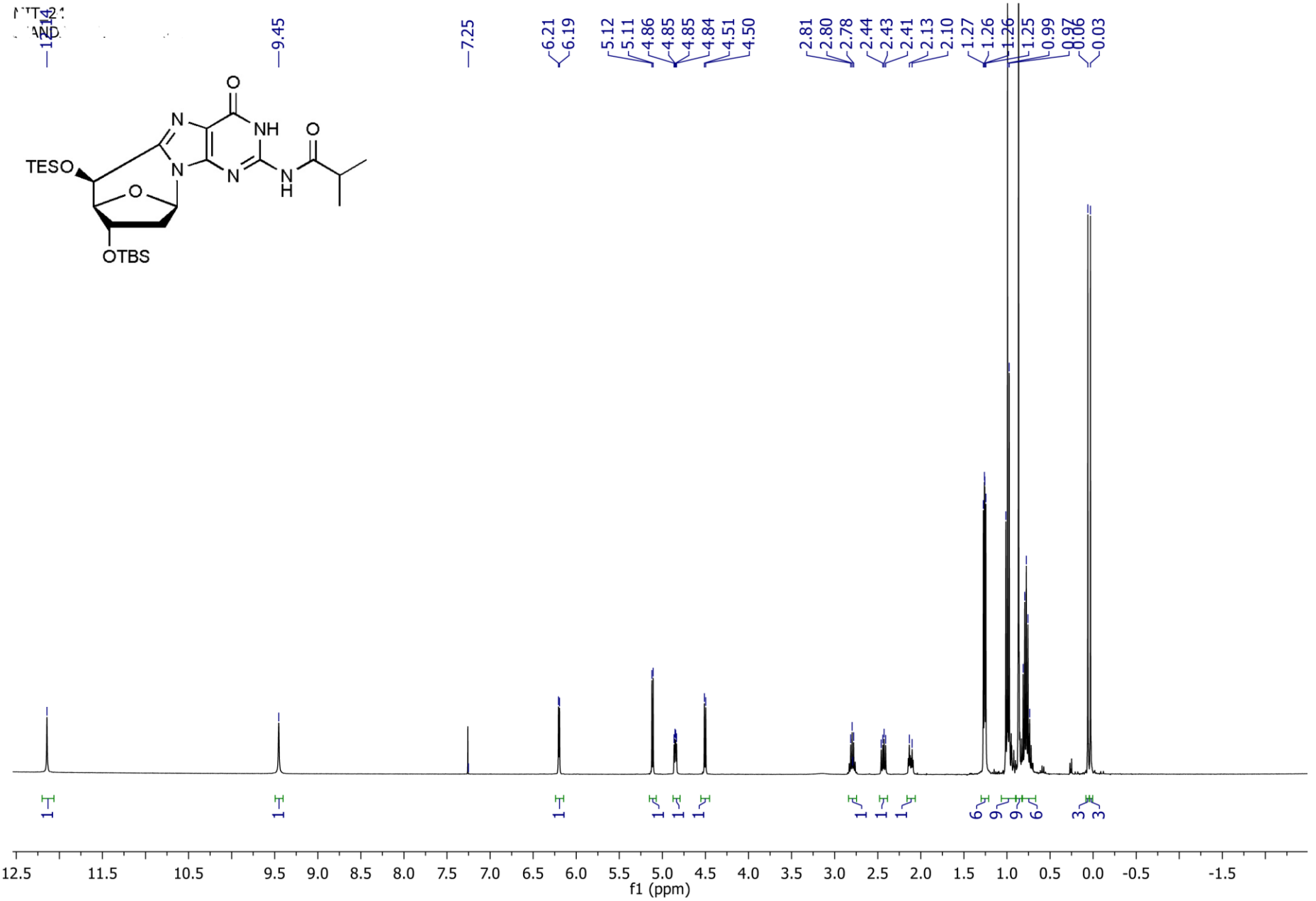


Figure S3. Analytical Page (15%) **1**:**ODN-1**, **2**: **ODN-2**, **3**: **ODN-3**, **4**: **ODN-4**, **5**: **ODN-5, 6**: **ODN-6**


TESO
OTBS
O
N
NH
O
N
N
N
H
12.14
9.45
7.25
6.21
6.19
5.12
5.11
4.86
4.85
4.85
4.84
4.51
4.50
2.81
2.80
2.78
2.44
2.43
2.41
2.13
2.10
1.27
1.26
1.26
1.25
0.99
0.97
0.06
0.03
1
1
1
1
1
1
1
1
6
9
9
6
3
3
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
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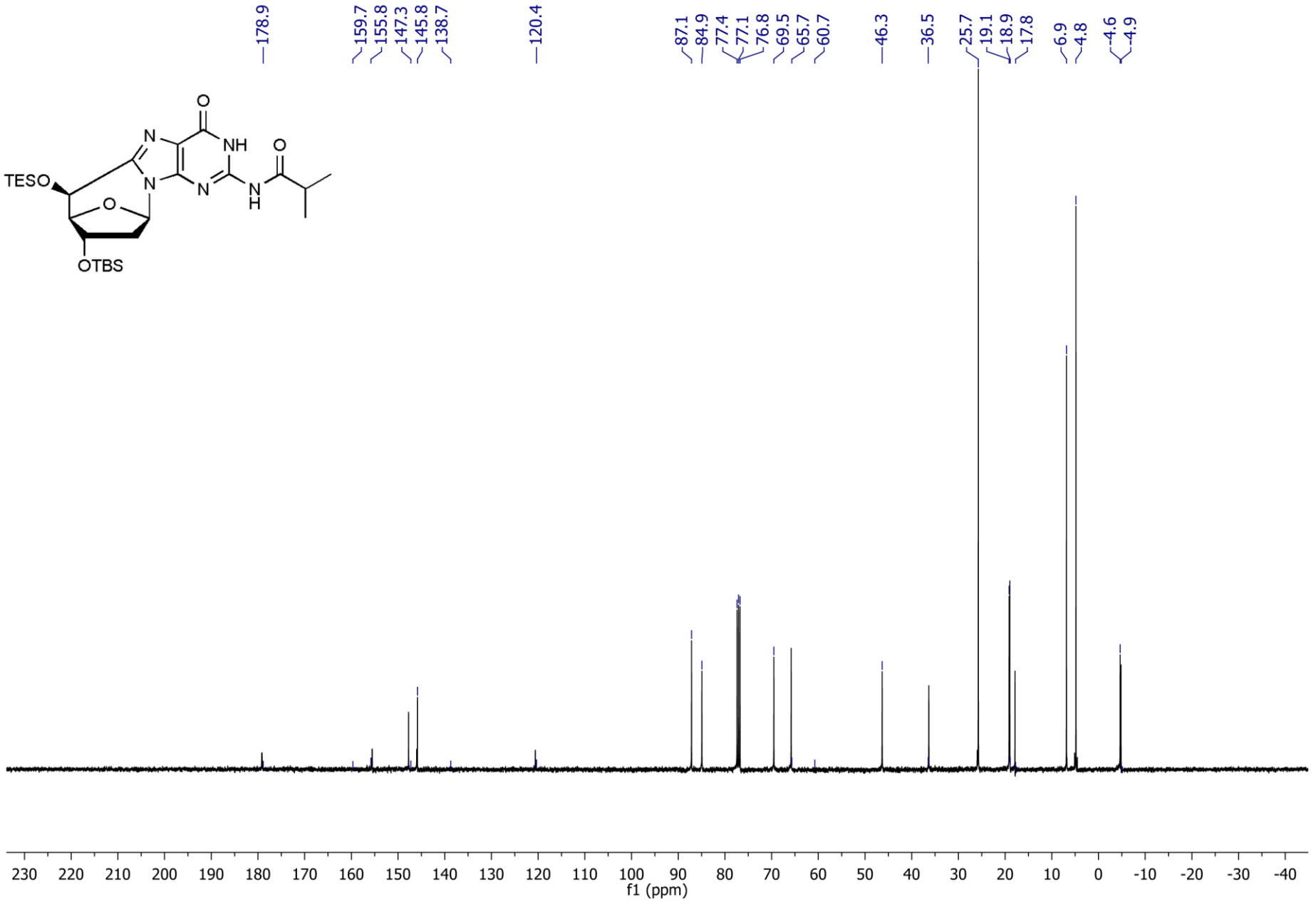
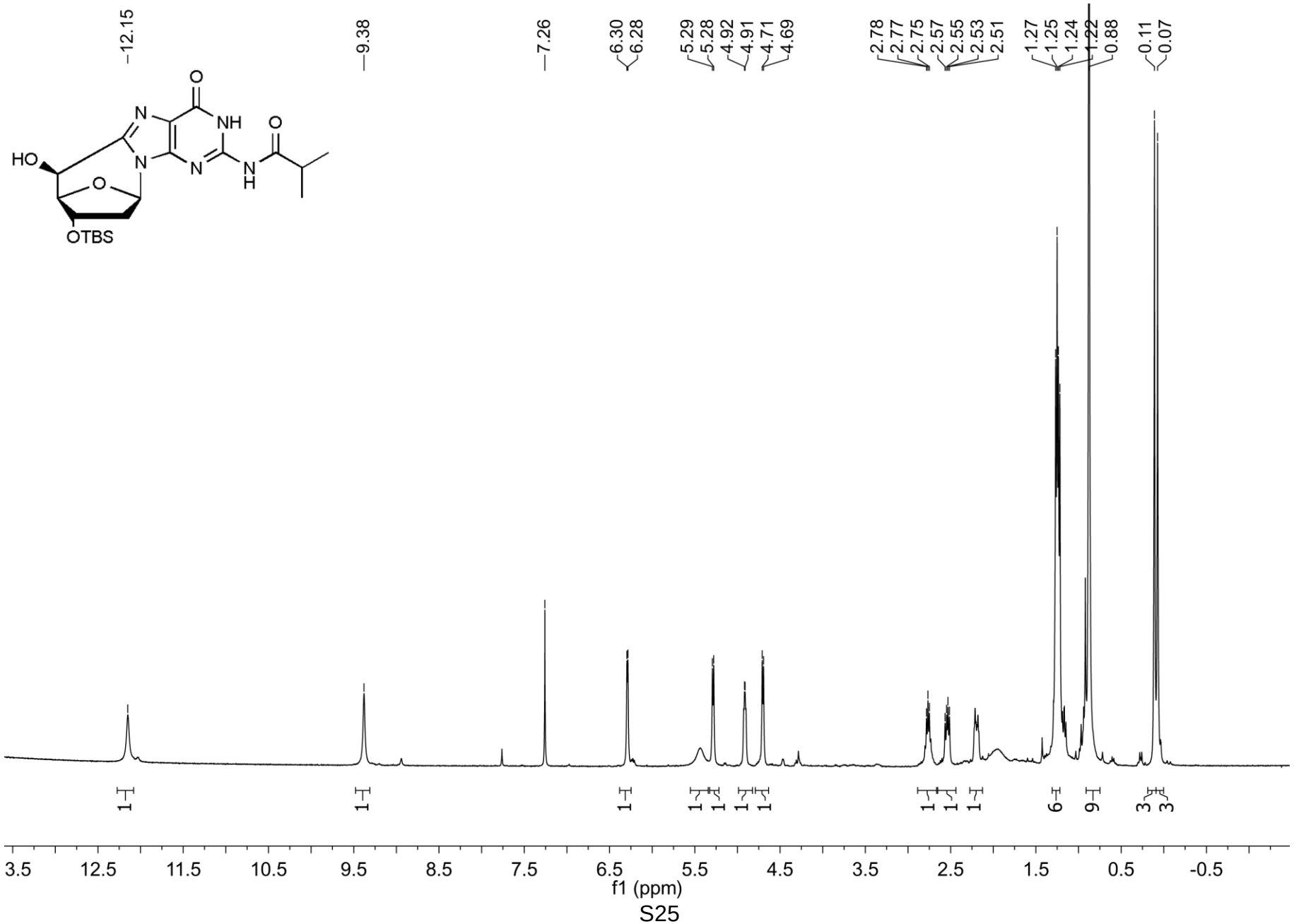
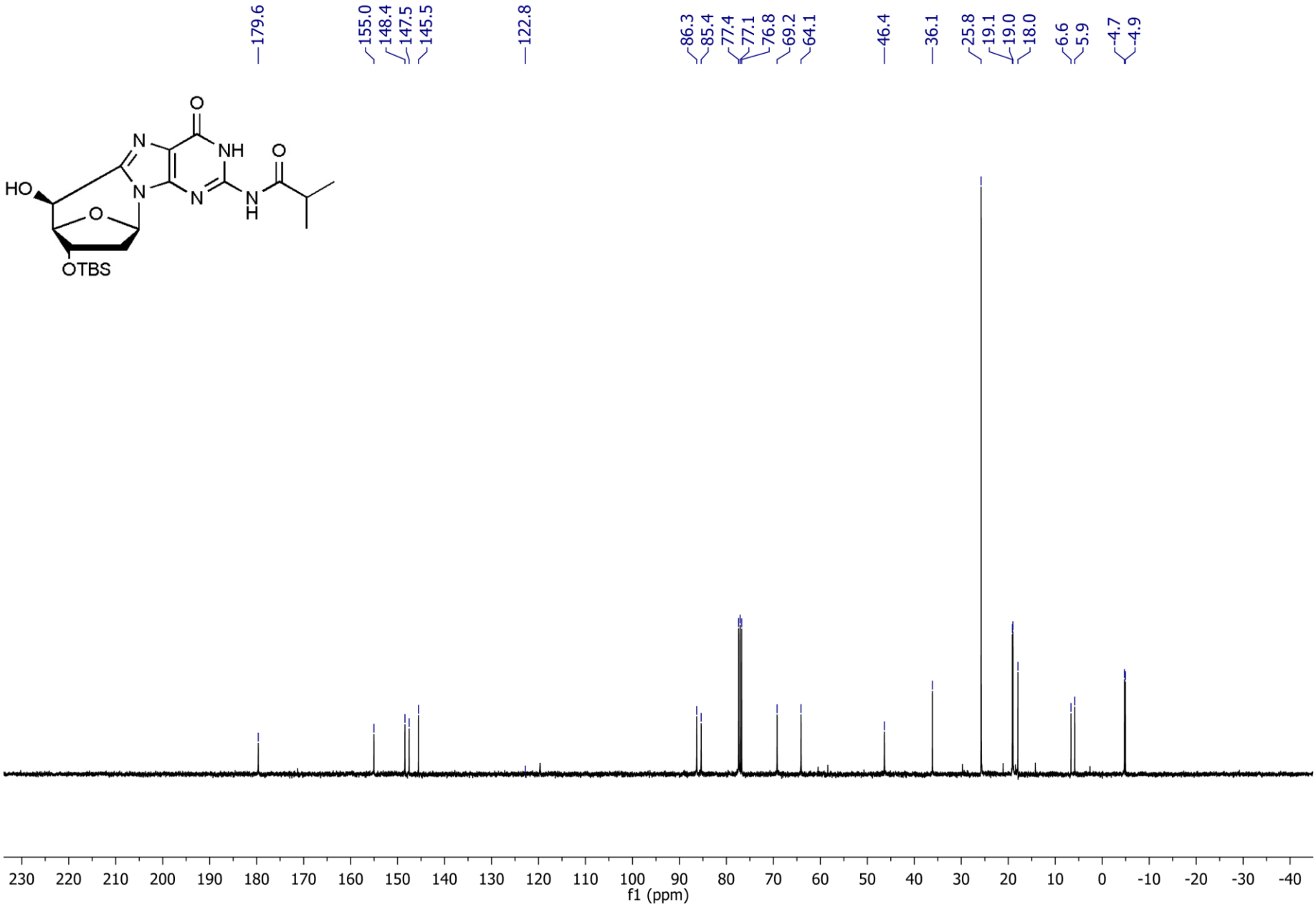
0.5
0.0
-0.5
-1.5
f1 (ppm)


178.9
159.7
155.8
147.3
145.8
138.7
120.4
87.1
84.9
77.4
77.1
76.8
69.5
65.7
60.7
46.3
36.5
25.7
19.1
18.9
17.8
6.9
4.8
-4.6
-4.9
TESO
OTBS
O
N
NH
O
N
N
N
H
O
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)


12.15
9.38
7.26
6.30
6.28
5.29
5.28
4.92
4.91
4.71
4.69
2.78
2.77
2.75
2.57
2.55
2.53
2.51
1.27
1.25
1.24
1.22
0.88
0.11
0.07
HO
O
N
NH
O
N
N
N
H
O
OTBS
1H
1H
1H
1H
1H
1H
1H
1H
1H
6H
9H
3H
3H
3.5
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)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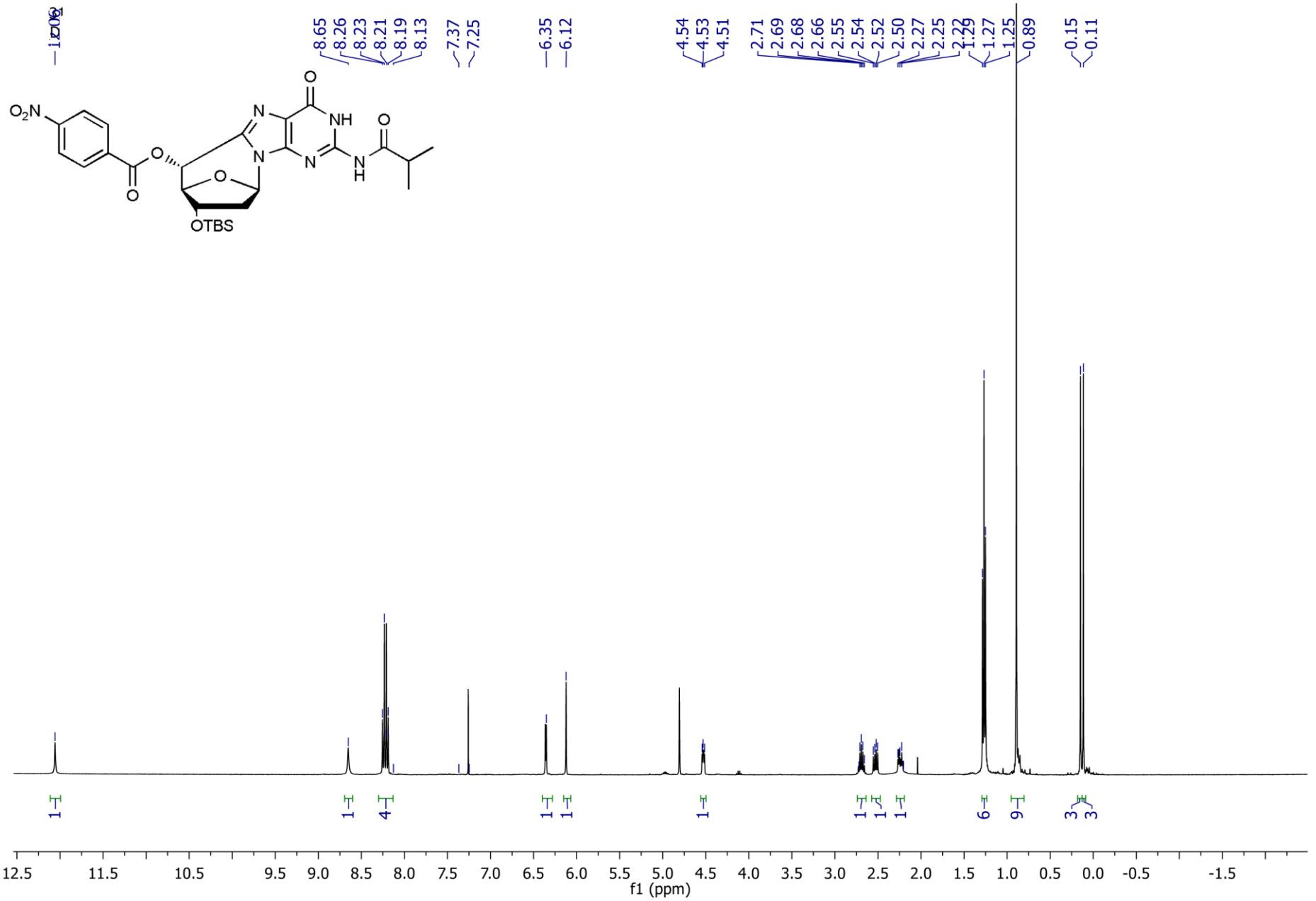
155.0
148.4
147.5
145.5
122.8
86.3
85.4
77.4
77.1
76.8
69.2
64.1
46.4
36.1
25.8
19.1
19.0
18.0
6.6
5.9
-4.7
-4.9
HO
O
N
NH
O
N
N
N
H
O
OTBS
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)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8.23
8.21
8.19
8.13
7.37
7.25
6.35
6.12
4.54
4.53
4.51
2.71
2.69
2.68
2.66
2.55
2.54
2.52
2.50
2.27
2.25
1.27
1.25
0.89
0.15
0.11
O2N
O
N
NH
O
N
N
N
H
O
O
O
OTBS
1
1
4
1
1
1
1
1
1
6
9
3
3
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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2.5
2.0
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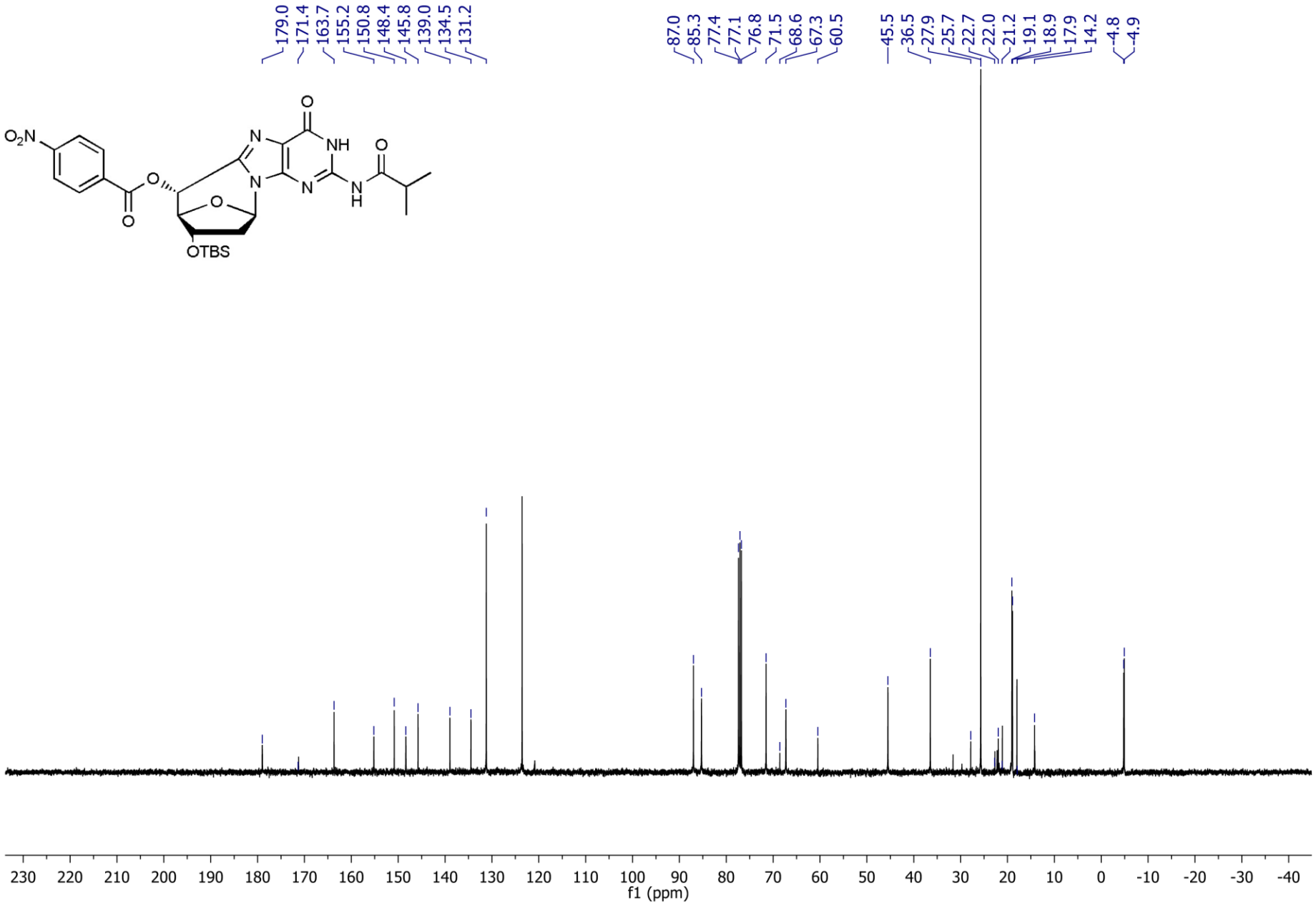
0.0
-0.5
-1.5
f1 (ppm)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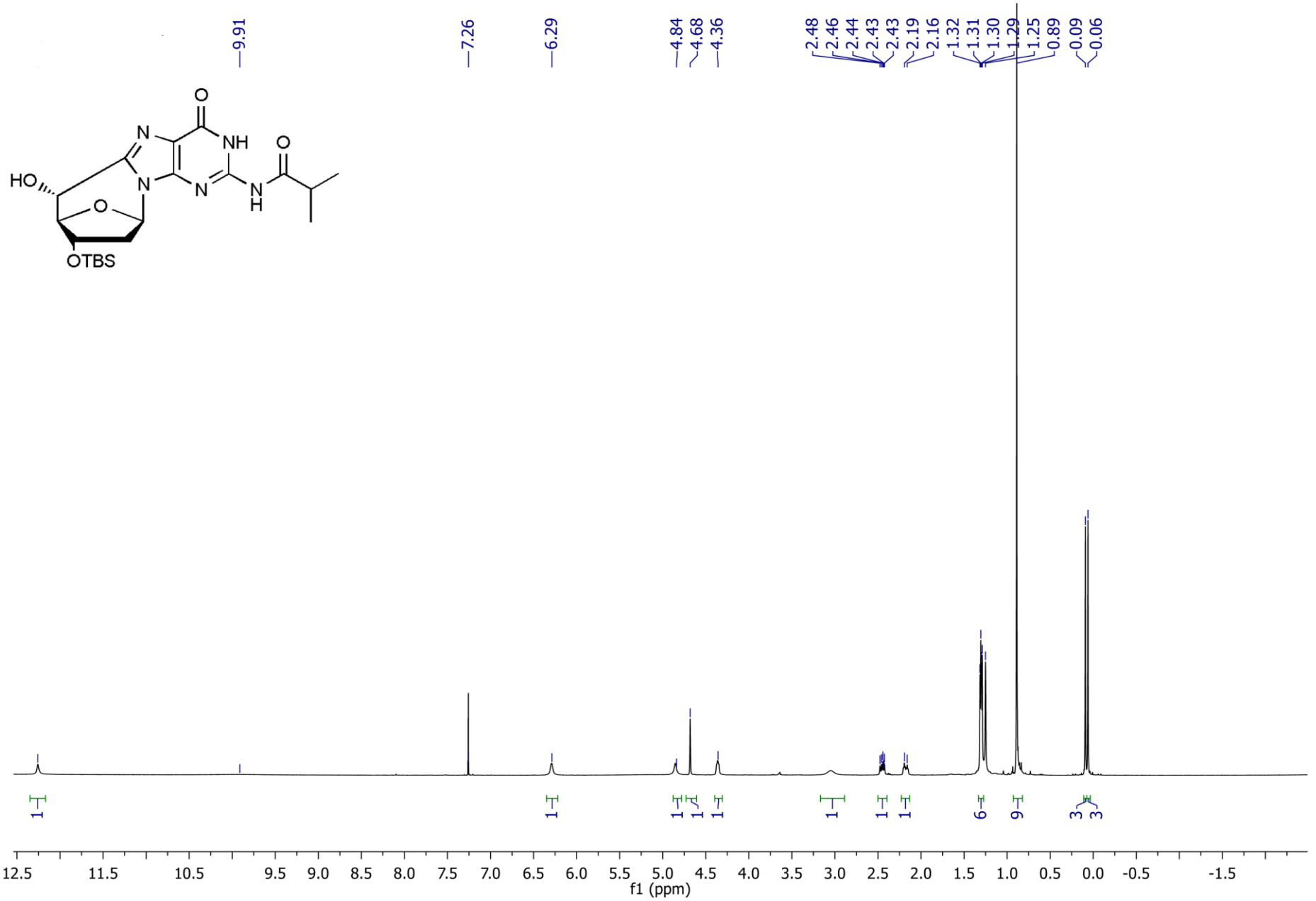
155.2
150.8
148.4
145.8
139.0
134.5
131.2
87.0
85.3
77.4
77.1
76.8
71.5
68.6
67.3
60.5
45.5
36.5
27.9
25.7
22.7
22.0
21.2
19.1
18.9
17.9
14.2
-4.8
-4.9
O2N
O
NH
N
OTBS
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)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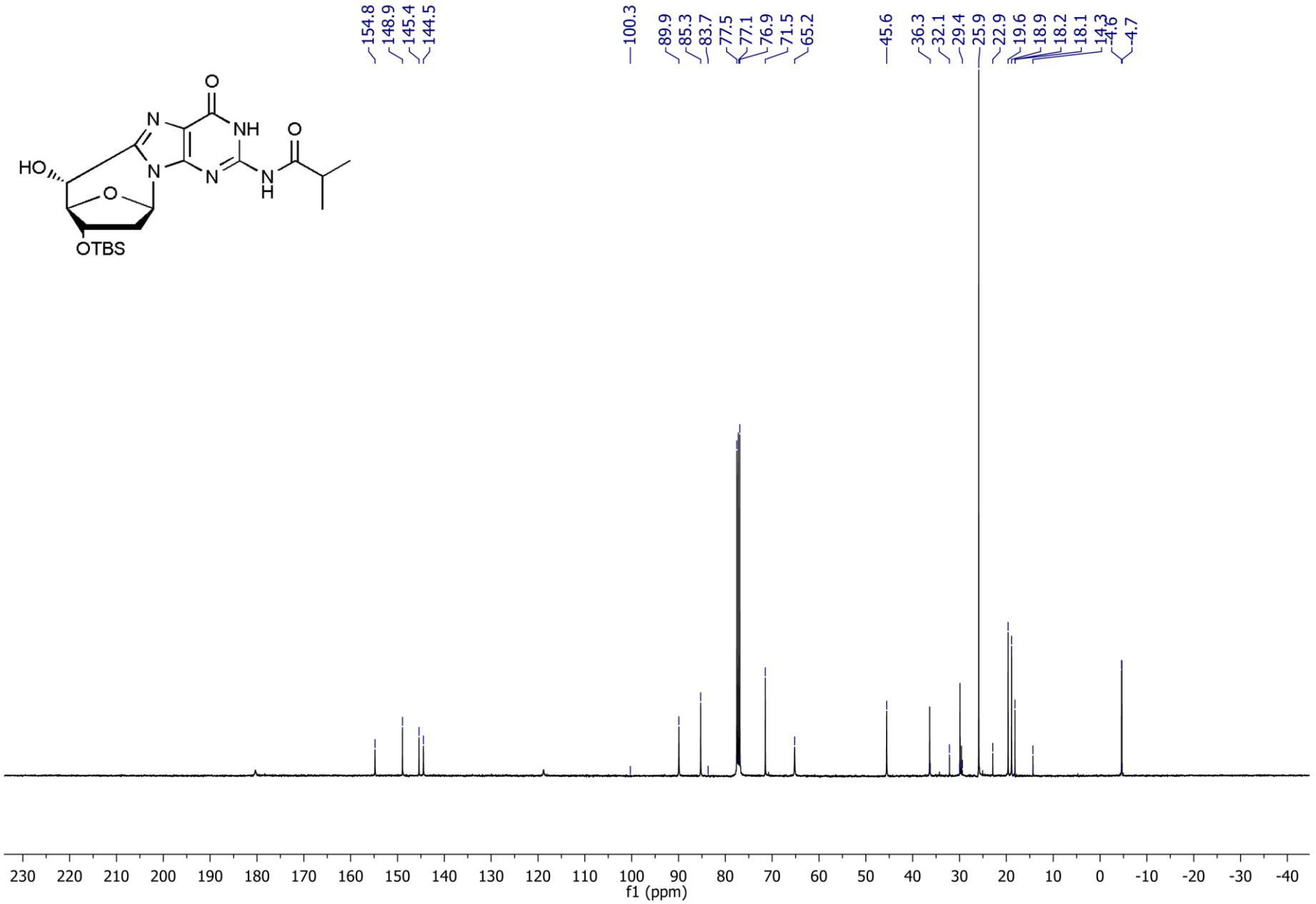
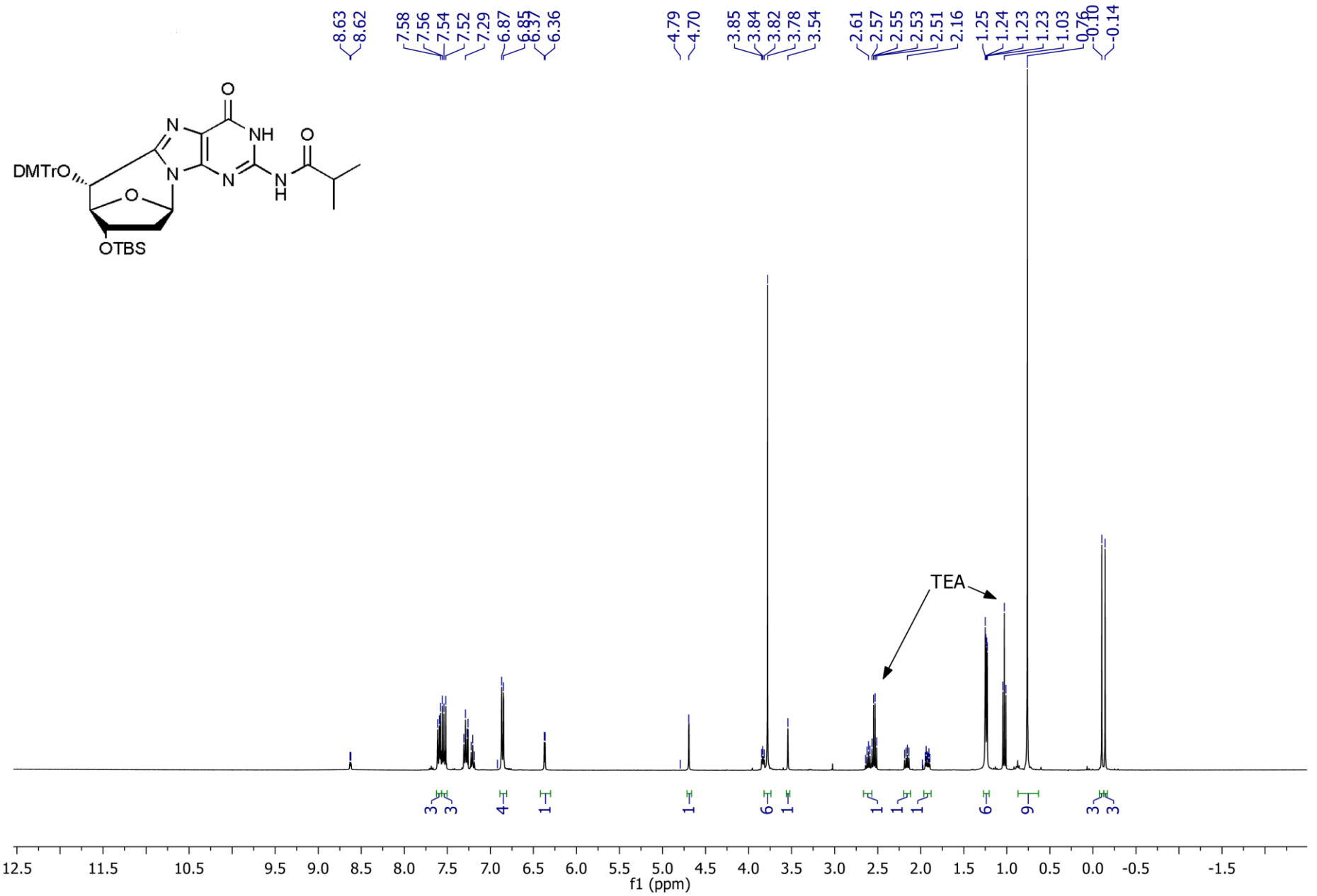
4.68
4.36
2.48
2.46
2.44
2.43
2.43
2.19
2.16
1.32
1.31
1.30
1.29
1.25
0.89
0.09
0.06
HO
O
N
NH
O
N
N
N
H
O
OTBS
1
1
1
1
1
1
1
6
9
3
3
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.5
f1 (ppm)


