Electronic Supplementary Information

Synthesis of Purine Derivatives of Me-TaNA and Properties of Me-TaNA-modified Oligonucleotides

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