154.8
148.9
145.4
144.5
100.3
89.9
85.3
83.7
77.5
77.1
76.9
71.5
65.2
45.6
36.3
32.1
29.4
25.9
22.9
19.6
18.9
18.2
18.1
14.3
14.6
-4.7
HO
O
N
NH
O
N
N
N
H
O
OTBS
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)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6.36
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3.85
3.84
3.82
3.78
3.54
2.61
2.57
2.55
2.53
2.51
2.16
1.25
1.24
1.23
1.23
1.03
0.76
0.10
-0.14
DMTrO
O
N
NH
O
N
N
N
H
O
OTBS
TEA
3
3
4
1
1
6
1
1
1
1
6
9
3
3
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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2.5
2.0
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0.0
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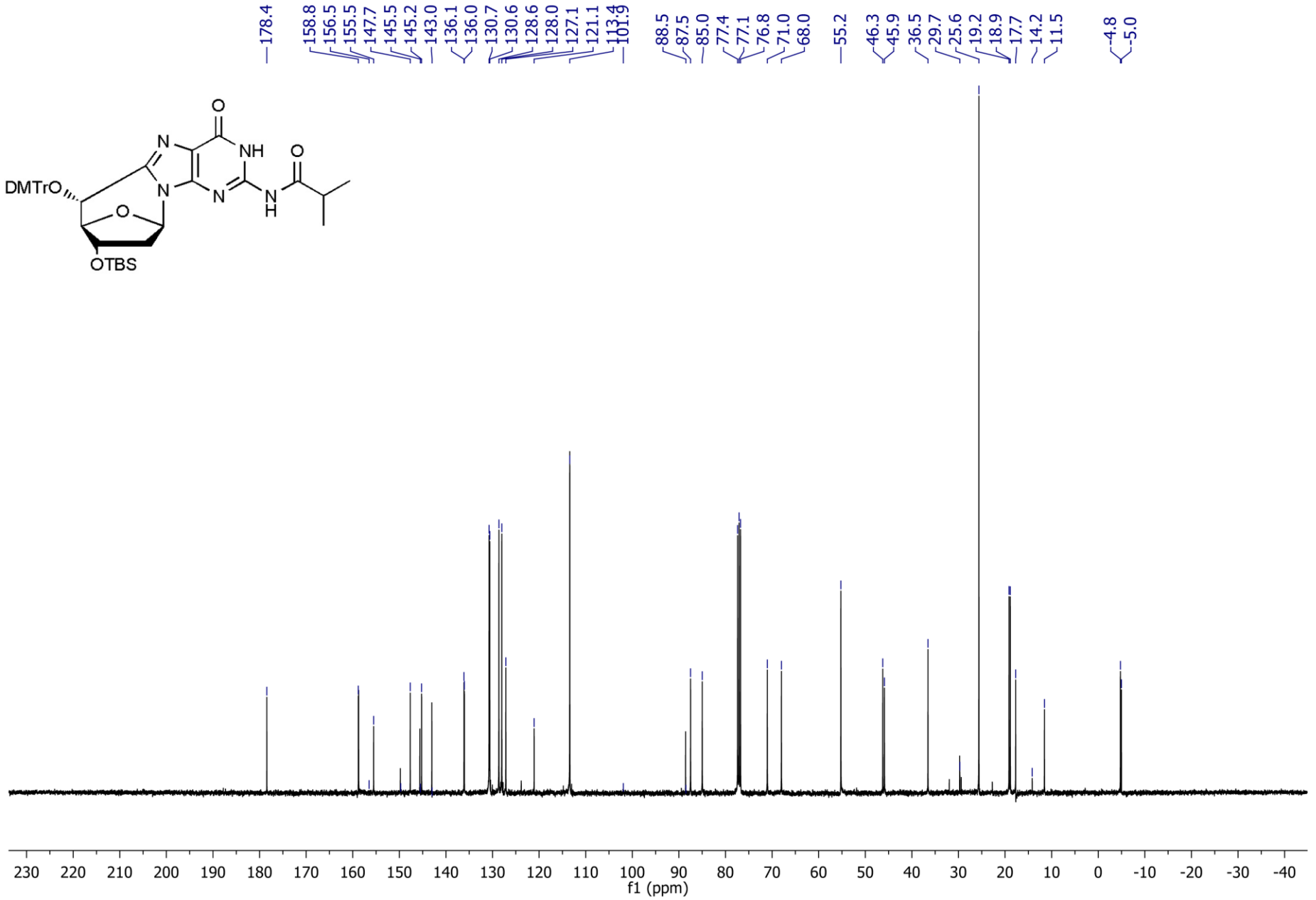
-1.5
f1 (ppm)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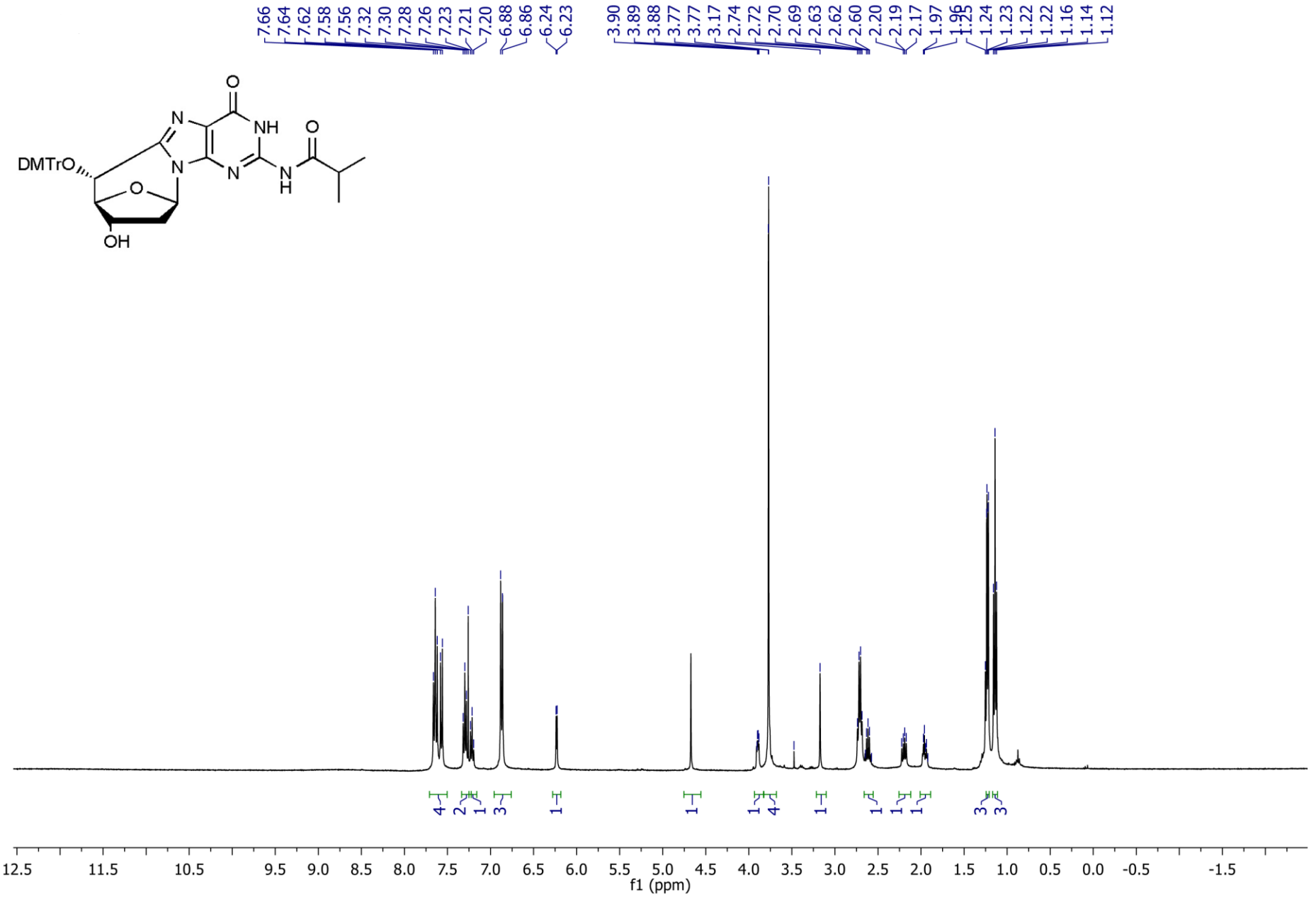
145.5
145.2
143.0
136.1
136.0
130.7
130.6
128.6
128.0
127.1
121.1
113.4
101.9
88.5
87.5
85.0
77.4
77.1
76.8
71.0
68.0
55.2
46.3
45.9
36.5
29.7
25.6
19.2
18.9
17.7
14.2
11.5
-4.8
-5.0
DMTrO
O
N
NH
O
N
N
N
H
O
OTBS
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)


7.66
7.64
7.62
7.58
7.56
7.32
7.30
7.28
7.26
7.23
7.21
7.20
6.88
6.86
6.24
6.23
3.90
3.89
3.88
3.77
3.77
3.17
2.74
2.72
2.70
2.69
2.63
2.62
2.60
2.20
2.19
2.17
1.97
1.96
1.25
1.24
1.23
1.22
1.22
1.16
1.14
1.12
DMTrO
O
N
NH
O
N
N
N
H
O
OH
4
2
1
3
1
1
1
4
1
1
1
1
3
3
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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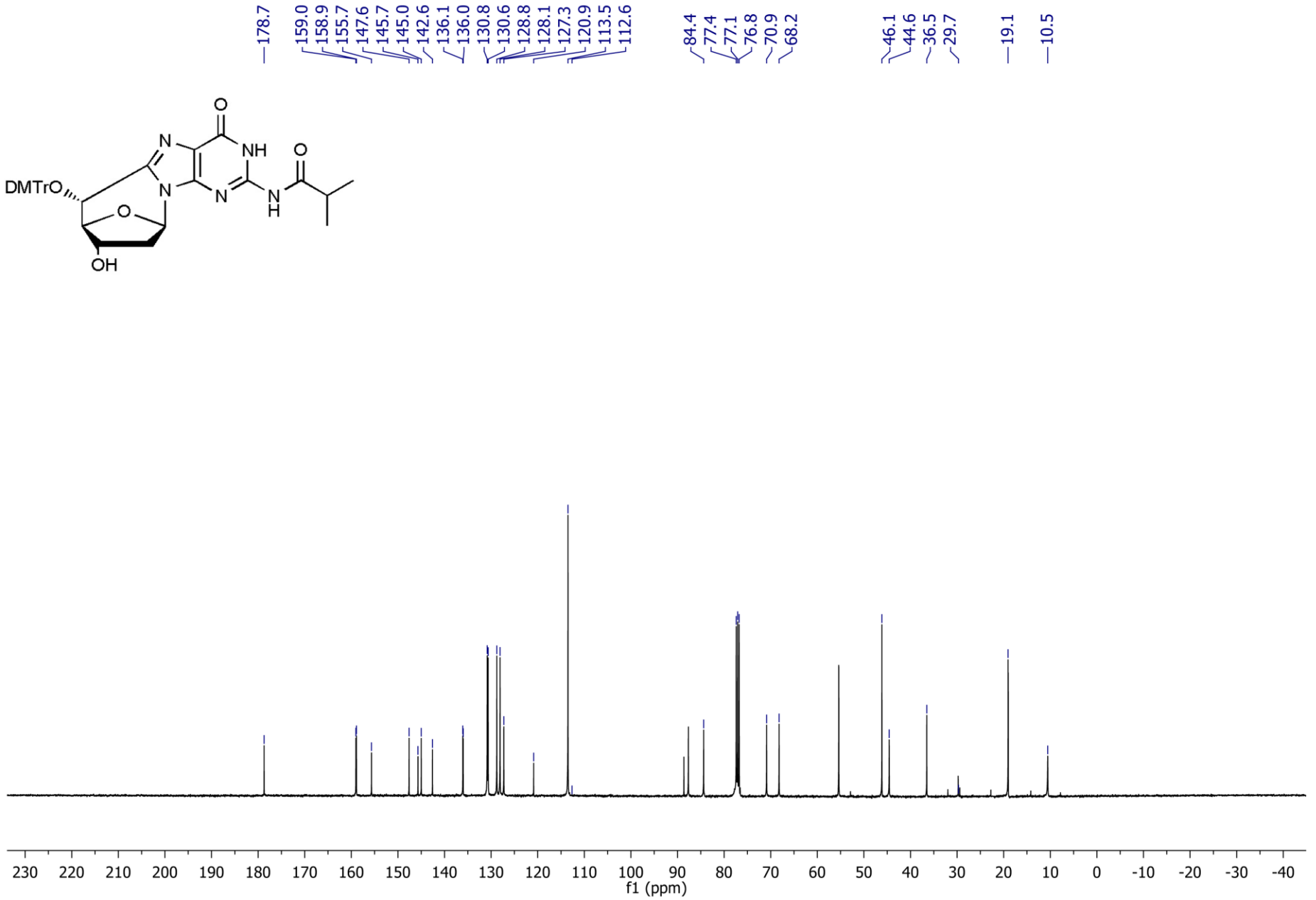
2.0
1.5
1.0
0.5
0.0
-0.5
-1.5
f1 (ppm)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155.7
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142.6
136.1
136.0
130.8
130.6
128.8
128.1
127.3
120.9
113.5
112.6
84.4
77.4
77.1
76.8
70.9
68.2
46.1
44.6
36.5
29.7
19.1
10.5
DMTrO
O
N
NH
O
N
N
N
H
O
OH
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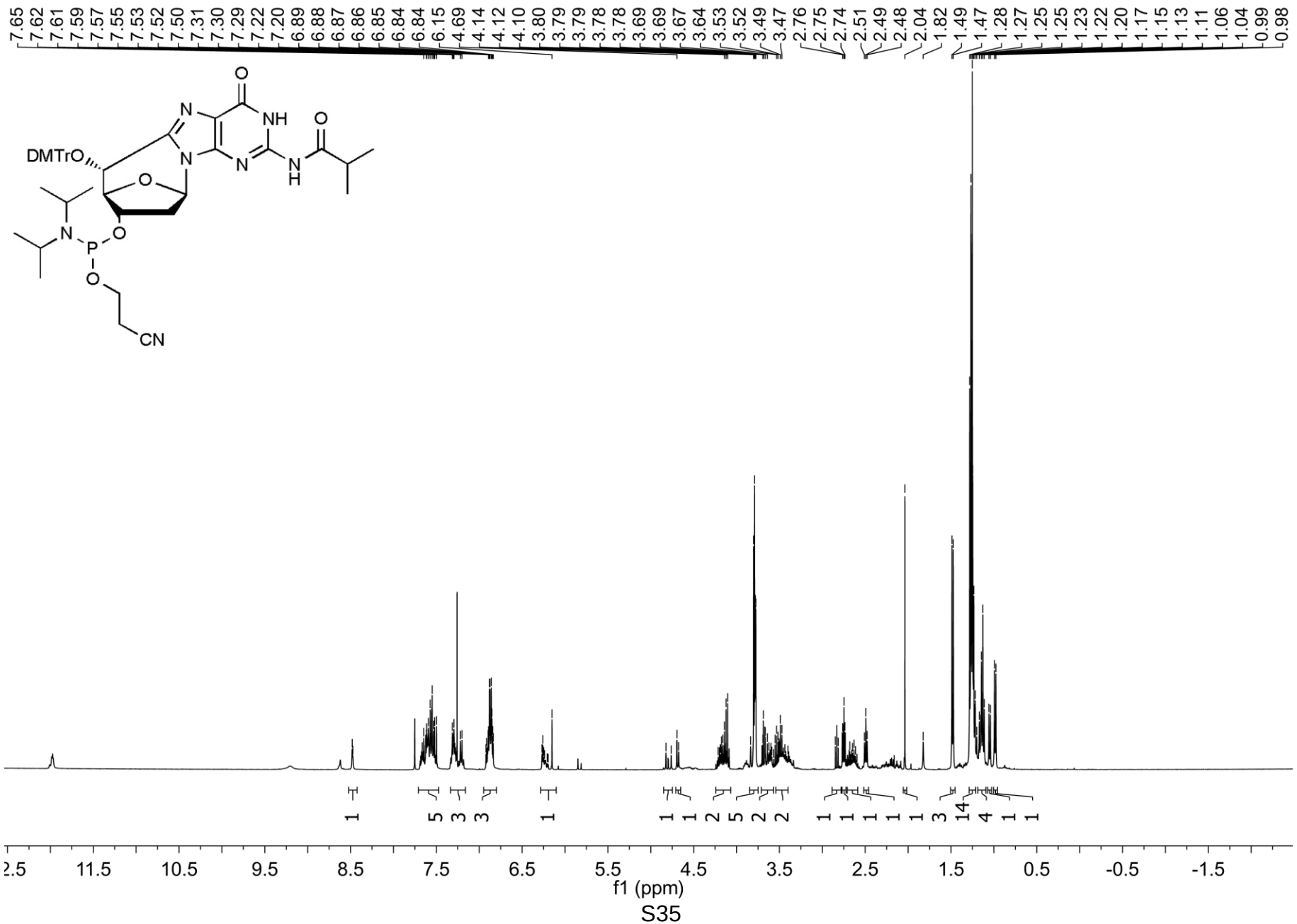
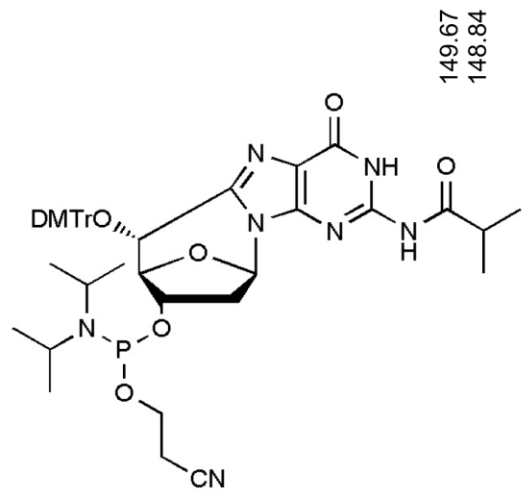
f1 (ppm)


7.65 7.62 7.61 7.59 7.57 7.55 7.53 7.52 7.50 7.31 7.30 7.29 7.22 7.20 6.89 6.88 6.87 6.86 6.85 6.84 6.84 6.15 4.69 4.14 4.12 4.10 3.80 3.79 3.79 3.78 3.78 3.69 3.69 3.67 3.64 3.53 3.52 3.49 3.47 2.76 2.75 2.74 2.51 2.49 2.48 2.04 1.82 1.49 1.47 1.28 1.27 1.25 1.25 1.23 1.22 1.20 1.17 1.15 1.13 1.11 1.06 1.04 0.99 0.98
DMTrO
O
N
NH
O
N
N
N
O
N
P
O
O
CN
1 5 3 3 1 1 1 2 5 2 2 1 1 1 1 1 3 14 4 1 1
2.5 11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5 -0.5 -1.5
f1 (ppm)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N
H
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P
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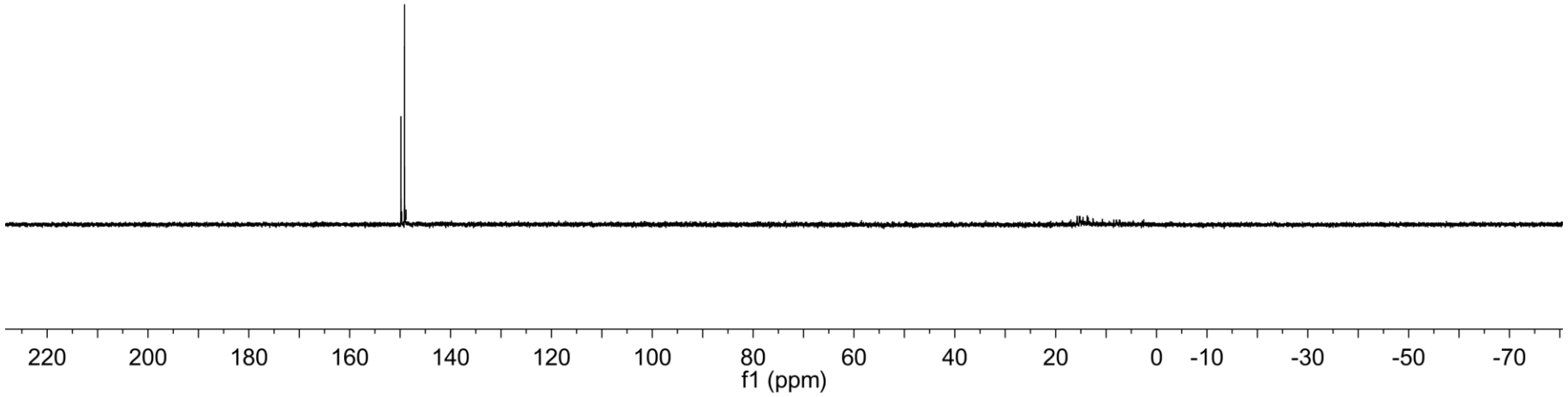
O
CN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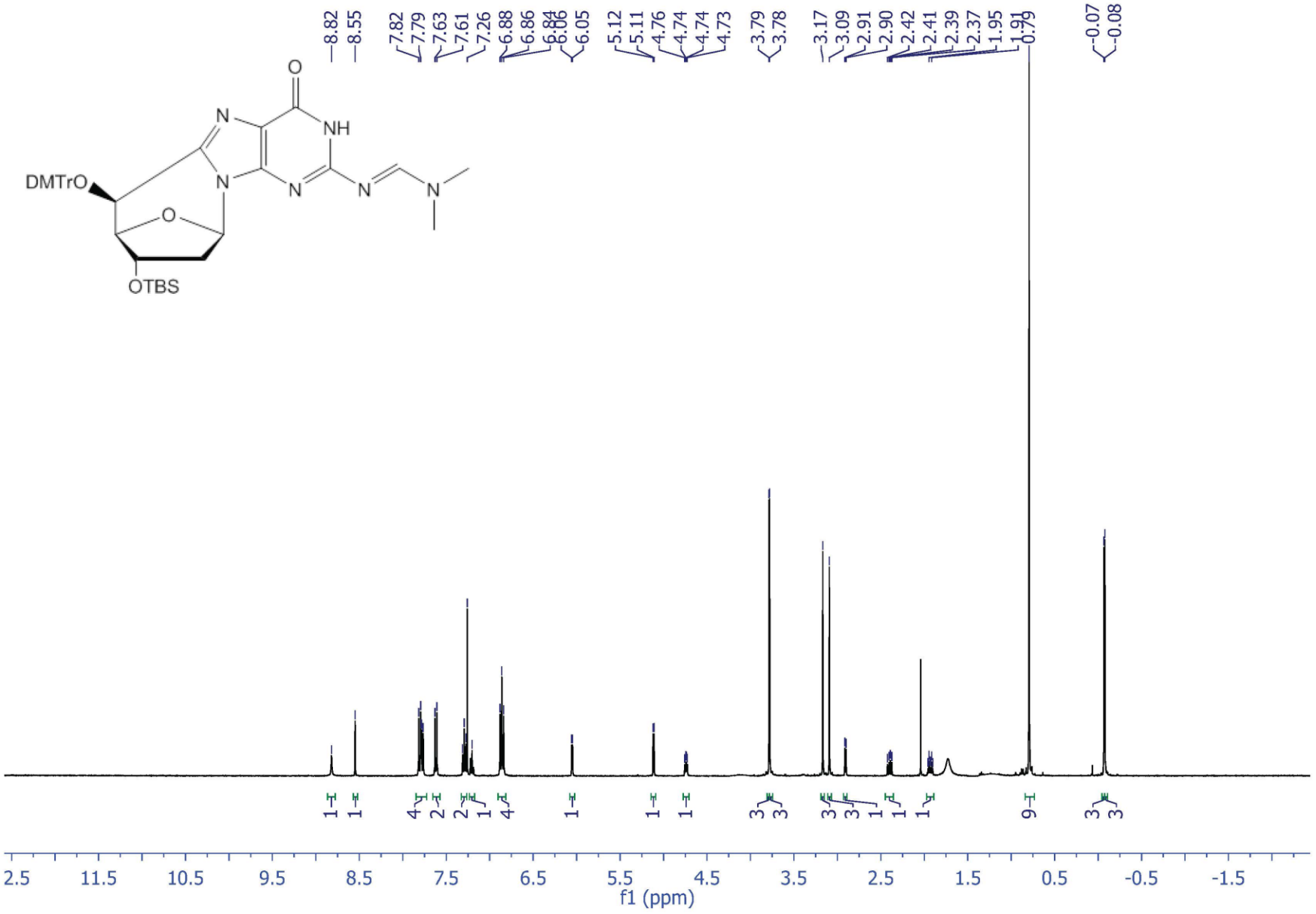
200
180
160
140
120
100
80
60
40
20
0
-10
-30
-50
-70
f1 (ppm)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7.82
7.79
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7.61
7.26
6.88
6.86
6.84
6.06
6.05
5.12
5.11
4.76
4.74
4.74
4.73
3.79
3.78
3.17
3.09
2.91
2.90
2.42
2.41
2.39
2.37
1.95
1.91
0.79
-0.07
-0.08
DMTrO
O
N
NH
N
N
N
N
O
OTBS
1
1
4
2
2
1
4
1
1
1
3
3
3
3
1
1
1
9
3
3
2.5
11.5
10.5
9.5
8.5
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4.5
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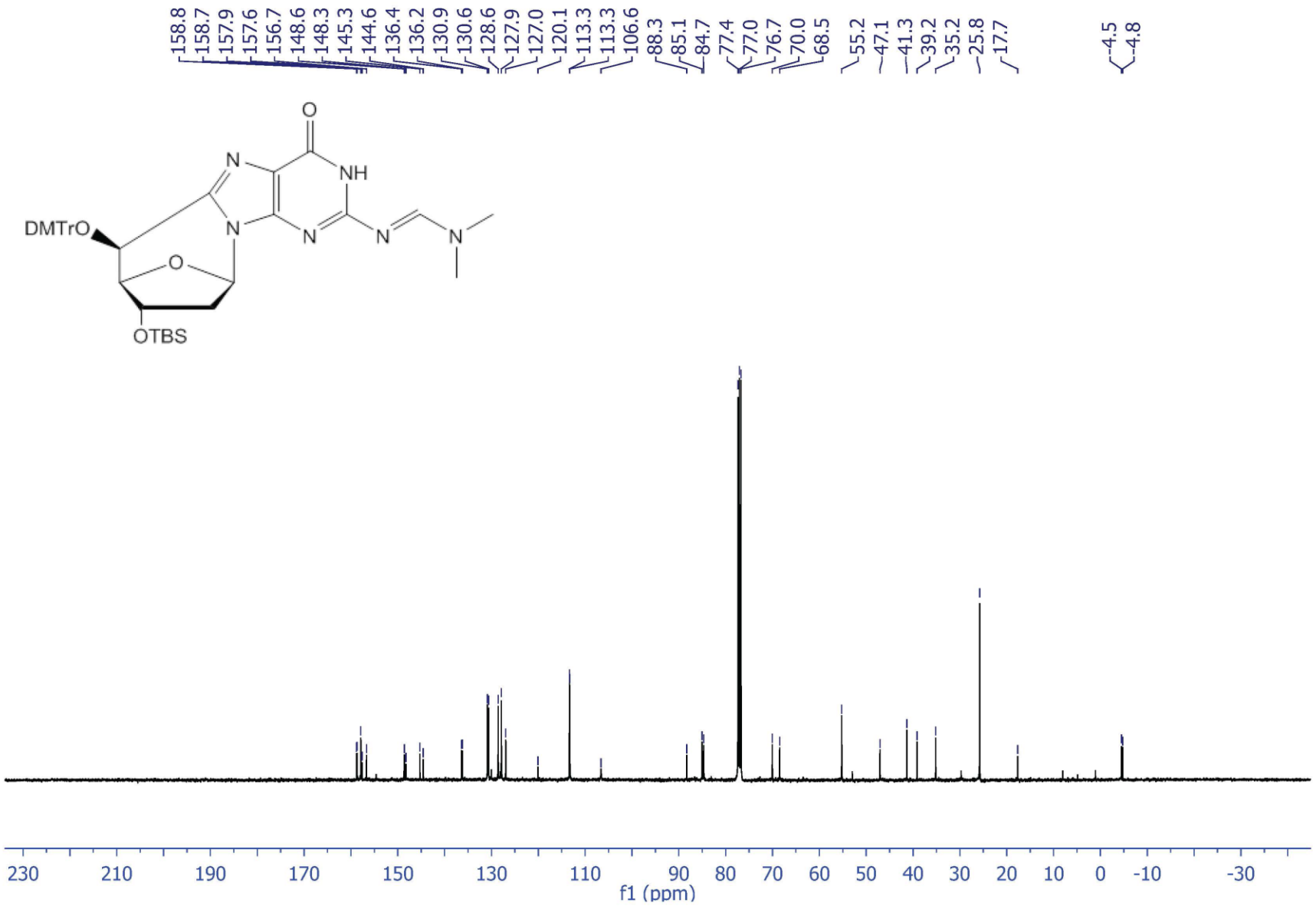
0.5
-0.5
-1.5
f1 (ppm)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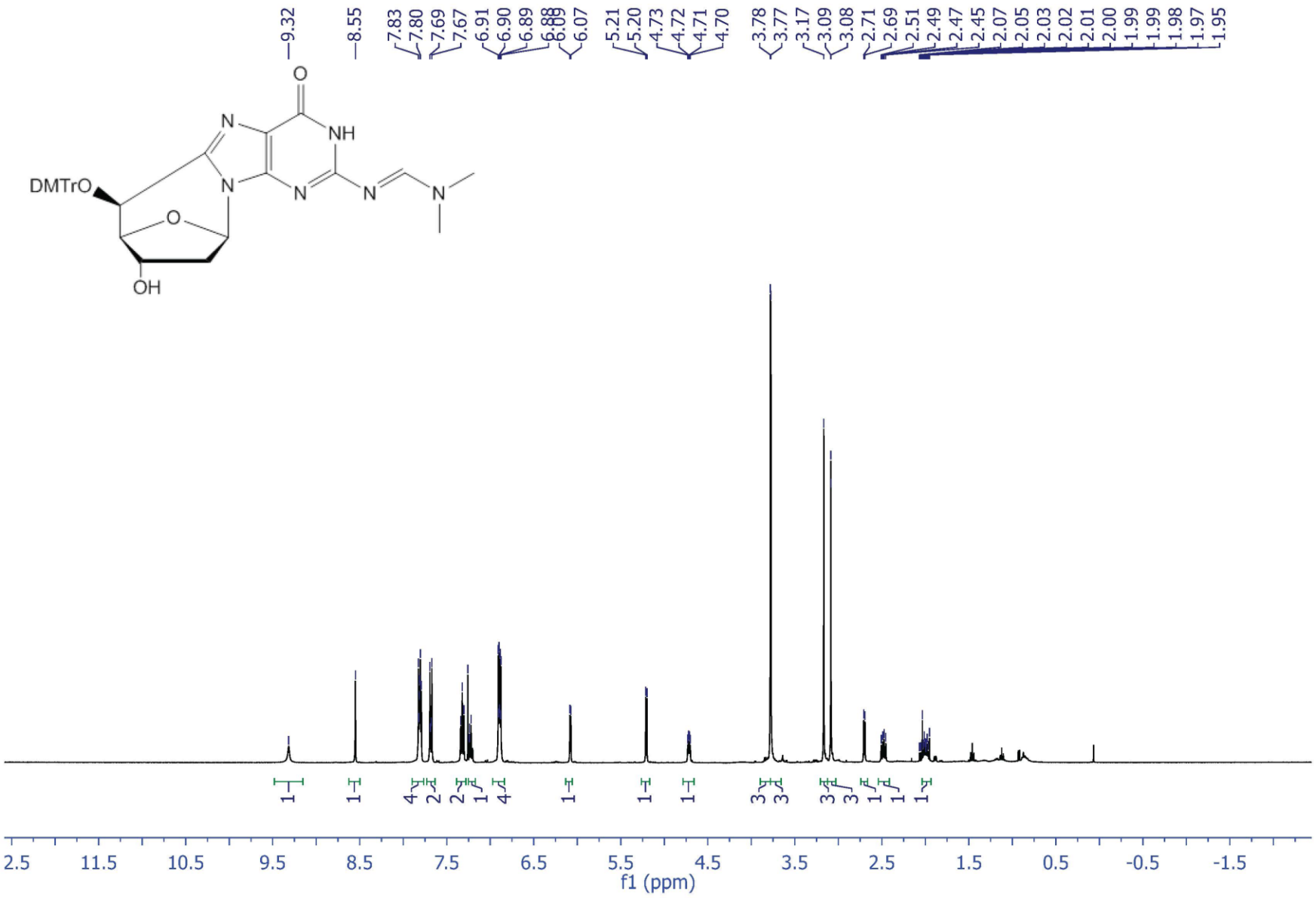
145.3
144.6
136.4
136.2
130.9
130.6
128.6
127.9
127.0
120.1
113.3
113.3
106.6
88.3
85.1
84.7
77.4
77.0
76.7
70.0
68.5
55.2
47.1
41.3
39.2
35.2
25.8
17.7
-4.5
-4.8
O
NH
N
N
N
N
N
DMTrO
O
OTBS
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)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4.72
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4.70
3.78
3.77
3.17
3.09
3.08
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2.69
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2.49
2.47
2.45
2.07
2.05
2.03
2.02
2.01
2.00
1.99
1.99
1.98
1.97
1.95
DMTrO
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NH
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OH
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3
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3
3
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2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
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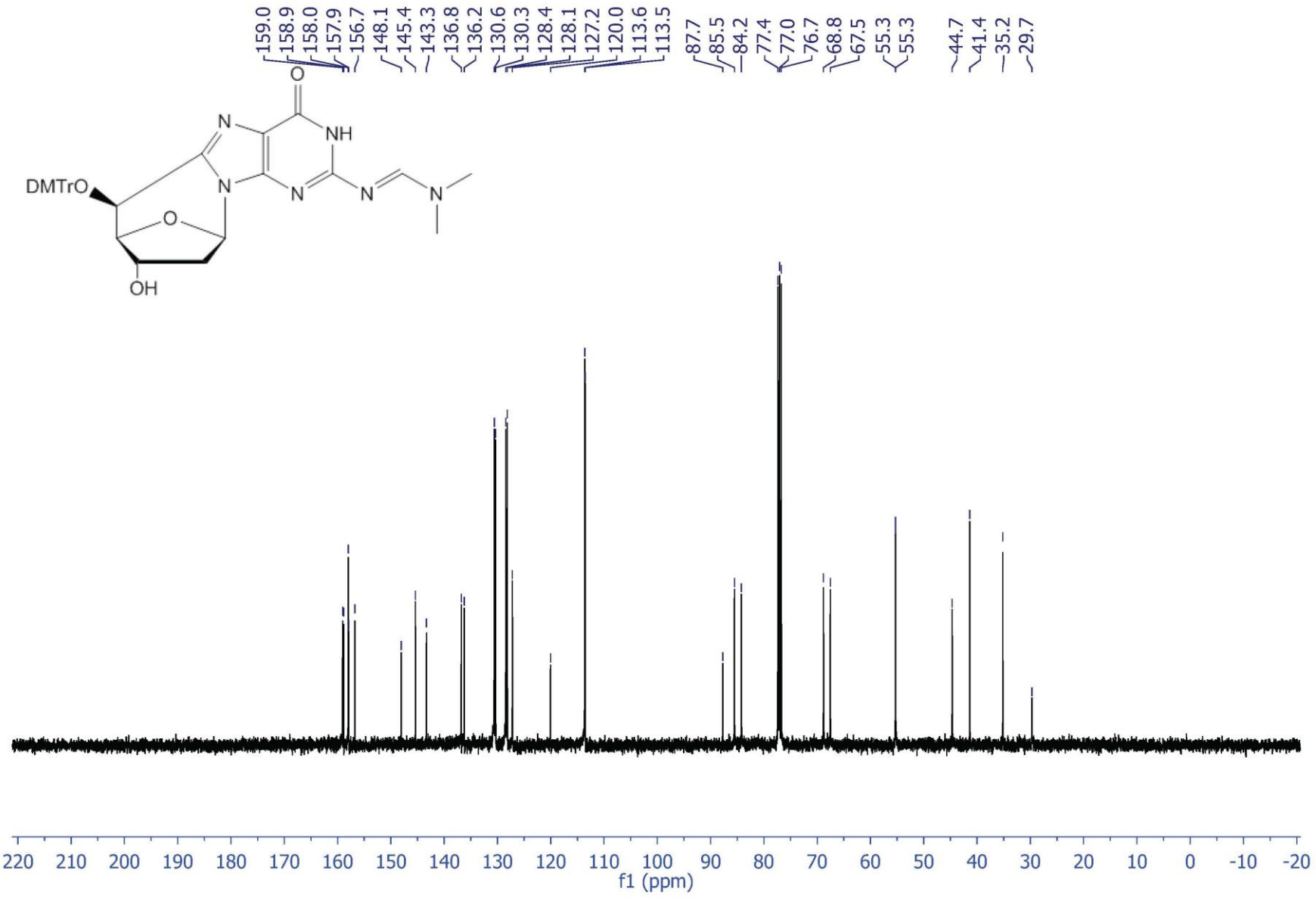
0.5
-0.5
-1.5
f1 (ppm)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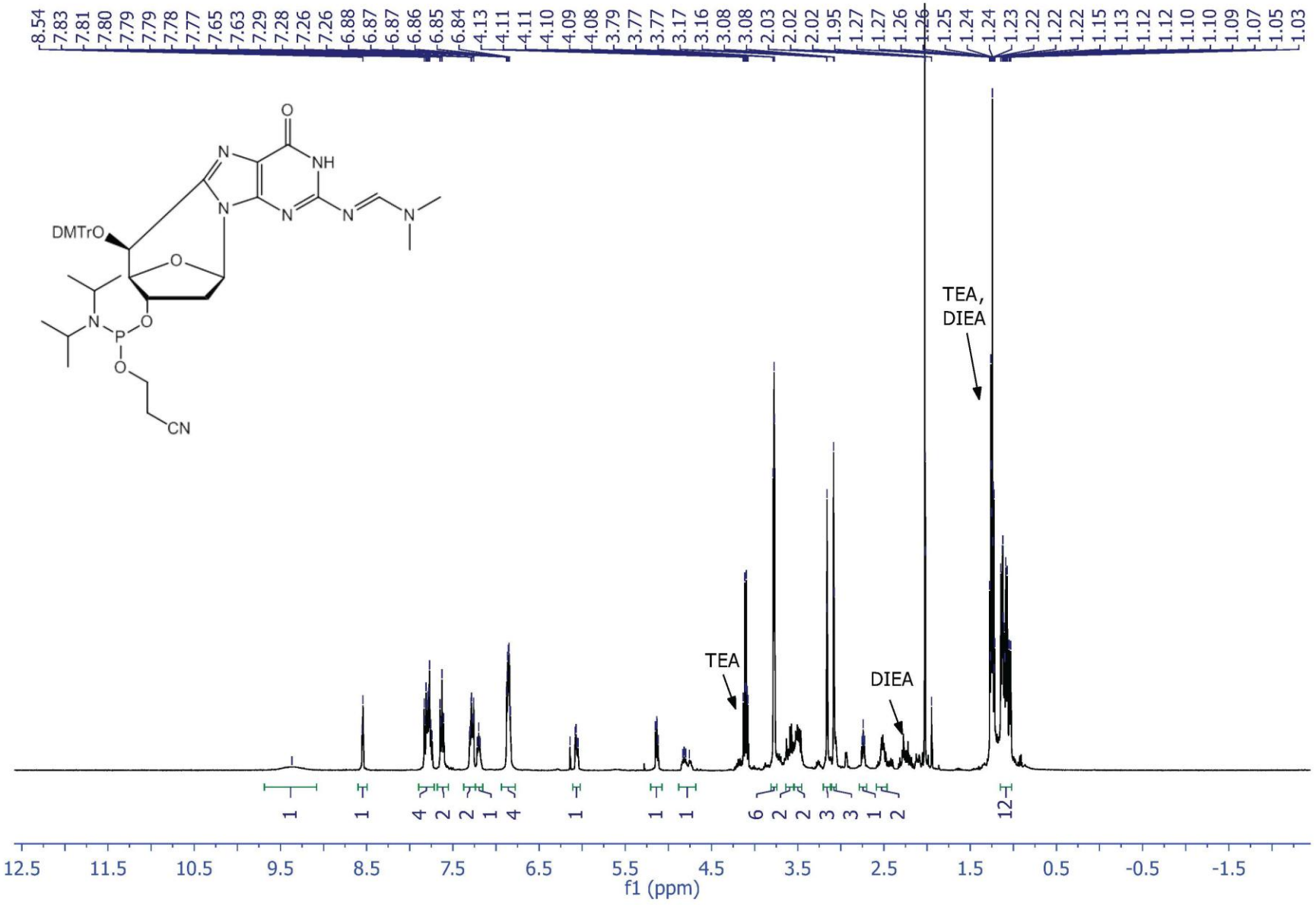
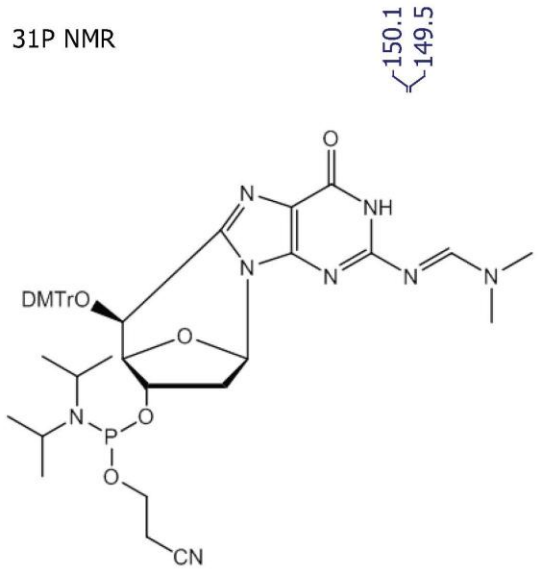
158.9
158.0
157.9
156.7
148.1
145.4
143.3
136.8
136.2
130.6
130.3
128.4
128.1
127.2
120.0
113.6
113.5
87.7
85.5
84.2
77.4
77.0
76.7
68.8
67.5
55.3
55.3
44.7
41.4
35.2
29.7
O
NH
N
N
N
N
N
DMTrO
O
OH
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


8.54 7.83 7.81 7.80 7.79 7.79 7.78 7.77 7.65 7.63 7.29 7.28 7.26 7.26 6.88 6.87 6.87 6.86 6.85 6.84 4.13 4.11 4.11 4.10 4.09 4.08 3.79 3.77 3.77 3.17 3.16 3.08 3.08 2.03 2.02 2.02 1.95 1.27 1.27 1.26 1.26 1.25 1.24 1.24 1.23 1.22 1.22 1.22 1.15 1.13 1.12 1.12 1.10 1.10 1.09 1.07 1.05 1.03
DMTrO
O
N
NH
N
N
N
N
O
N
P
O
O
CN
TEA,
DIEA
TEA
DIEA
1 1 4 2 2 1 4 1 1 1 6 2 2 3 3 1 2 12
12.5 11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5 -0.5 -1.5
f1 (ppm)

31P NMR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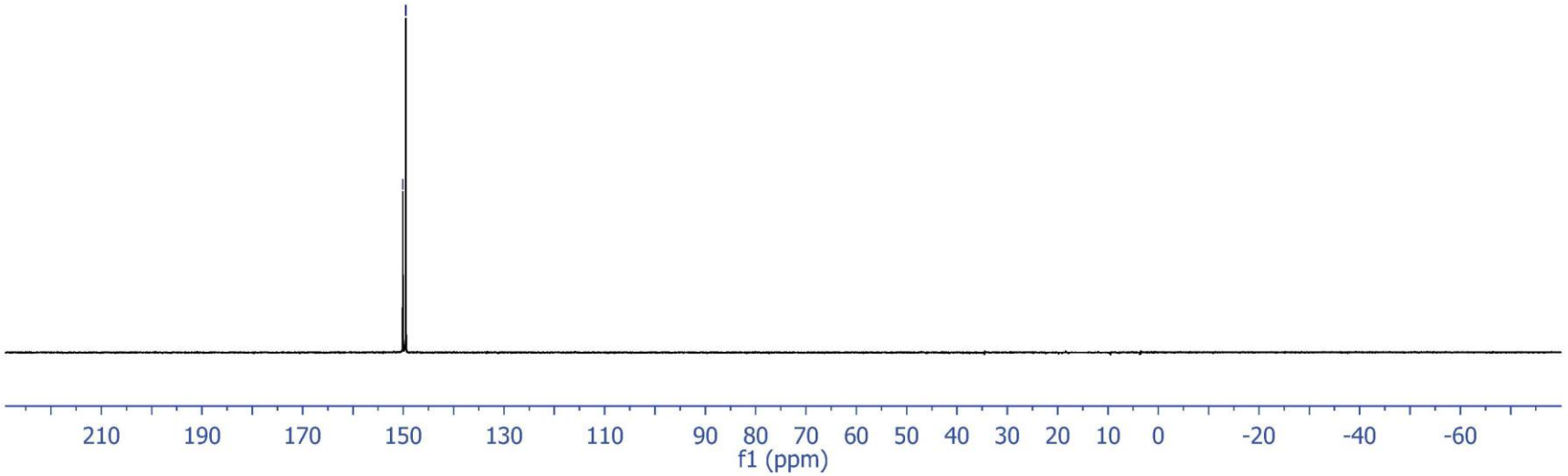
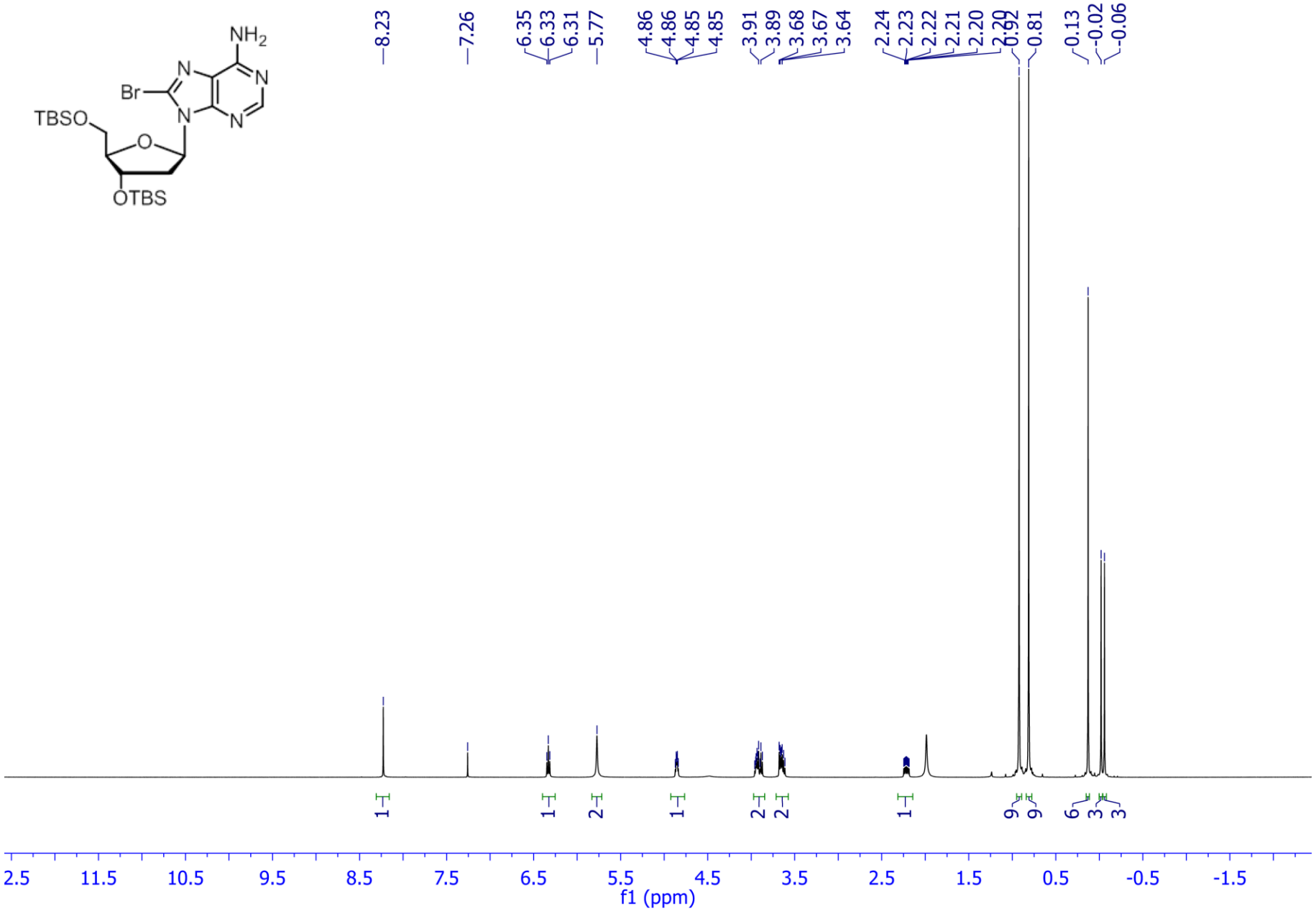
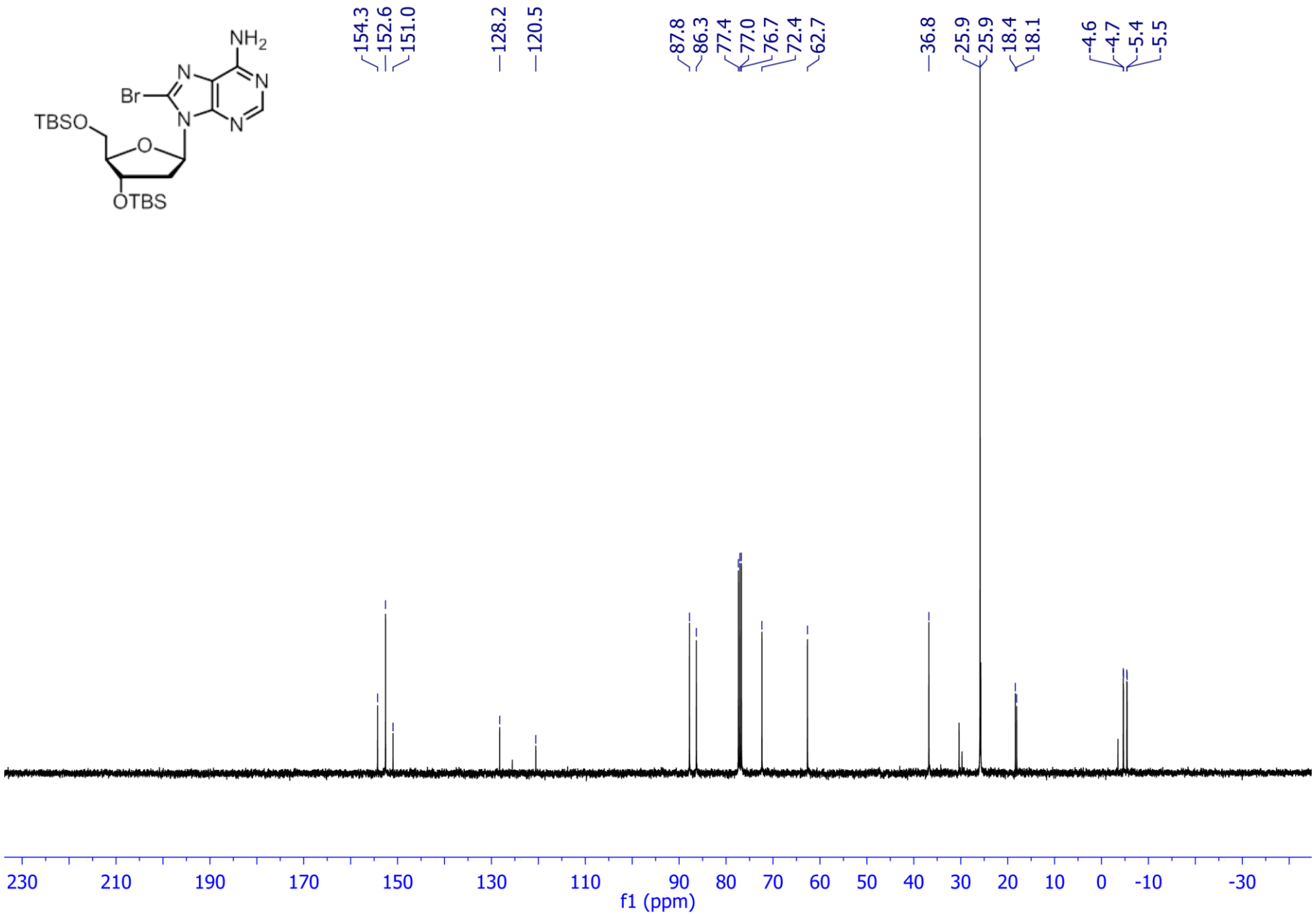
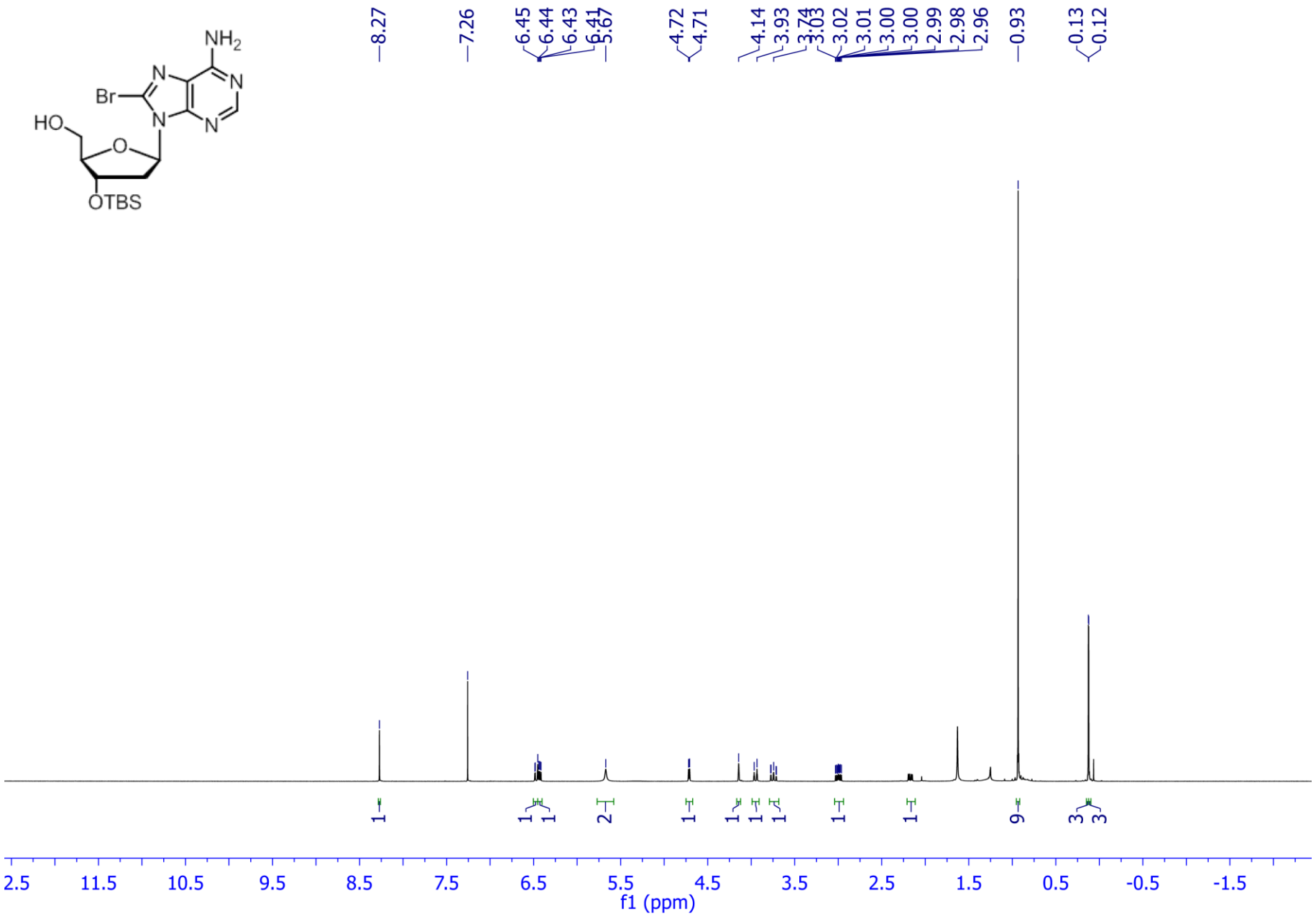
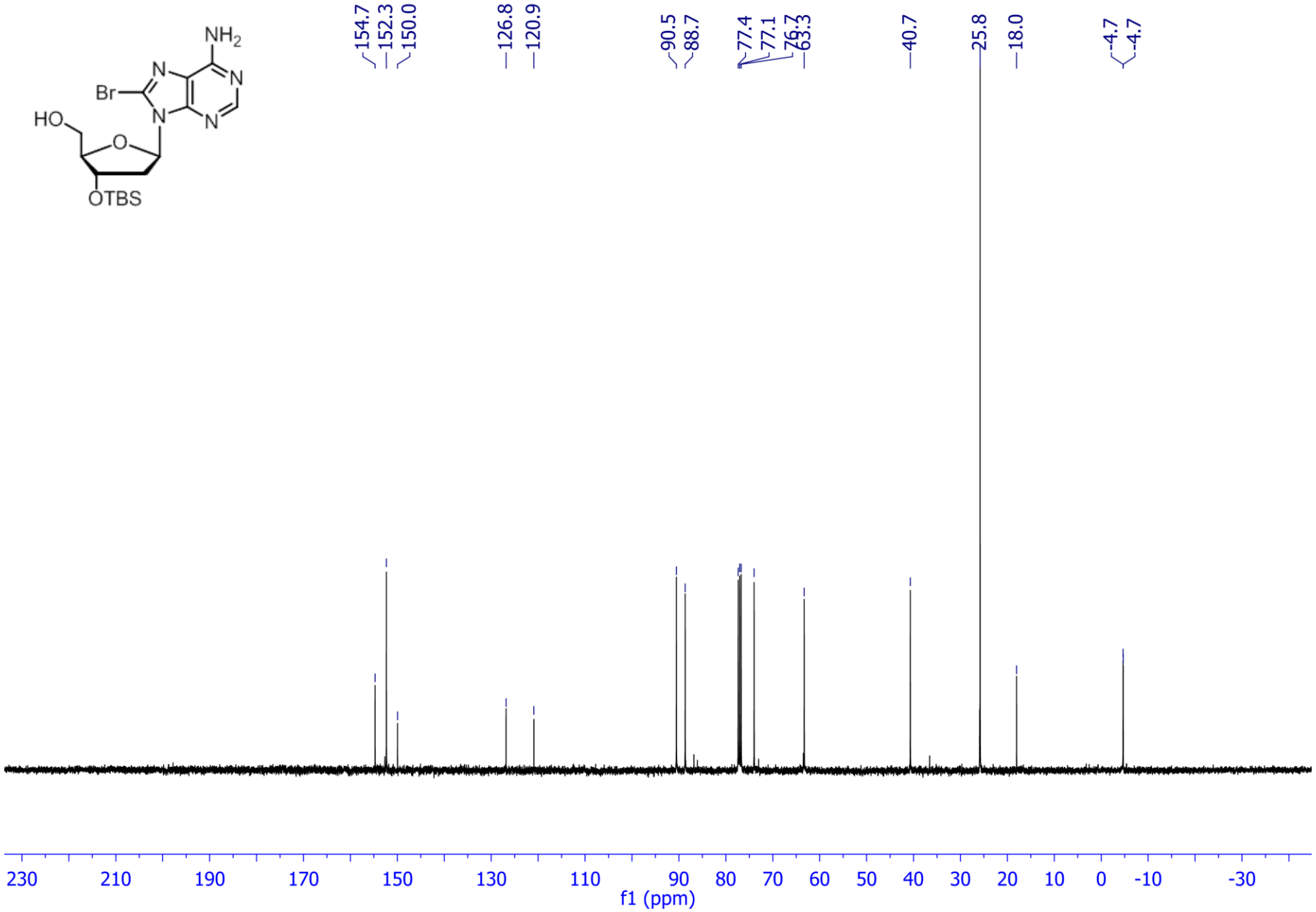
149.5
O
N
NH
N
N
N
N
DMTrO
O
N
P
O
O
CN

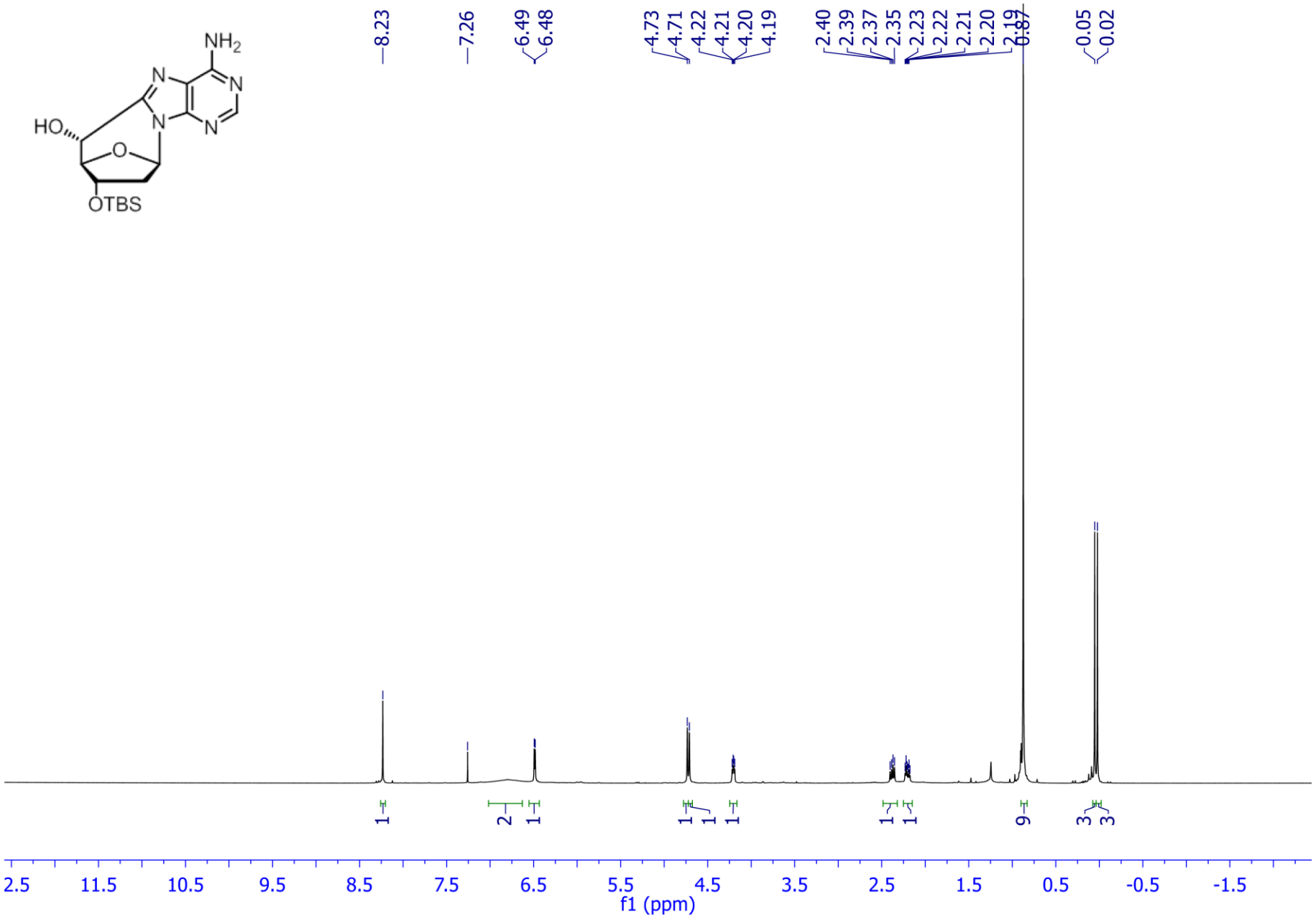

210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-20
-40
-60
f1 (ppm)

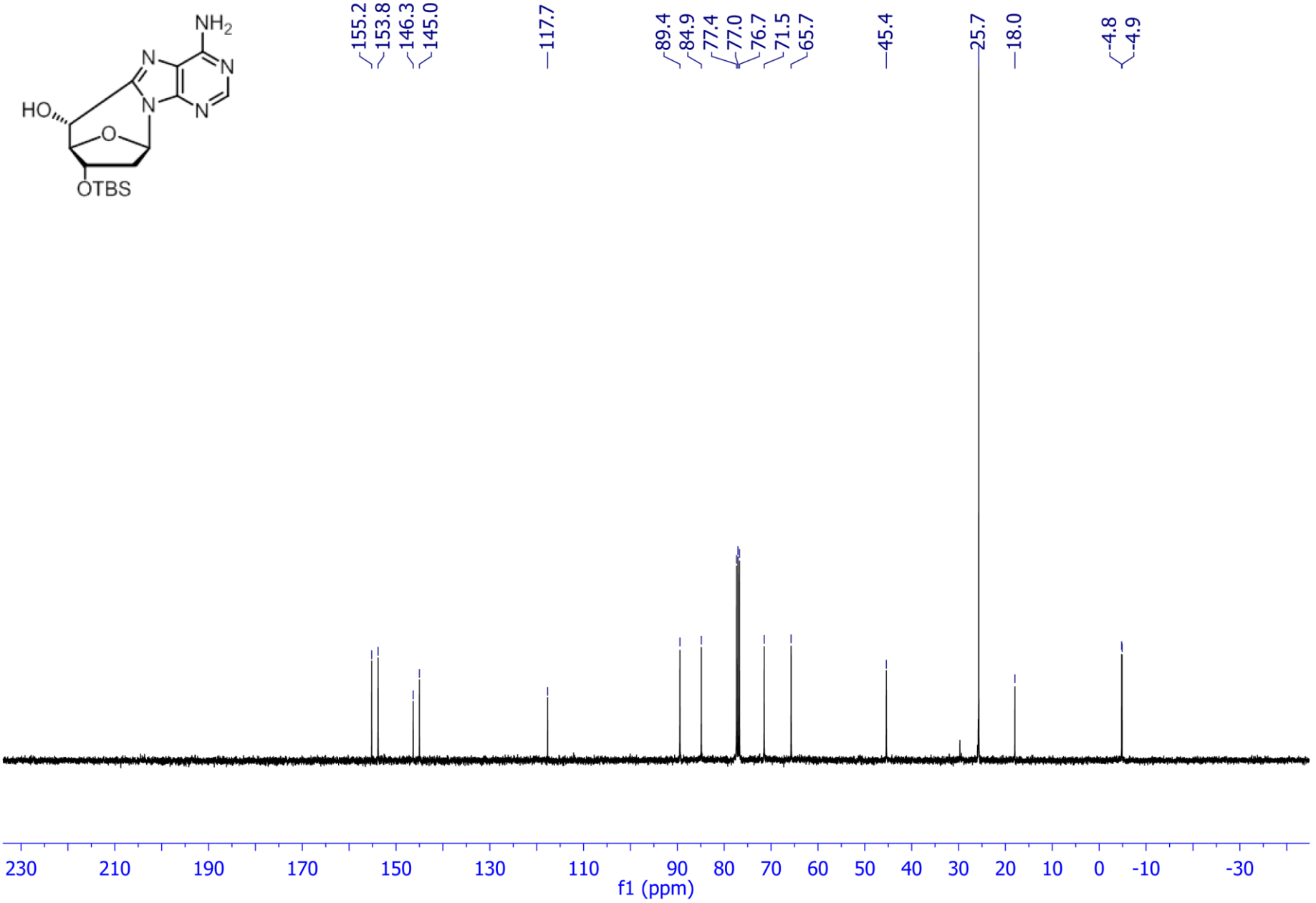

NH2
N
N
Br
N
N
TBSO
O
OTBS
8.23
7.26
6.35
6.33
6.31
5.77
4.86
4.86
4.85
4.85
3.91
3.89
3.68
3.67
3.64
2.24
2.23
2.22
2.21
2.20
0.92
0.81
0.13
-0.02
-0.06
1
1
2
1
2
2
1
9
9
6
3
3
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

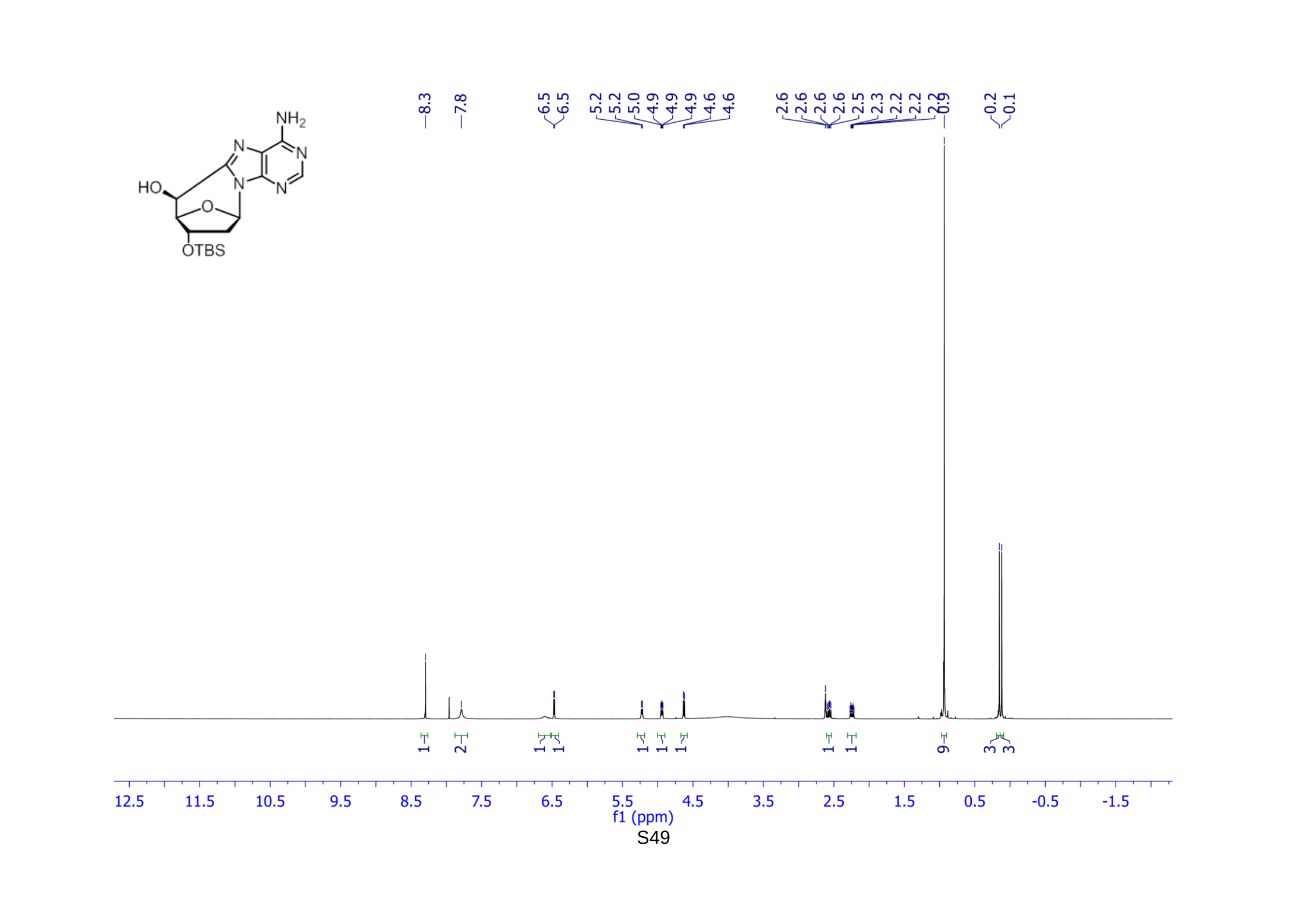

NH2
N
N
Br
N
N
TBSO
O
OTBS
154.3
152.6
151.0
128.2
120.5
87.8
86.3
77.4
77.0
76.7
72.4
62.7
36.8
25.9
25.9
18.4
18.1
-4.6
-4.7
-5.4
-5.5
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

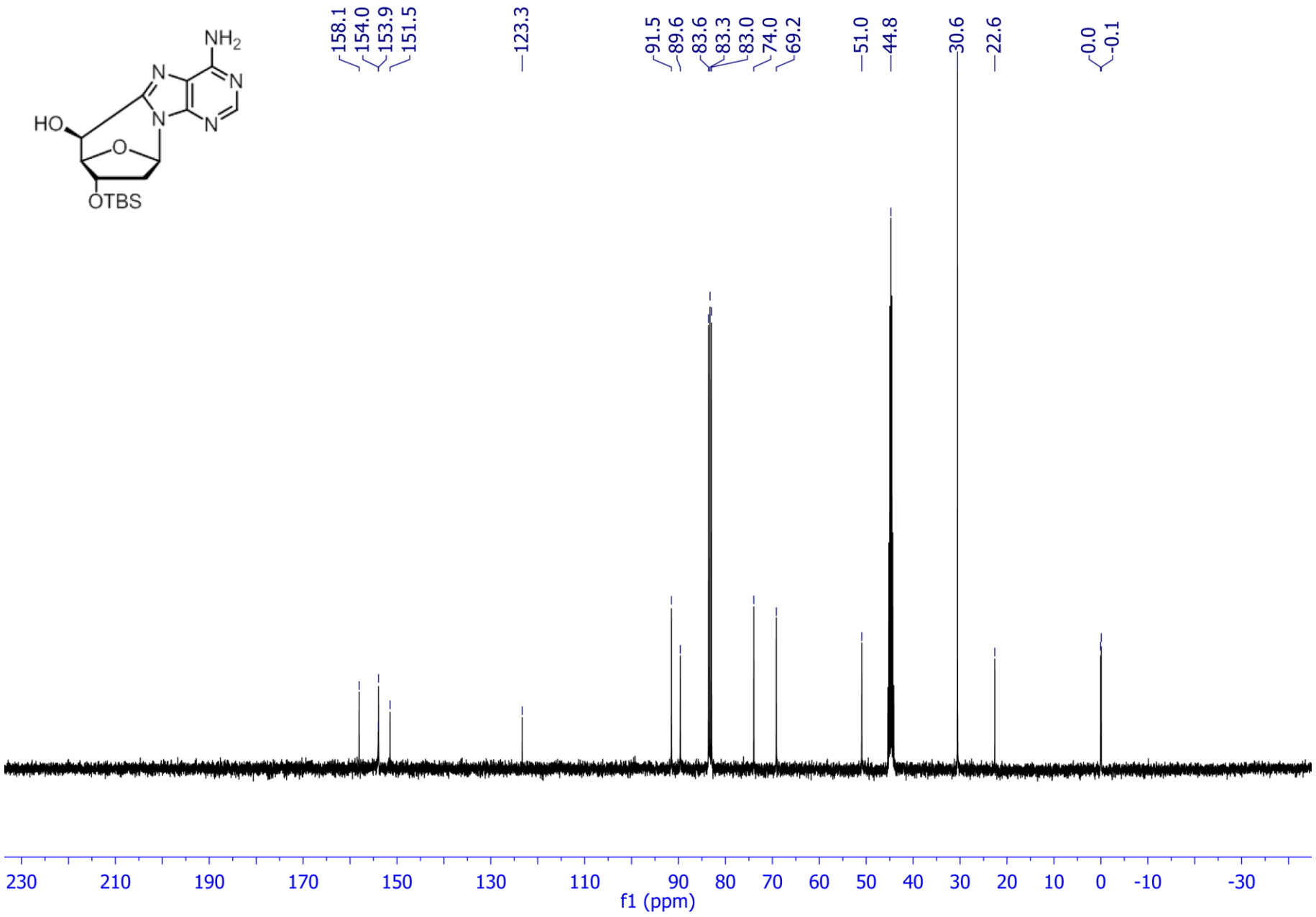

NH2
N
N
Br
N
N
HO
O
OTBS
8.27
7.26
6.45
6.44
6.43
6.41
6.37
4.72
4.71
4.14
3.93
3.74
3.03
3.02
3.01
3.00
3.00
2.99
2.98
2.96
0.93
0.13
0.12
1
1
1
2
1
1
1
1
1
1
9
3
3
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

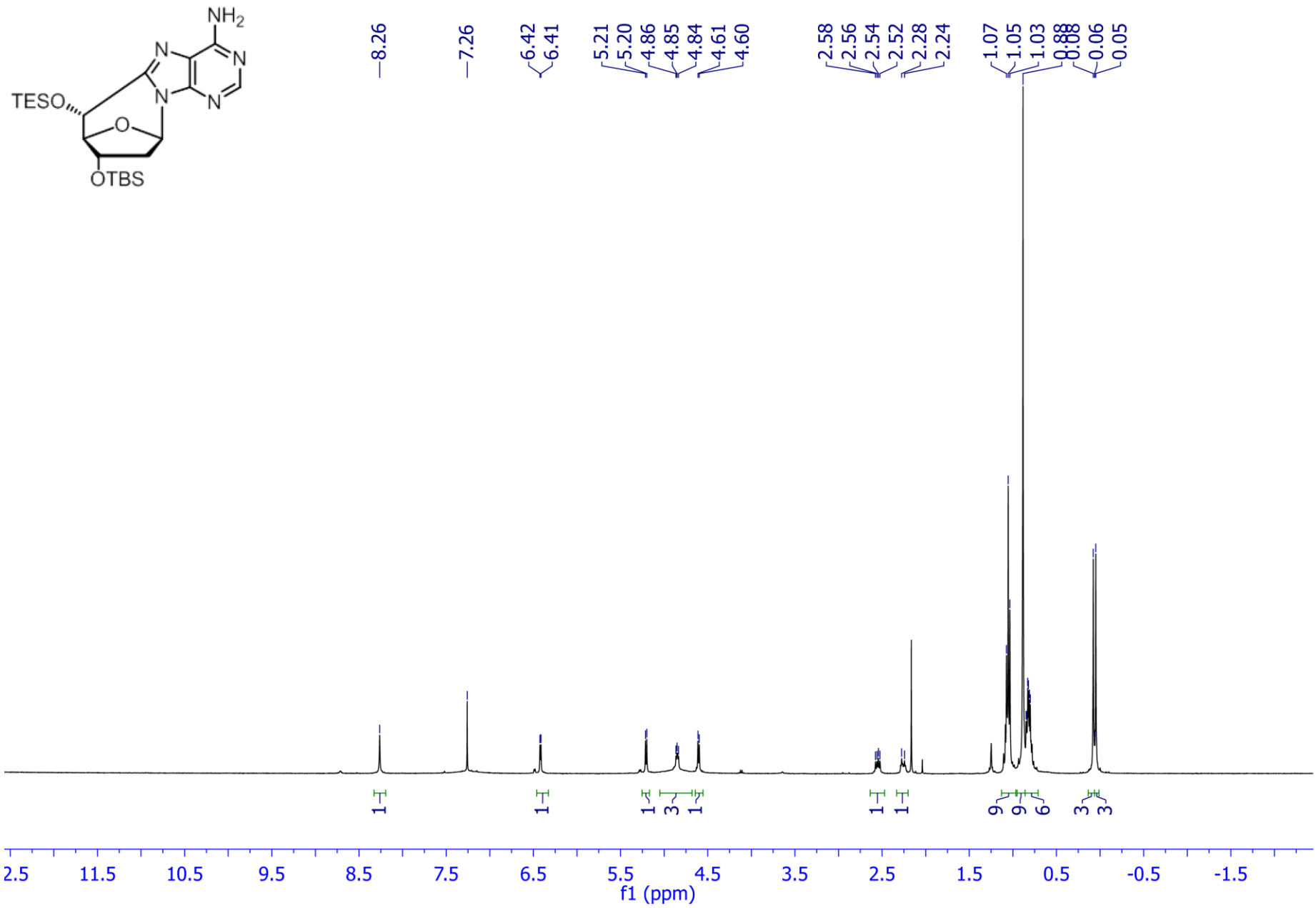

NH2
N
N
Br
N
N
HO
O
OTBS
154.7
152.3
150.0
126.8
120.9
90.5
88.7
77.4
77.1
76.7
63.3
40.7
25.8
18.0
-4.7
-4.7
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

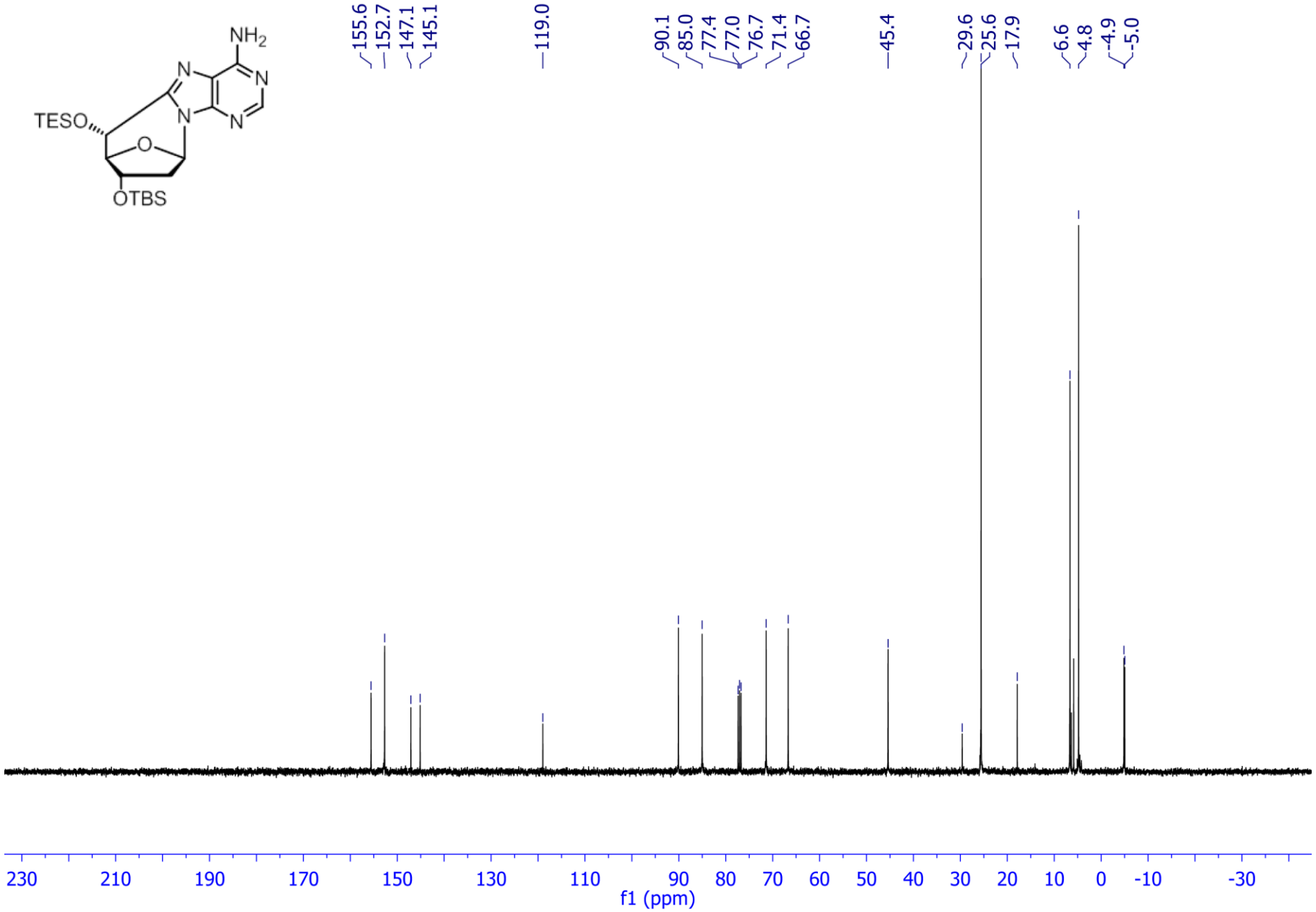

NH2
N
N
N
N
HO
O
OTBS
8.23
7.26
6.49
6.48
4.73
4.71
4.22
4.21
4.20
4.19
2.40
2.39
2.37
2.35
2.23
2.22
2.21
2.20
2.19
0.87
0.05
0.02
1
2
1
1
1
1
1
1
9
3
3
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

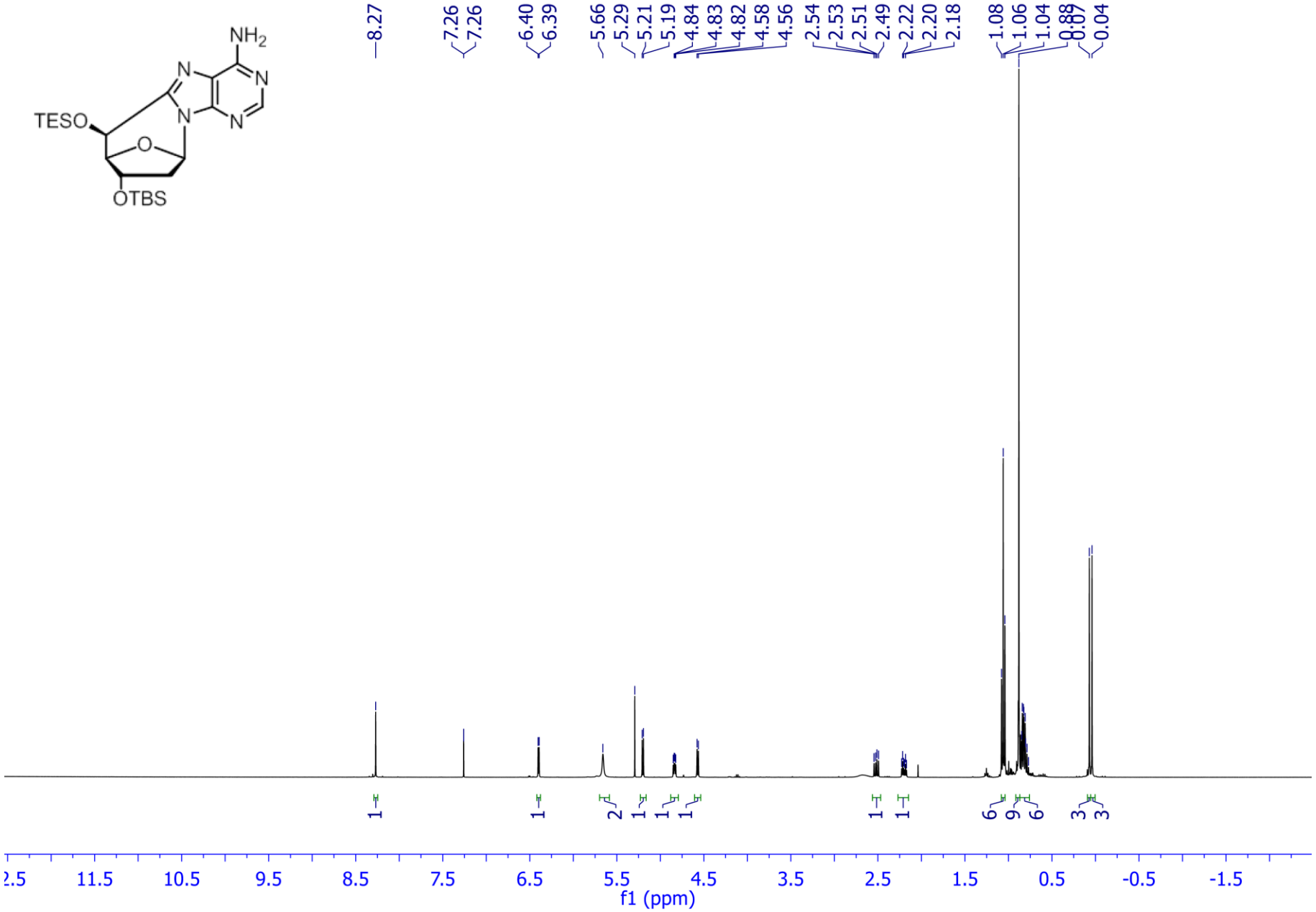

NH2
N
N
N
N
HO
O
OTBS
155.2
153.8
146.3
145.0
117.7
89.4
84.9
77.4
77.0
76.7
71.5
65.7
45.4
25.7
18.0
-4.8
-4.9
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

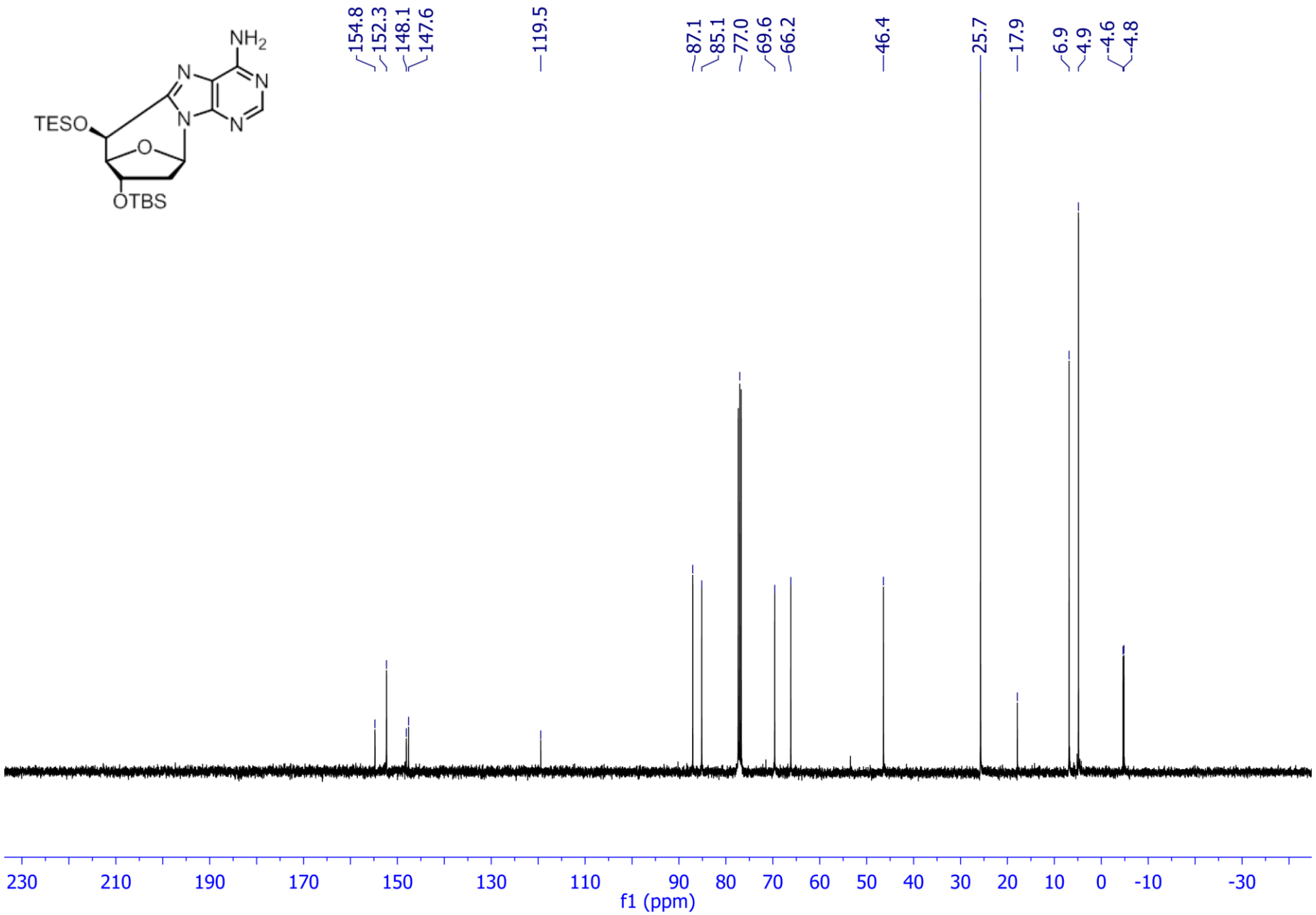

NH2
N
N
N
N
HO
O
OTBS
8.3
7.8
6.5
6.5
5.2
5.2
5.0
4.9
4.9
4.9
4.6
4.6
2.6
2.6
2.6
2.6
2.5
2.3
2.2
2.2
2.2
0.9
0.2
0.1
1
2
1
1
1
1
1
1
1
9
3
3
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

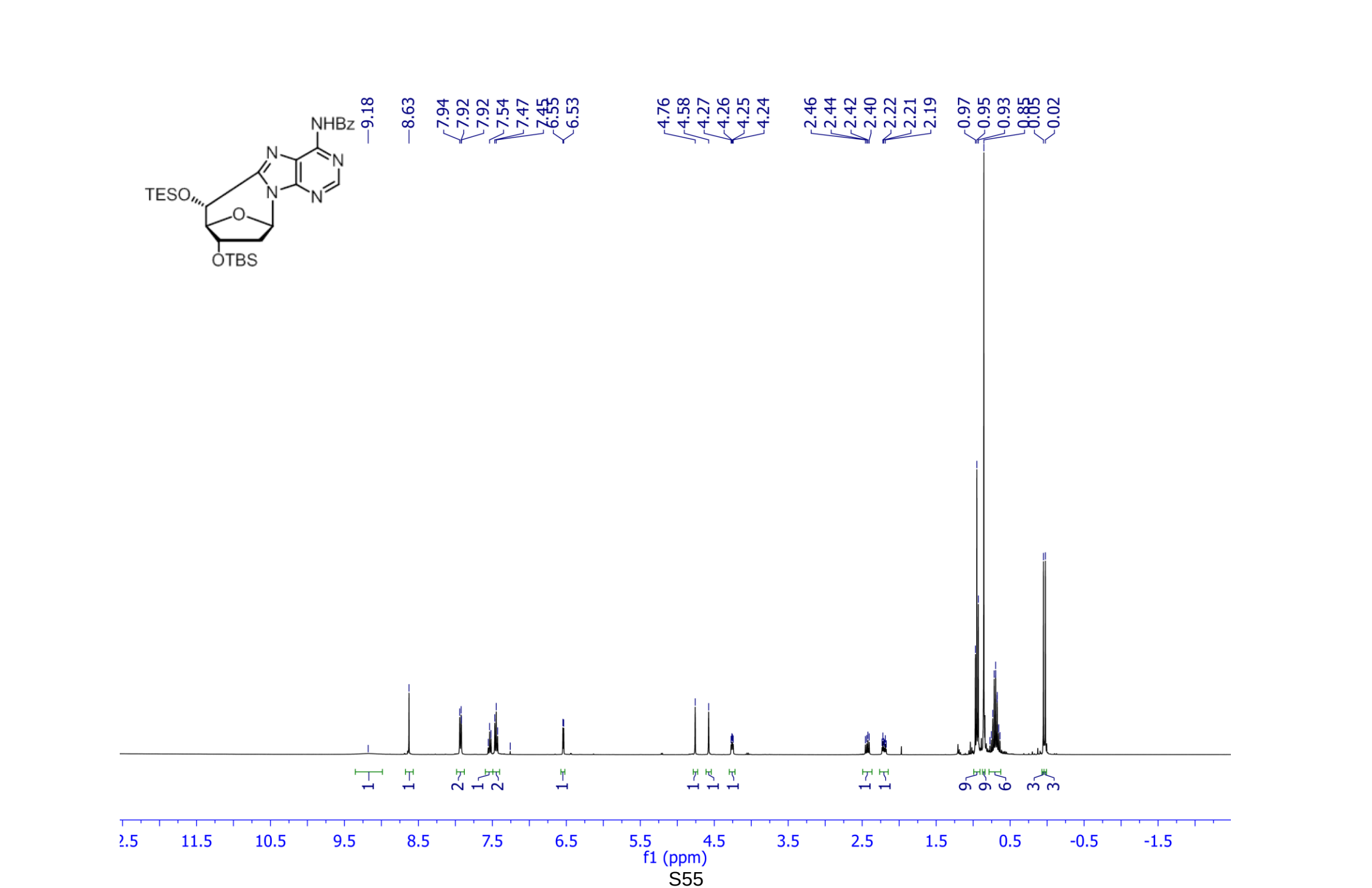

158.1
154.0
153.9
151.5
123.3
91.5
89.6
83.6
83.3
83.0
74.0
69.2
51.0
44.8
30.6
22.6
0.0
-0.1
NH2
N
N
N
N
HO
O
OTBS
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

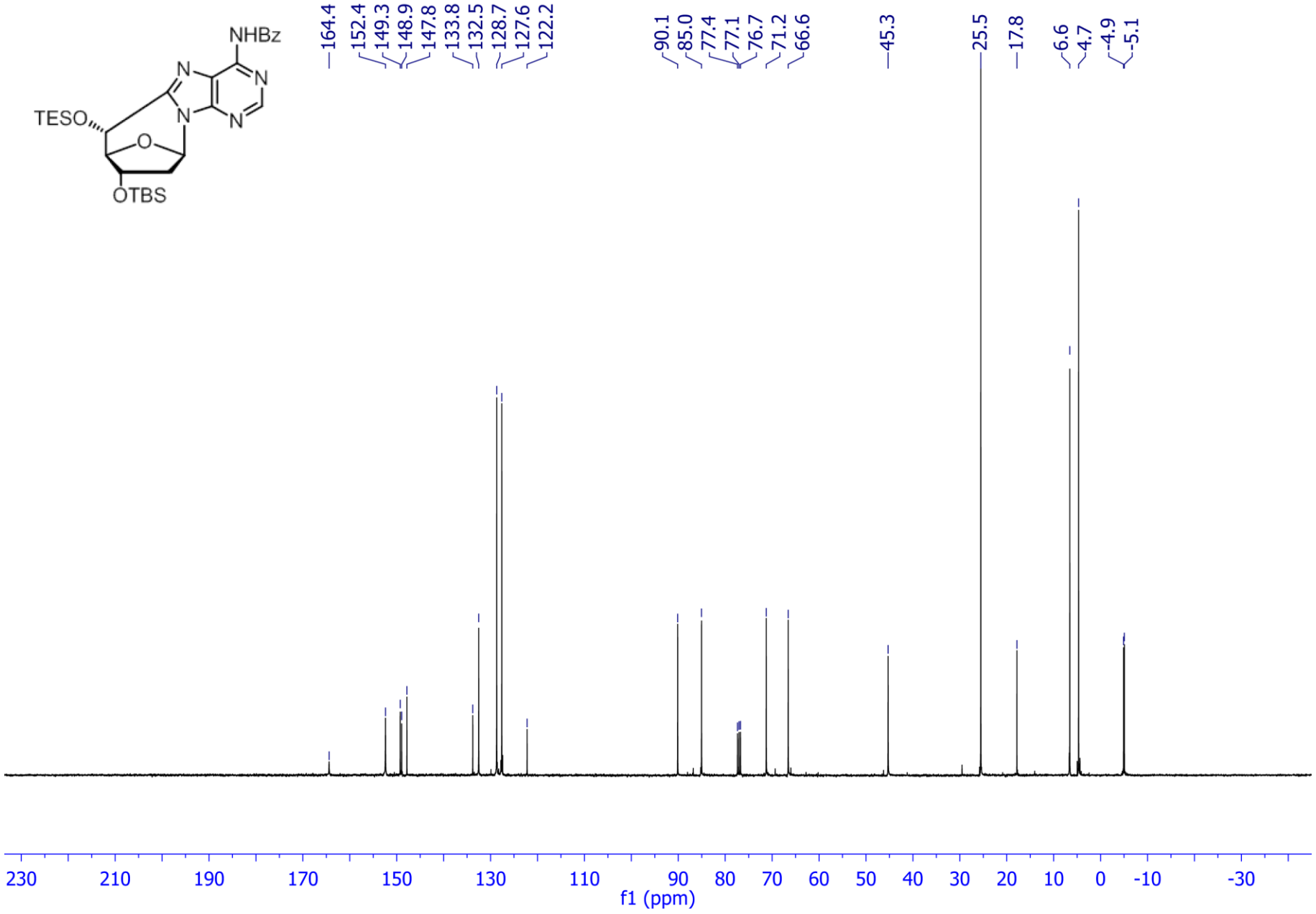

NH2
N
N
N
N
TESO
O
OTBS
8.26
7.26
6.42
6.41
5.21
5.20
4.86
4.85
4.84
4.61
4.60
2.58
2.56
2.54
2.52
2.28
2.24
1.07
1.05
1.03
0.06
0.05
1
1
1
3
1
1
1
9
9
6
3
3
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

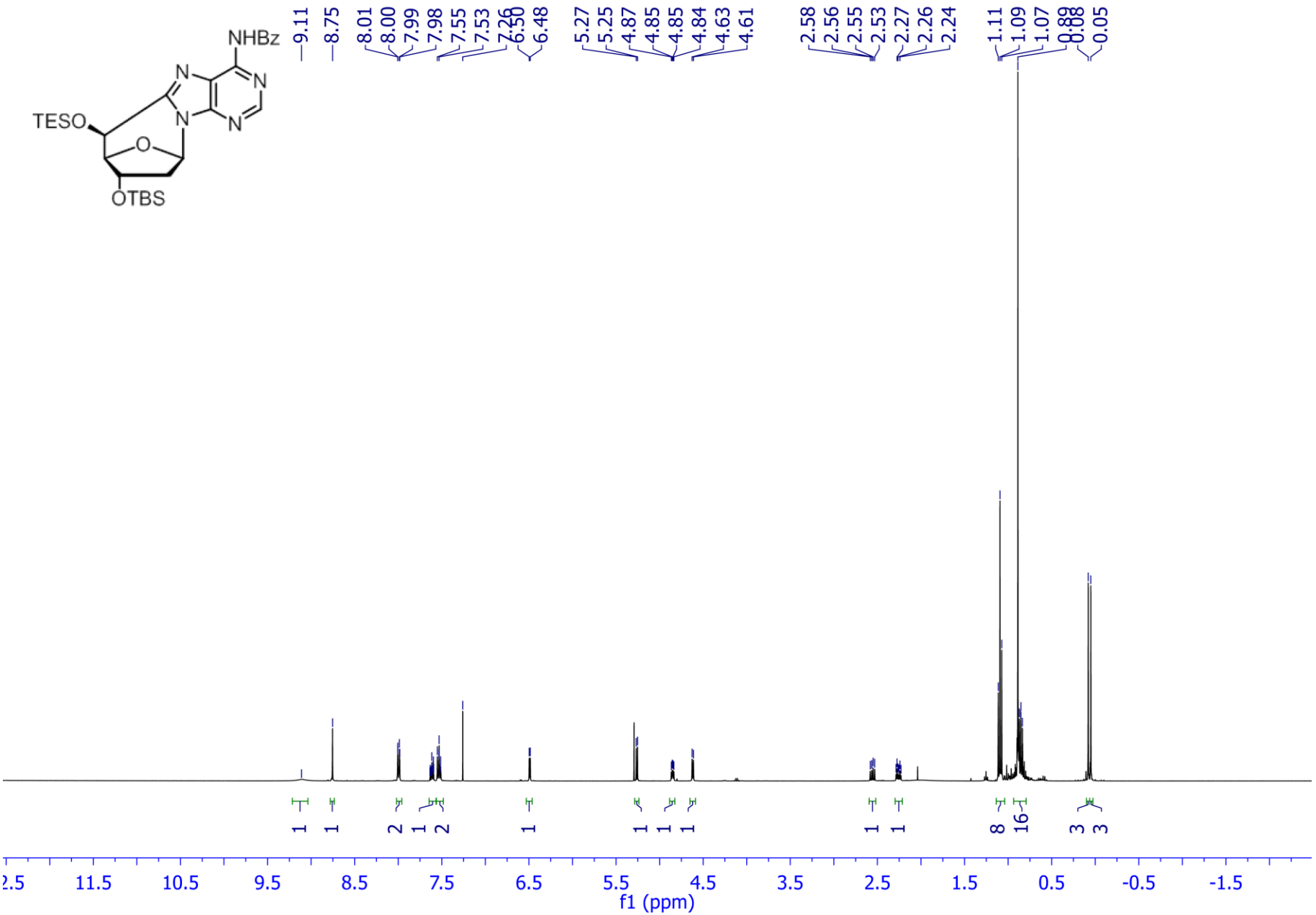

NH2
N
N
N
N
TESO
O
OTBS
155.6
152.7
147.1
145.1
119.0
90.1
85.0
77.4
77.0
76.7
71.4
66.7
45.4
29.6
25.6
17.9
6.6
4.8
-4.9
-5.0
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

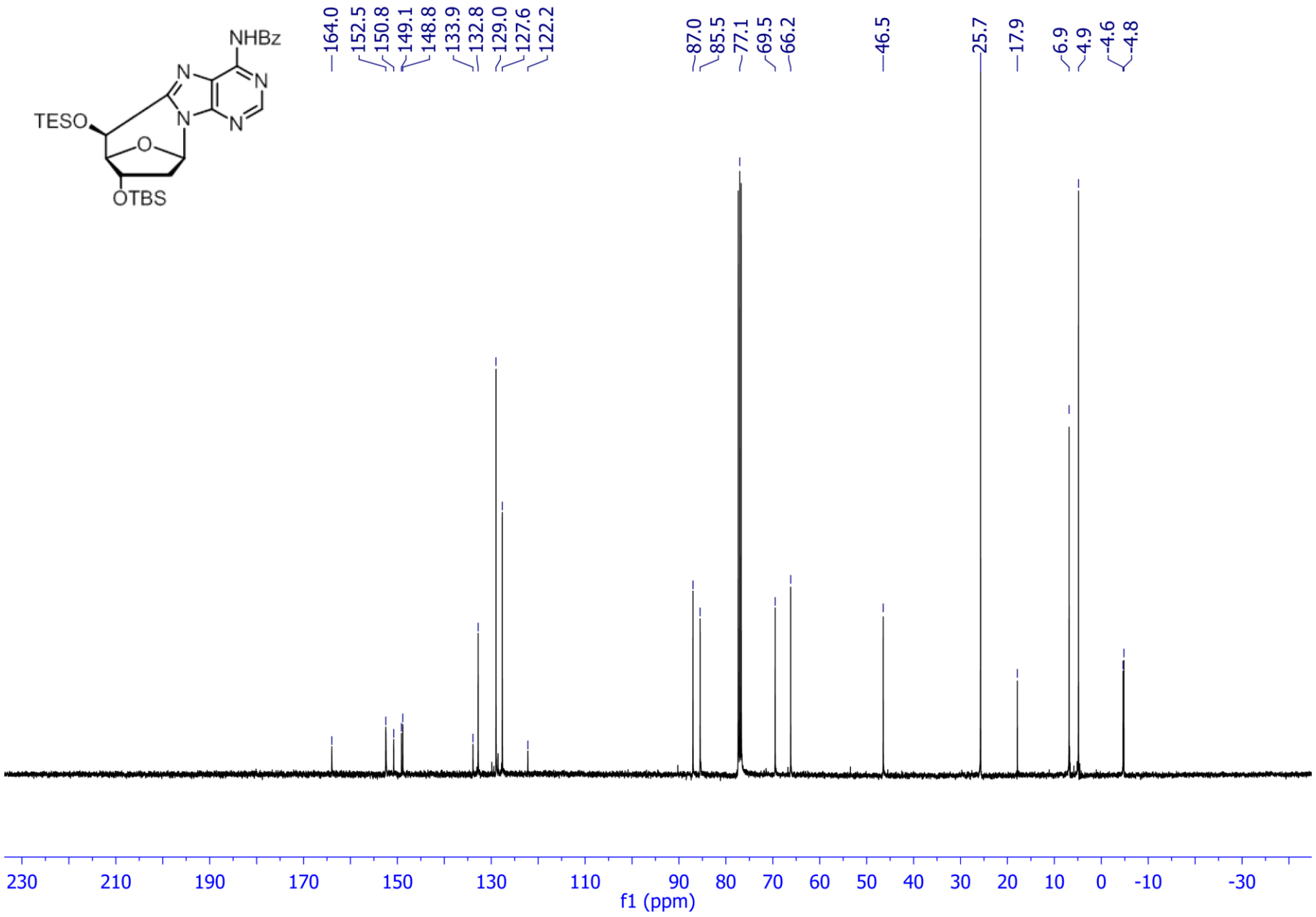

NH2
N
N
N
N
TESO
O
OTBS
8.27
7.26
7.26
6.40
6.39
5.66
5.29
5.21
5.19
4.84
4.83
4.82
4.58
4.56
2.54
2.53
2.51
2.49
2.22
2.20
2.18
1.08
1.06
1.04
0.88
0.87
0.04
1
1
2
1
1
1
1
1
6
9
6
3
3
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

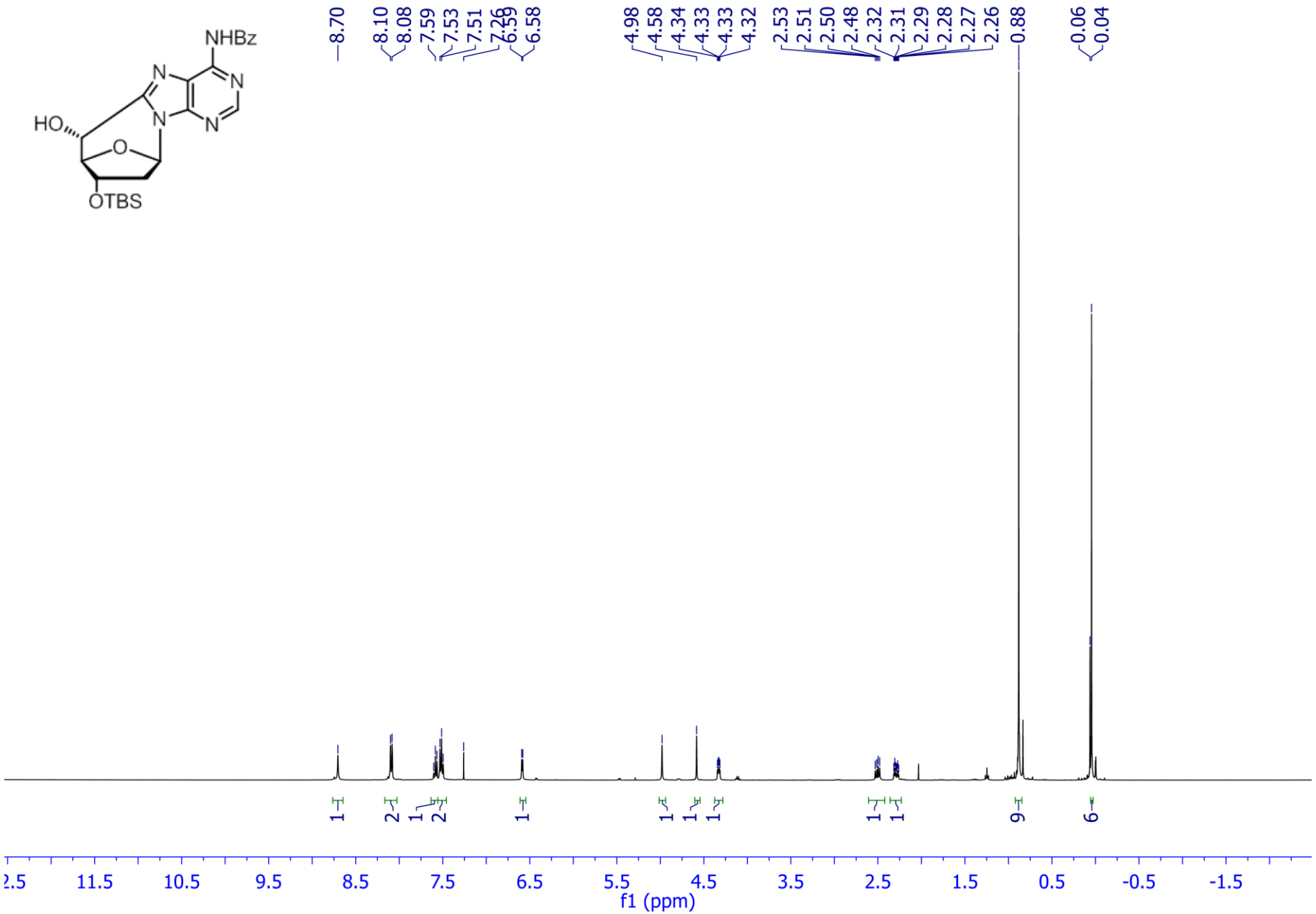

NH2
N
N
N
N
TESO
O
OTBS
154.8
152.3
148.1
147.6
119.5
87.1
85.1
77.0
69.6
66.2
46.4
25.7
17.9
6.9
4.9
-4.6
-4.8
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

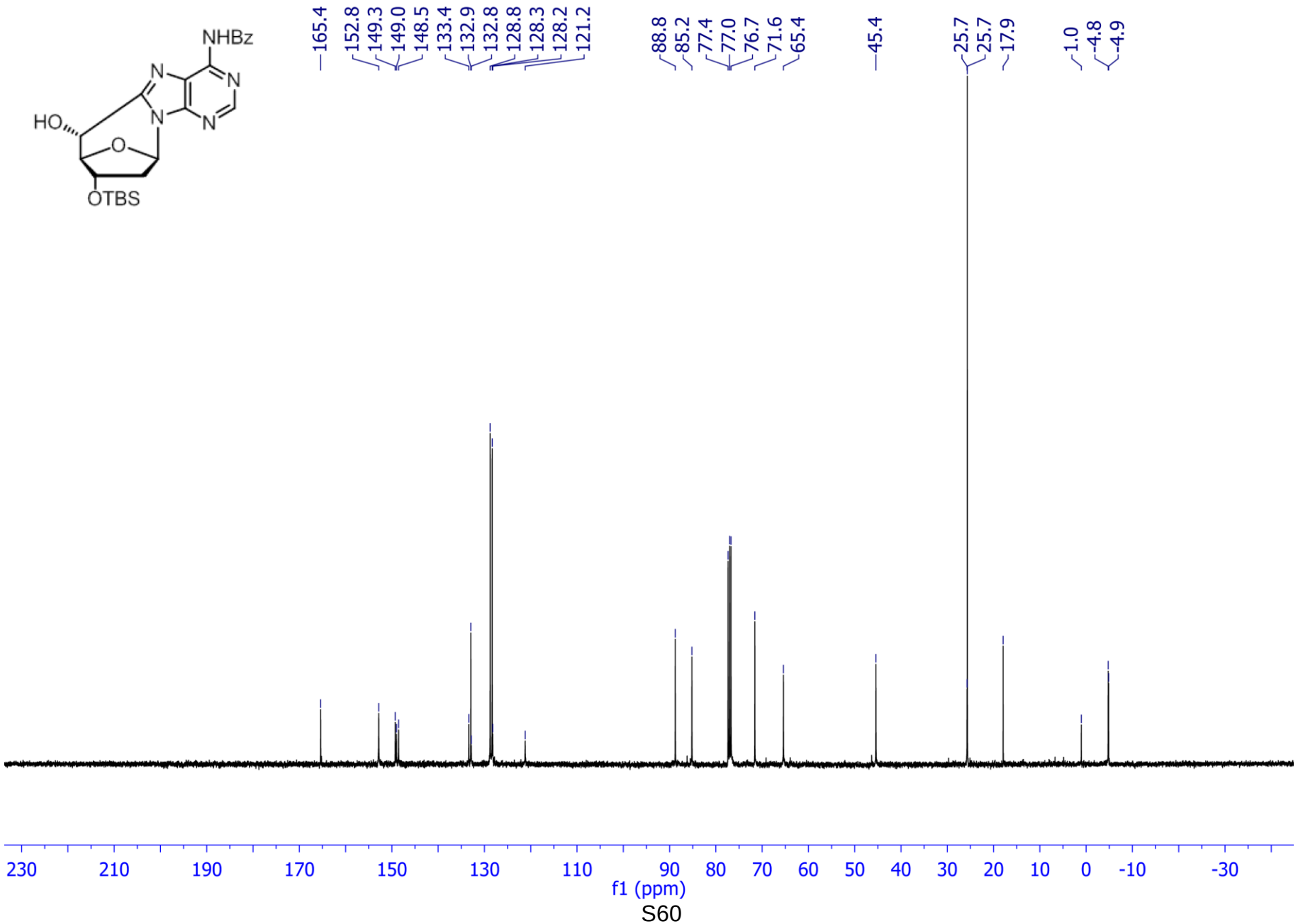

NHBz
N
N
N
N
TESO
O
OTBS
9.18
8.63
7.94
7.92
7.92
7.54
7.47
7.45
6.55
6.53
4.76
4.58
4.27
4.26
4.25
4.24
2.46
2.44
2.42
2.40
2.22
2.21
2.19
0.97
0.95
0.93
0.85
0.85
0.02
1
1
2
1
2
1
1
1
1
1
1
9
9
6
3
3
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

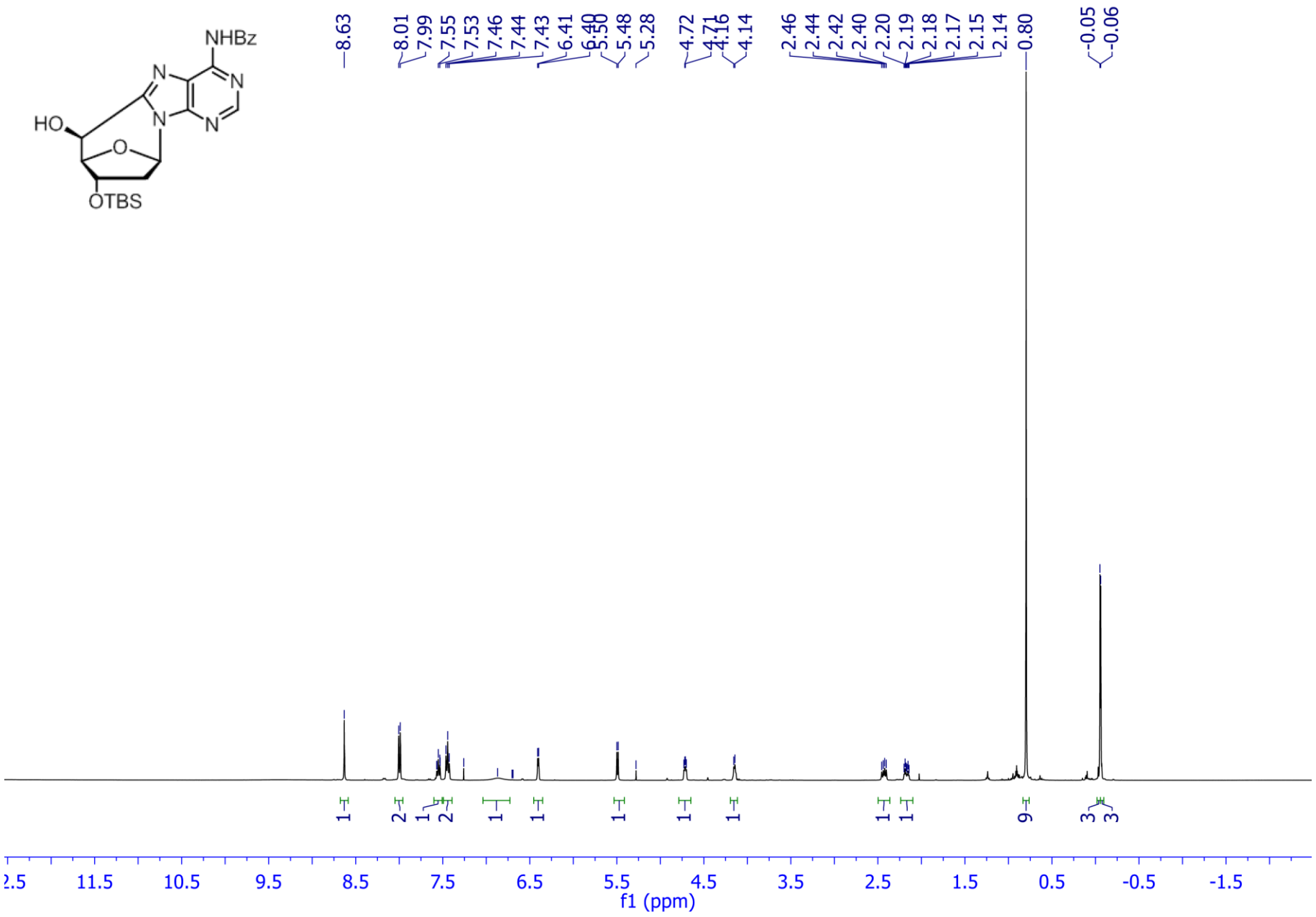

NHBz
N
N
N
N
TESO
O
OTBS
164.4
152.4
149.3
148.9
147.8
133.8
132.5
128.7
127.6
122.2
90.1
85.0
77.4
77.1
76.7
71.2
66.6
45.3
25.5
17.8
6.6
4.7
-4.9
-5.1
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)

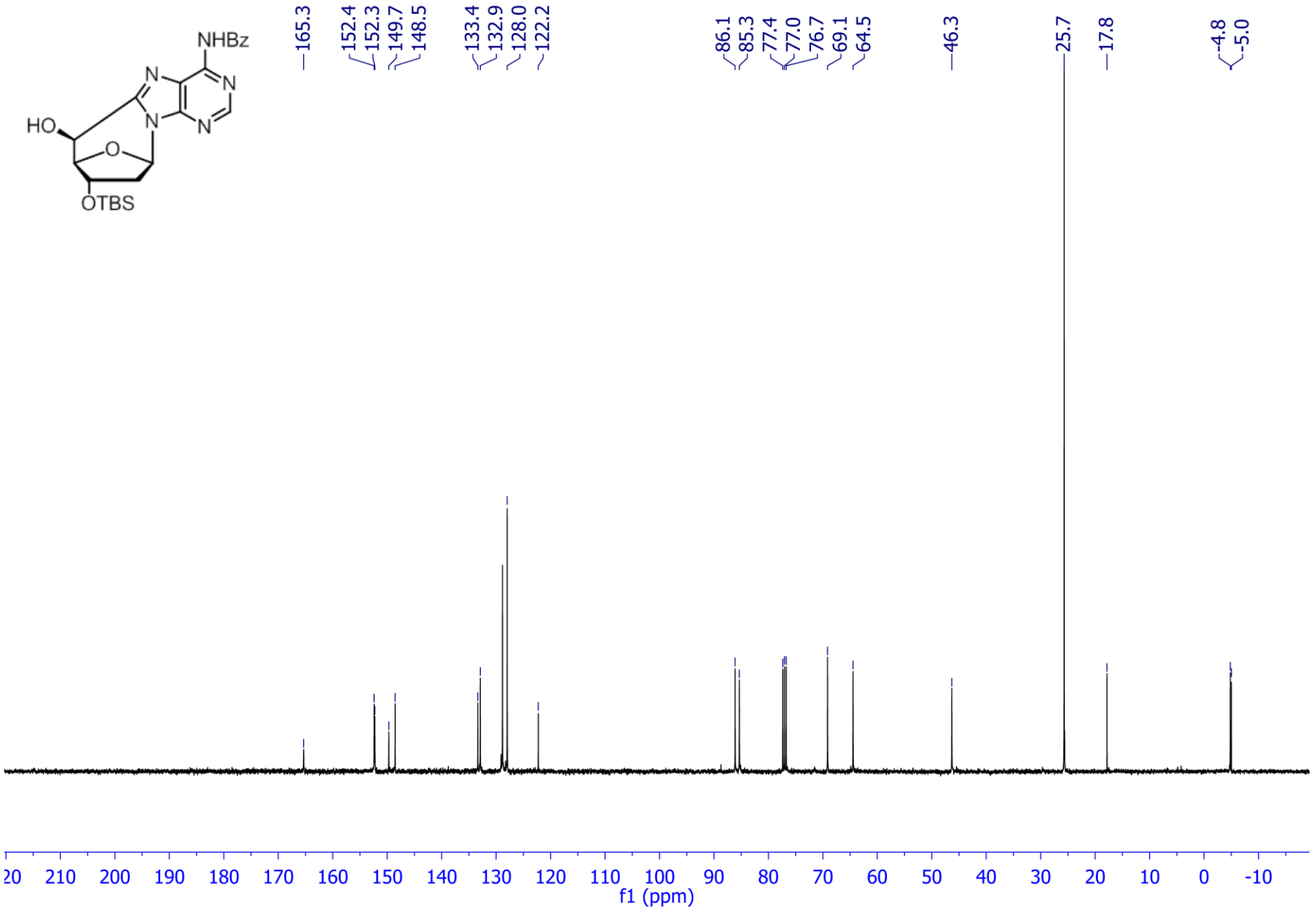

NHBz
N
N
N
N
TESO
O
OTBS
9.11
8.75
8.01
8.00
7.99
7.98
7.55
7.53
7.26
6.50
6.48
5.27
5.25
4.87
4.85
4.85
4.84
4.63
4.61
2.58
2.56
2.55
2.53
2.27
2.26
2.24
1.11
1.09
1.07
0.89
0.88
0.05
1
1
2
1
2
1
1
1
1
1
1
8
16
3
3
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)

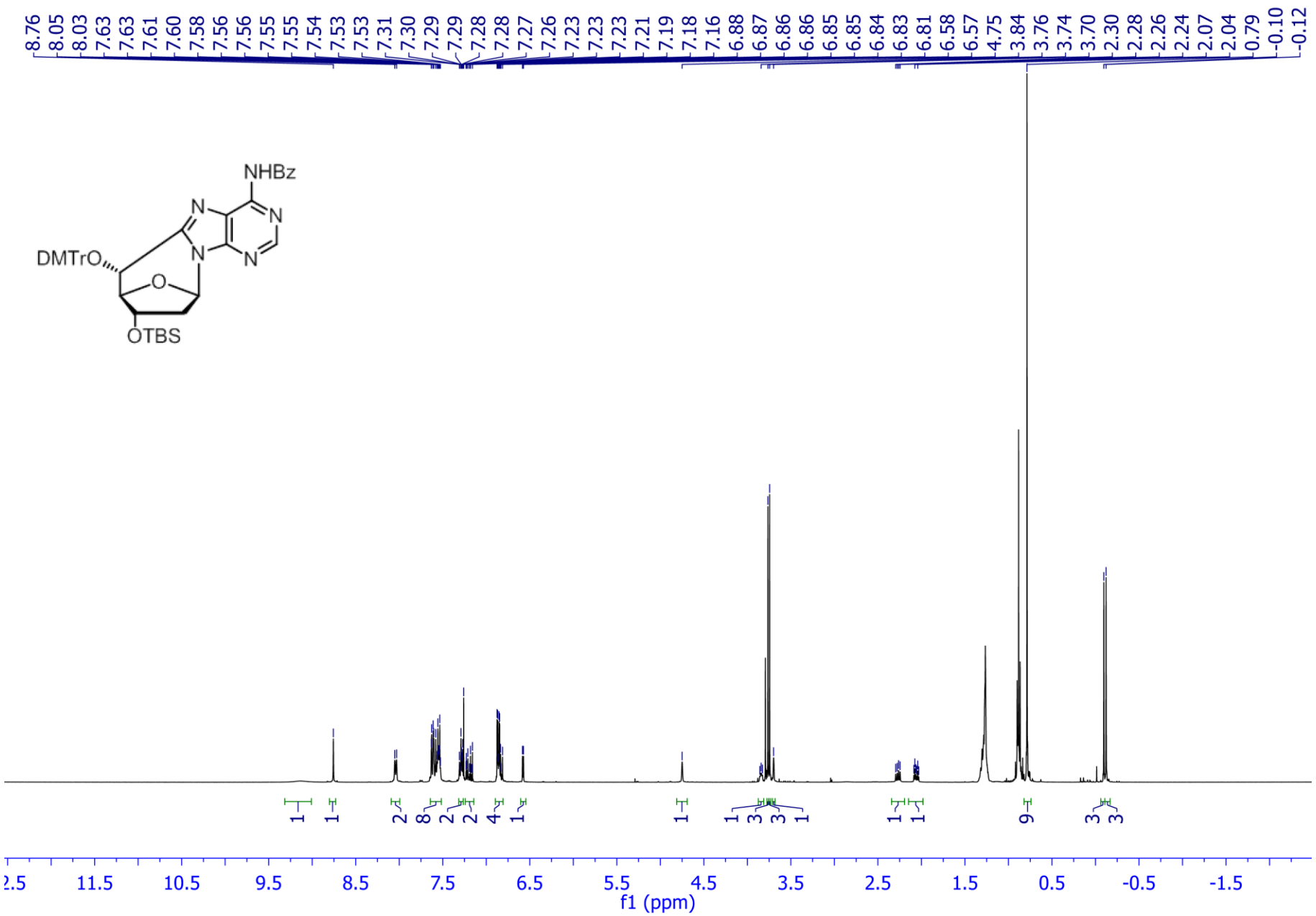

NHBz
N
N
N
N
TESO
O
OTBS
164.0
152.5
150.8
149.1
148.8
133.9
132.8
129.0
127.6
122.2
87.0
85.5
77.1
69.5
66.2
46.5
25.7
17.9
6.9
4.9
-4.6
-4.8
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)


NHBz
N
N
N
N
HO
O
OTBS
8.70
8.10
8.08
7.59
7.53
7.51
7.26
6.59
6.58
4.98
4.58
4.34
4.33
4.33
4.32
2.53
2.51
2.50
2.48
2.32
2.31
2.29
2.28
2.27
2.26
0.88
0.06
0.04
1
2
1
2
1
1
1
1
1
9
6
f1 (ppm)


NHBz
N
N
N
N
HO
O
OTBS
165.4
152.8
149.3
149.0
148.5
133.4
132.9
132.8
128.8
128.3
128.2
121.2
88.8
85.2
77.4
77.0
76.7
71.6
65.4
45.4
25.7
25.7
17.9
1.0
-4.8
-4.9
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)


NHBz
N
N
N
N
HO
O
OTBS
8.63
8.01
7.99
7.55
7.53
7.46
7.44
7.43
6.41
6.40
5.50
5.48
5.28
4.72
4.71
4.16
4.14
2.46
2.44
2.42
2.40
2.20
2.19
2.18
2.17
2.15
2.14
0.80
-0.05
-0.06
1
2
1
2
1
1
1
1
1
1
1
9
3
3
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)


NHBz
N
N
N
N
HO
O
OTBS
165.3
152.4
152.3
149.7
148.5
133.4
132.9
128.0
122.2
86.1
85.3
77.4
77.0
76.7
69.1
64.5
46.3
25.7
17.8
-4.8
-5.0
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.05
8.03
7.63
7.63
7.61
7.60
7.58
7.56
7.56
7.55
7.55
7.54
7.53
7.53
7.31
7.30
7.29
7.29
7.28
7.28
7.27
7.26
7.23
7.23
7.23
7.21
7.19
7.18
7.16
6.88
6.87
6.86
6.86
6.85
6.85
6.84
6.83
6.81
6.58
6.57
4.75
3.84
3.76
3.74
3.70
2.30
2.28
2.26
2.24
2.07
2.04
0.79
-0.10
-0.12
NHBz
N
N
N
N
DMTrO
O
OTBS
1
1
2
8
2
2
4
1
1
1
3
3
1
1
1
9
3
3
2.5
11.5
10.5
9.5
8.5
7.5
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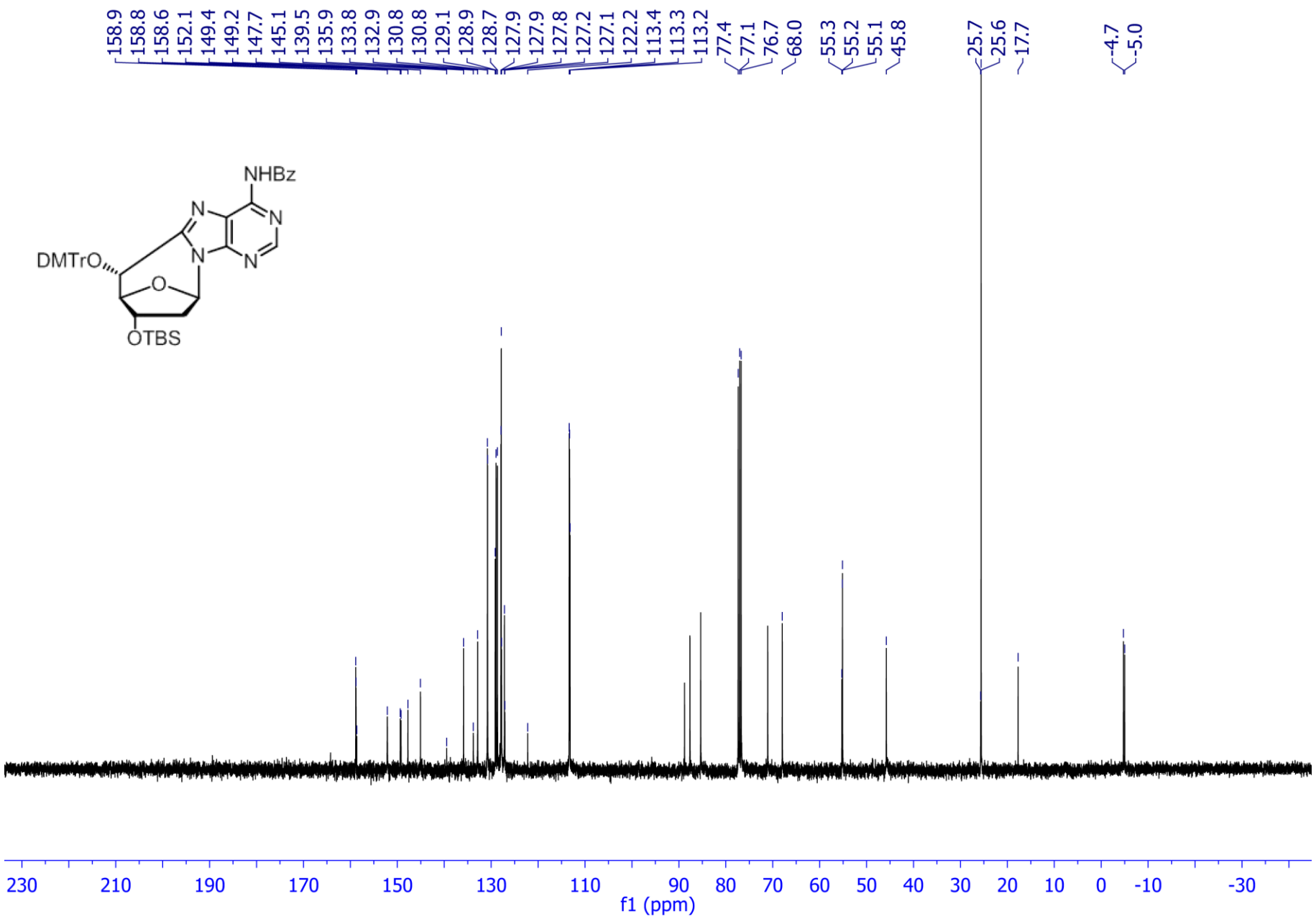
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)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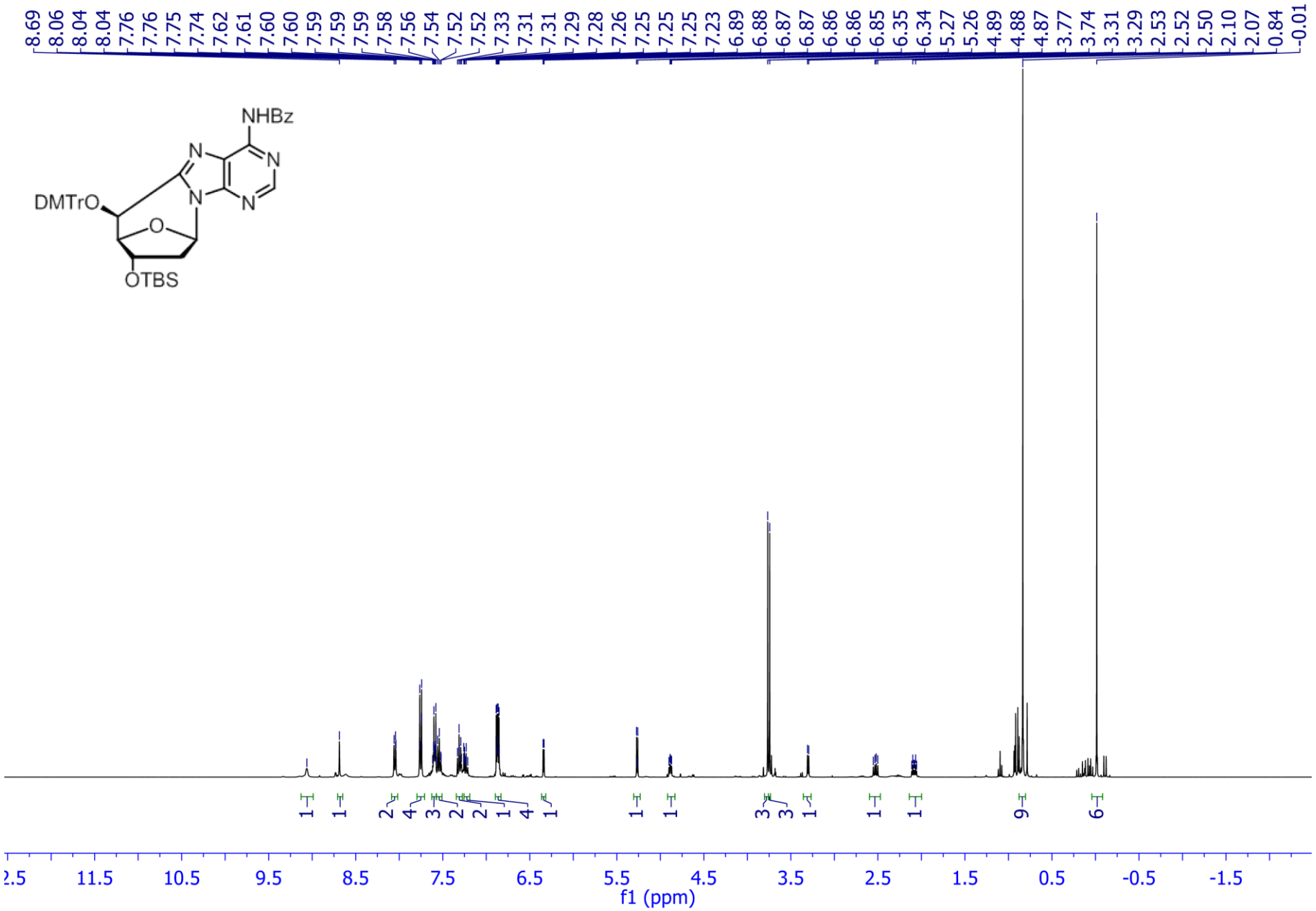
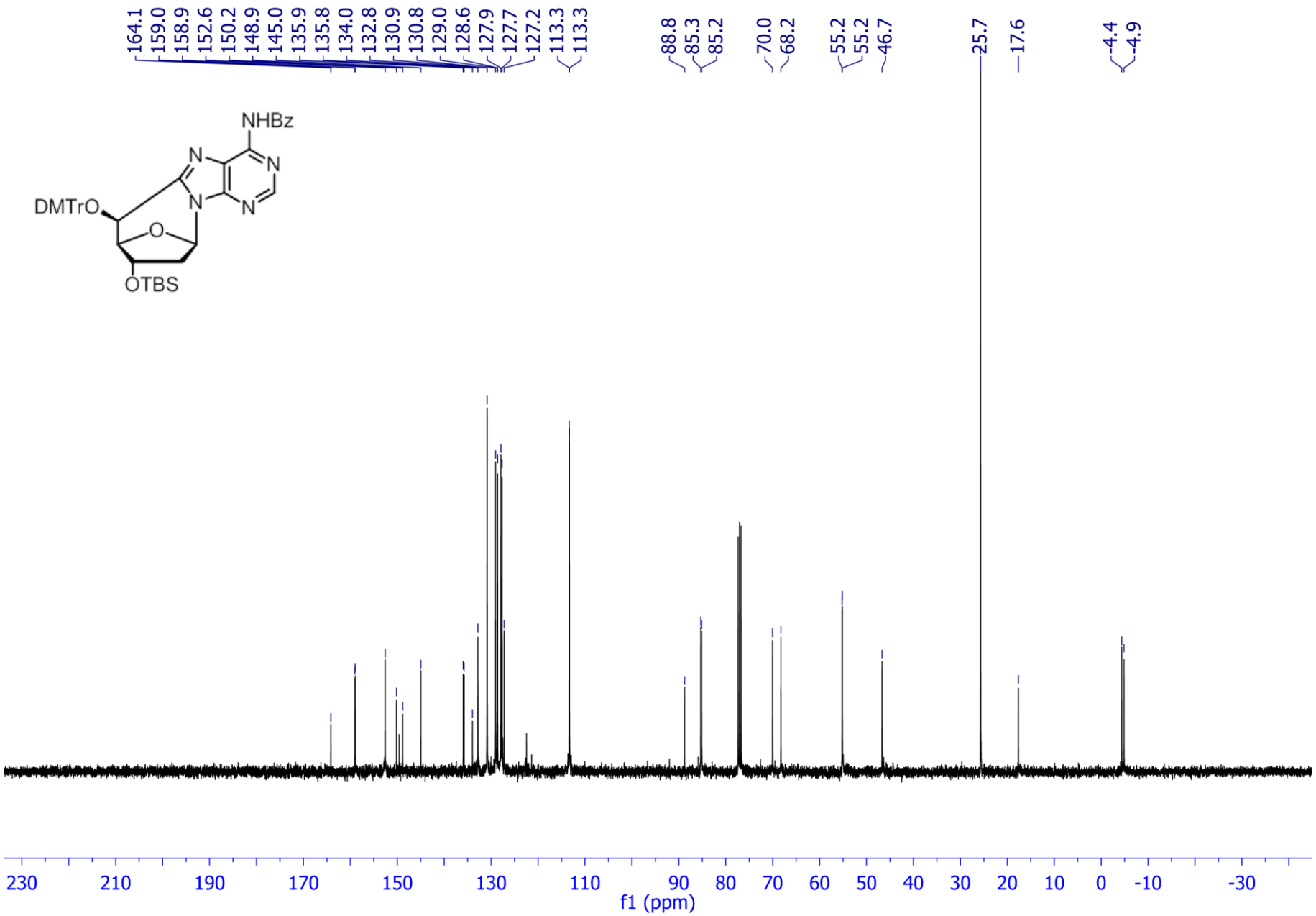
158.8
158.6
152.1
149.4
149.2
147.7
145.1
139.5
135.9
133.8
132.9
130.8
130.8
129.1
128.9
128.7
127.9
127.9
127.8
127.2
127.1
122.2
113.4
113.3
113.2
77.4
77.1
76.7
68.0
55.3
55.2
55.1
45.8
25.7
25.6
17.7
-4.7
-5.0
NHBz
N
N
N
N
DMTrO
O
OTBS
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)


8.69 8.06 8.04 8.04 7.76 7.76 7.75 7.74 7.62 7.61 7.60 7.60 7.59 7.59 7.59 7.58 7.56 7.54 7.52 7.52 7.33 7.31 7.31 7.29 7.28 7.26 7.25 7.25 7.25 7.23 6.89 6.88 6.87 6.87 6.86 6.86 6.85 6.35 6.34 5.27 5.26 4.89 4.88 4.87 3.77 3.74 3.31 3.29 2.53 2.52 2.50 2.10 2.07 0.84 -0.01
NHBz
N
N
N
N
DMTrO
O
OTBS
1 1 2 4 3 2 2 1 4 1 1 1 3 3 1 1 1 9 6
2.5 11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5 -0.5 -1.5
f1 (ppm)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159.0
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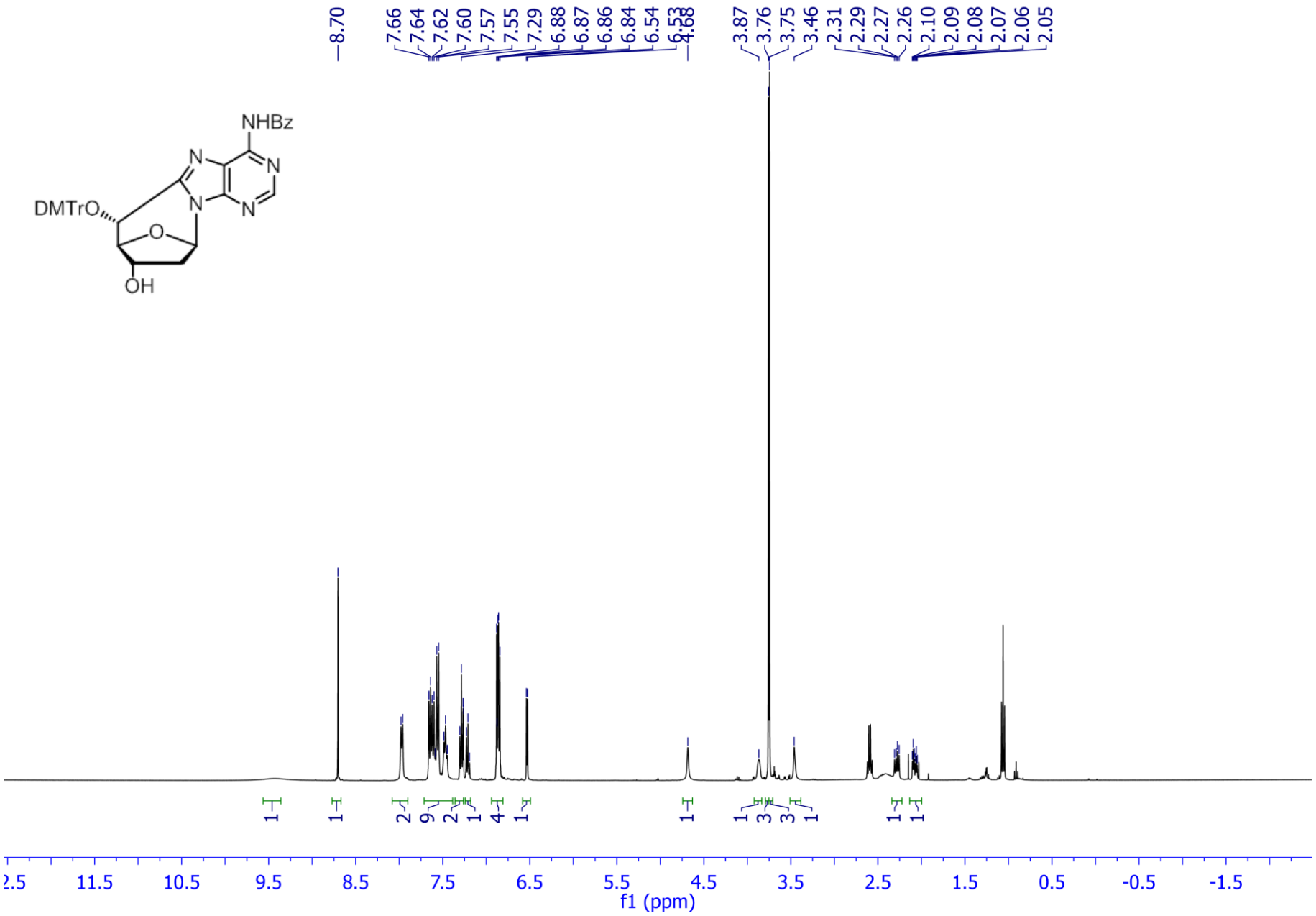
152.6
150.2
148.9
145.0
135.9
135.8
134.0
132.8
130.9
130.8
129.0
128.6
127.9
127.7
127.2
113.3
113.3
88.8
85.3
85.2
70.0
68.2
55.2
55.2
46.7
25.7
17.6
-4.4
-4.9
NHBz
N
N
N
N
DMTrO
O
OTBS
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)


8.70
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7.64
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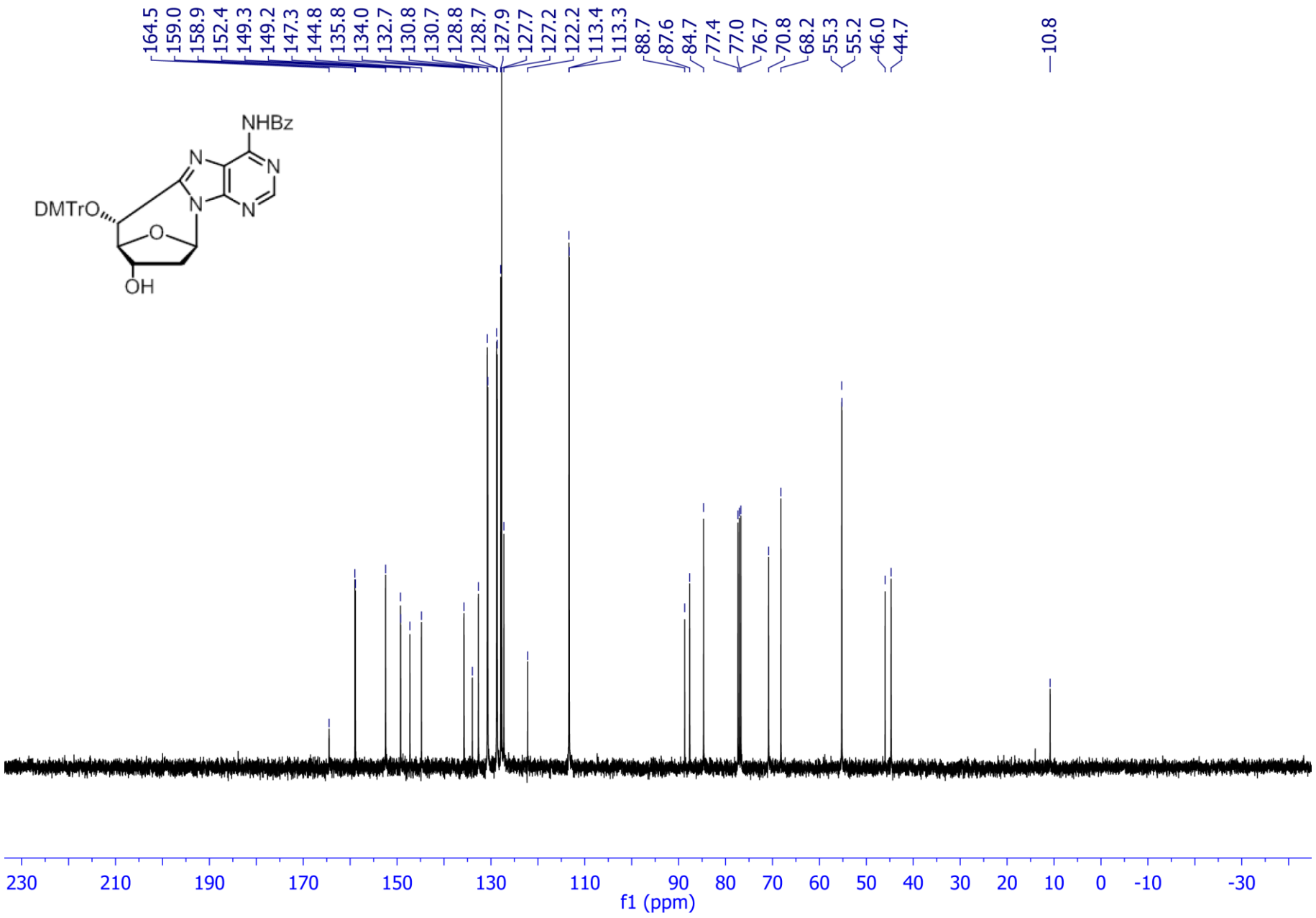
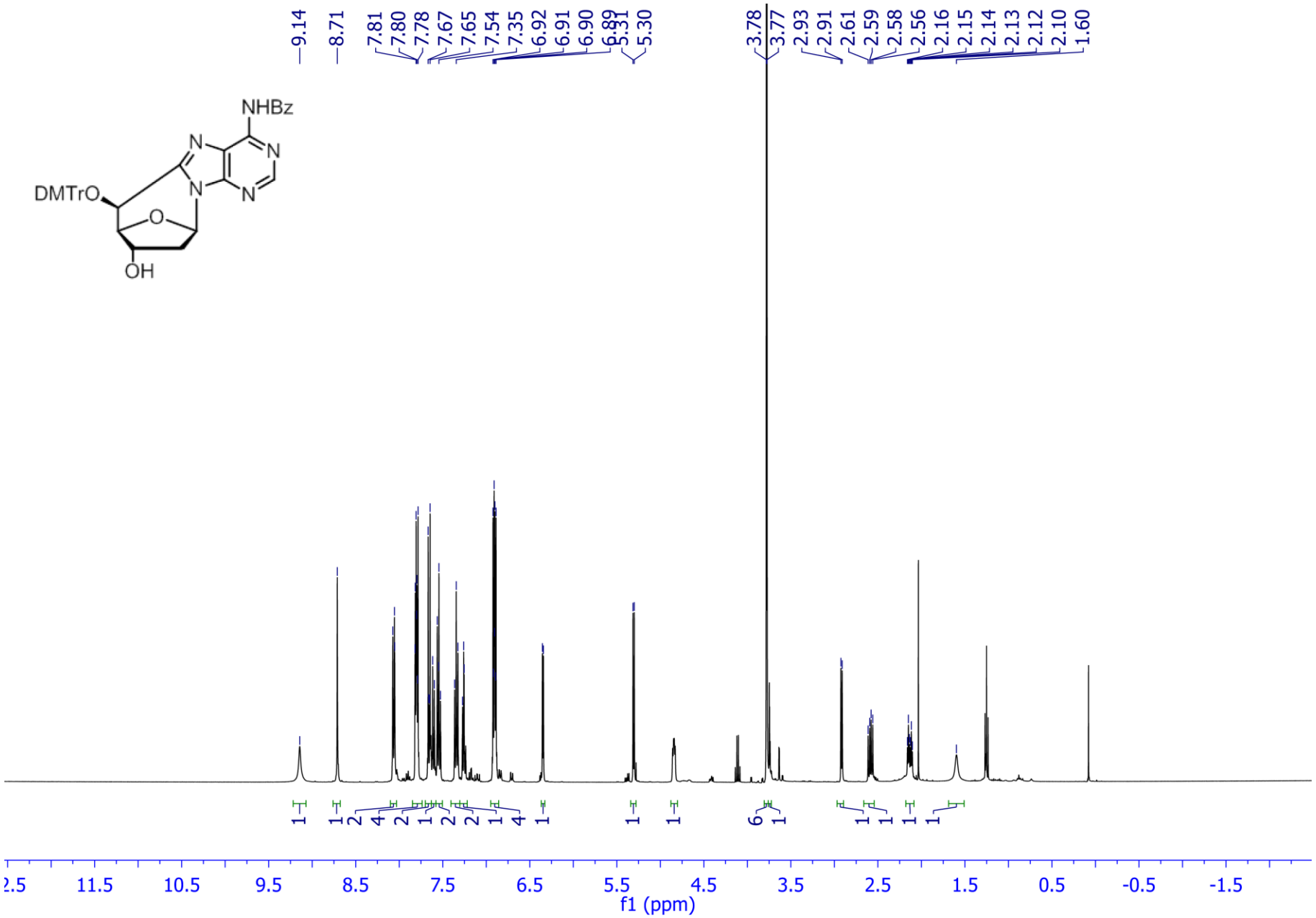
7.57
7.55
7.29
6.88
6.87
6.86
6.84
6.54
6.53
4.68
3.87
3.76
3.75
3.46
2.31
2.29
2.27
2.26
2.10
2.09
2.08
2.07
2.06
2.05
NHBz
N
N
N
N
DMTrO
O
OH
1
1
2
9
2
1
4
1
1
1
3
3
1
1
1
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)


164.5
159.0
158.9
152.4
149.3
149.2
147.3
144.8
135.8
134.0
132.7
130.8
130.7
128.8
128.7
127.9
127.7
127.2
122.2
113.4
113.3
88.7
87.6
84.7
77.4
77.0
76.7
70.8
68.2
55.3
55.2
46.0
44.7
10.8
NHBz
N
N
N
N
DMTrO
O
OH
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)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8.71
7.81
7.80
7.78
7.67
7.65
7.54
7.35
6.92
6.91
6.90
6.89
5.31
5.30
3.78
3.77
2.93
2.91
2.61
2.59
2.58
2.56
2.16
2.15
2.14
2.13
2.12
2.10
1.60
NHBz
N
N
N
N
DMTrO
O
OH
1
1
2
4
2
1
2
2
1
4
1
1
1
6
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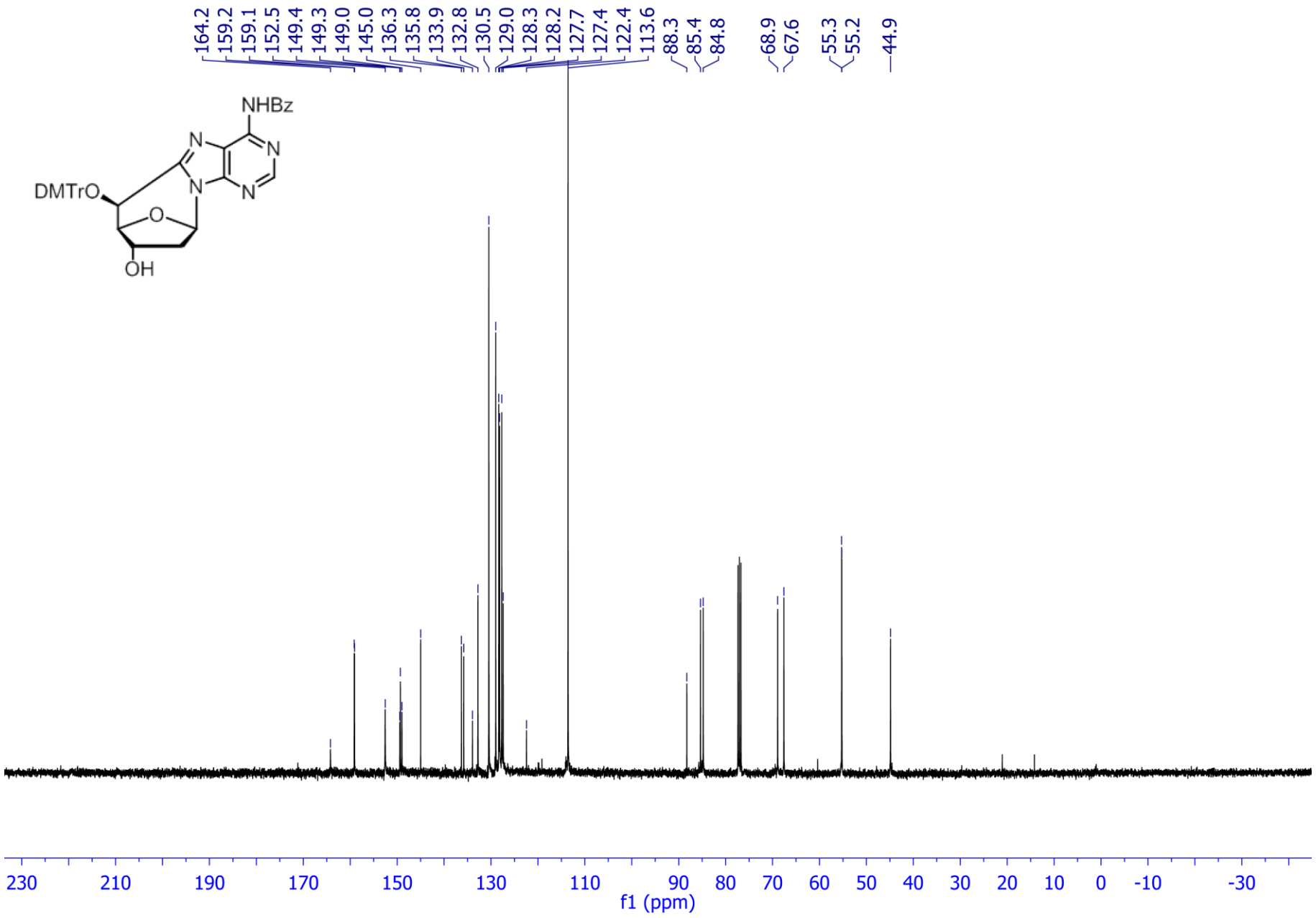
1
1
1
1
2.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
f1 (ppm)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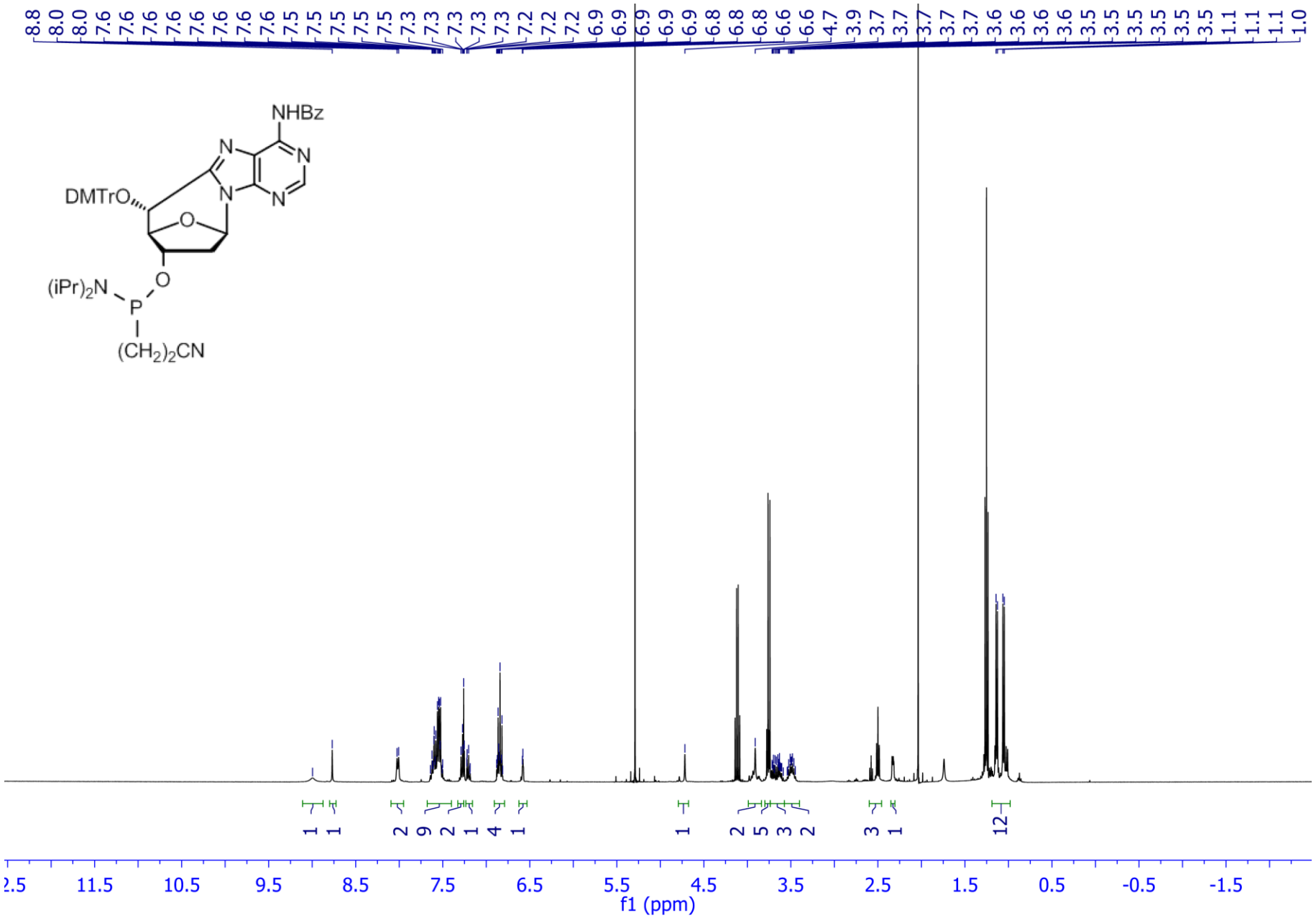
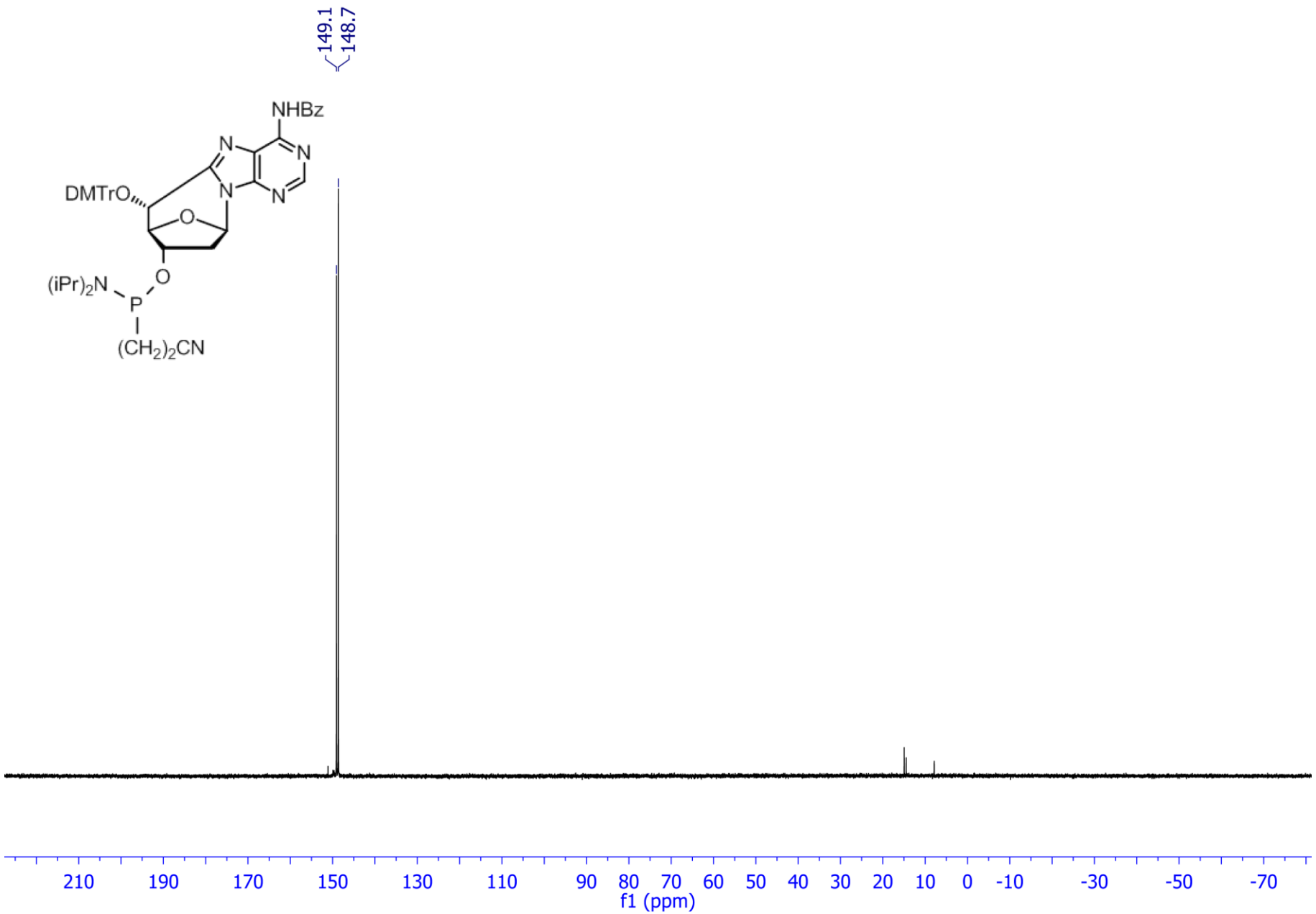
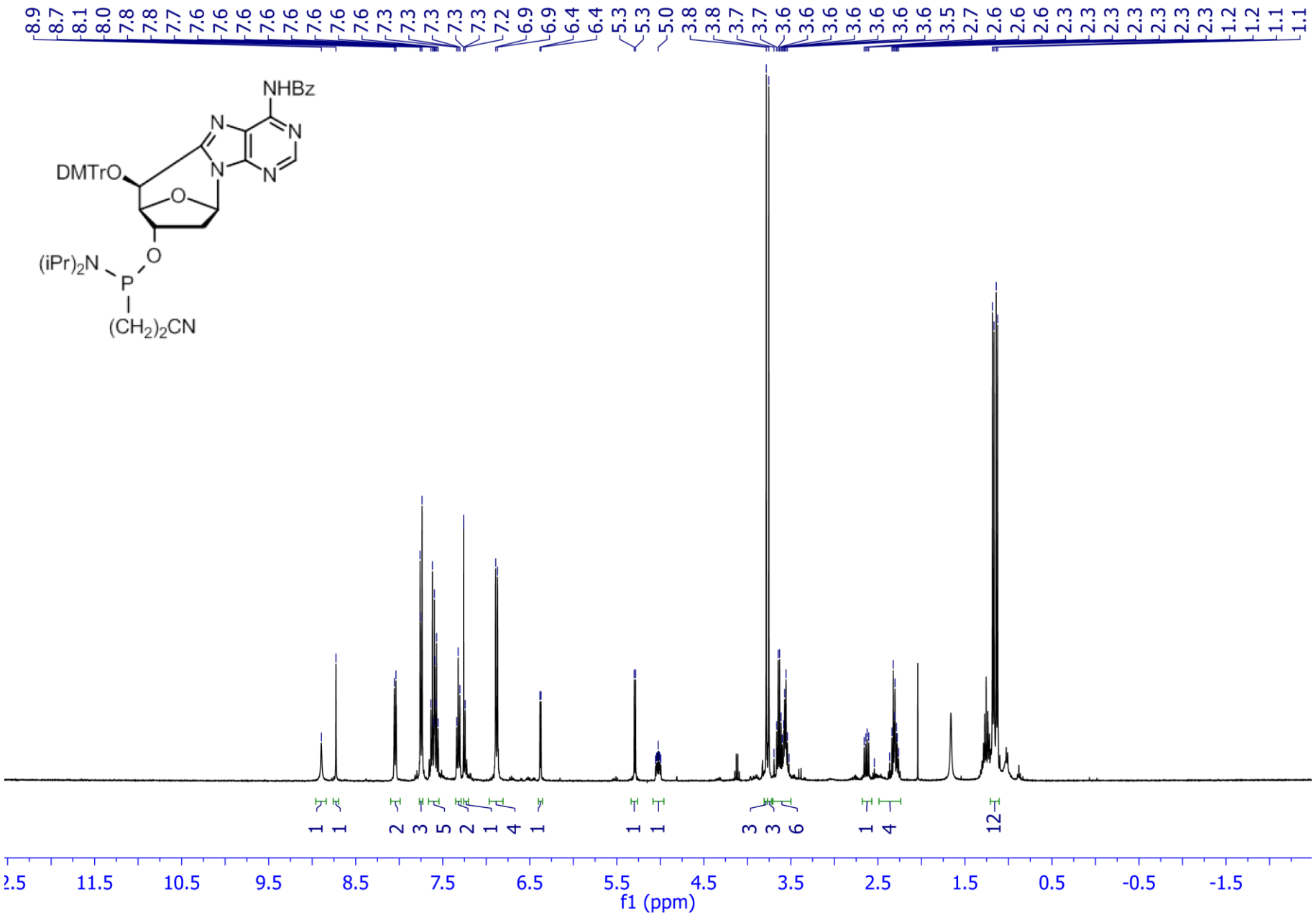
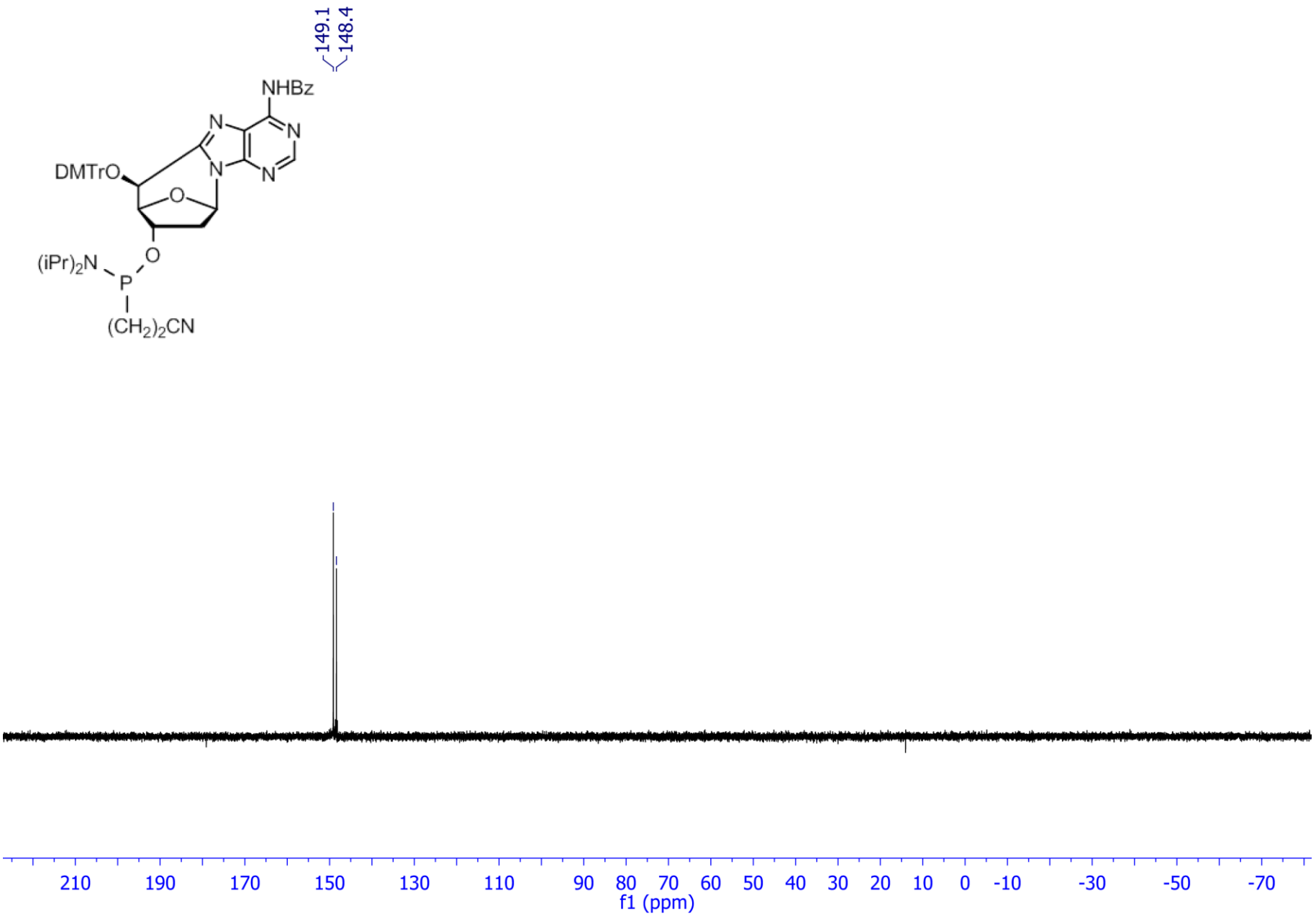
159.2
159.1
152.5
149.4
149.3
149.0
145.0
136.3
135.8
133.9
132.8
130.5
129.0
128.3
128.2
127.7
127.4
122.4
113.6
88.3
85.4
84.8
68.9
67.6
55.3
55.2
44.9
NHBz
N
N
N
N
DMTrO
O
OH
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
f1 (ppm)


NHBz
DMTrO
(iPr)2N
P
(CH2)2CN
f1 (ppm)


149.1
148.7
NHBz
N
N
N
N
DMTrO
O
O
(iPr)2N
P
(CH2)2CN
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
-50
-70
f1 (ppm)


NHBz
N
N
N
N
DMTrO
O
O
(iPr)2N
P
(CH2)2CN
f1 (ppm)


149.1
148.4
NHBz
N
N
N
N
DMTrO
O
O
(iPr)2N
P
(CH2)2CN
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-30
-50
-70
f1 (ppm)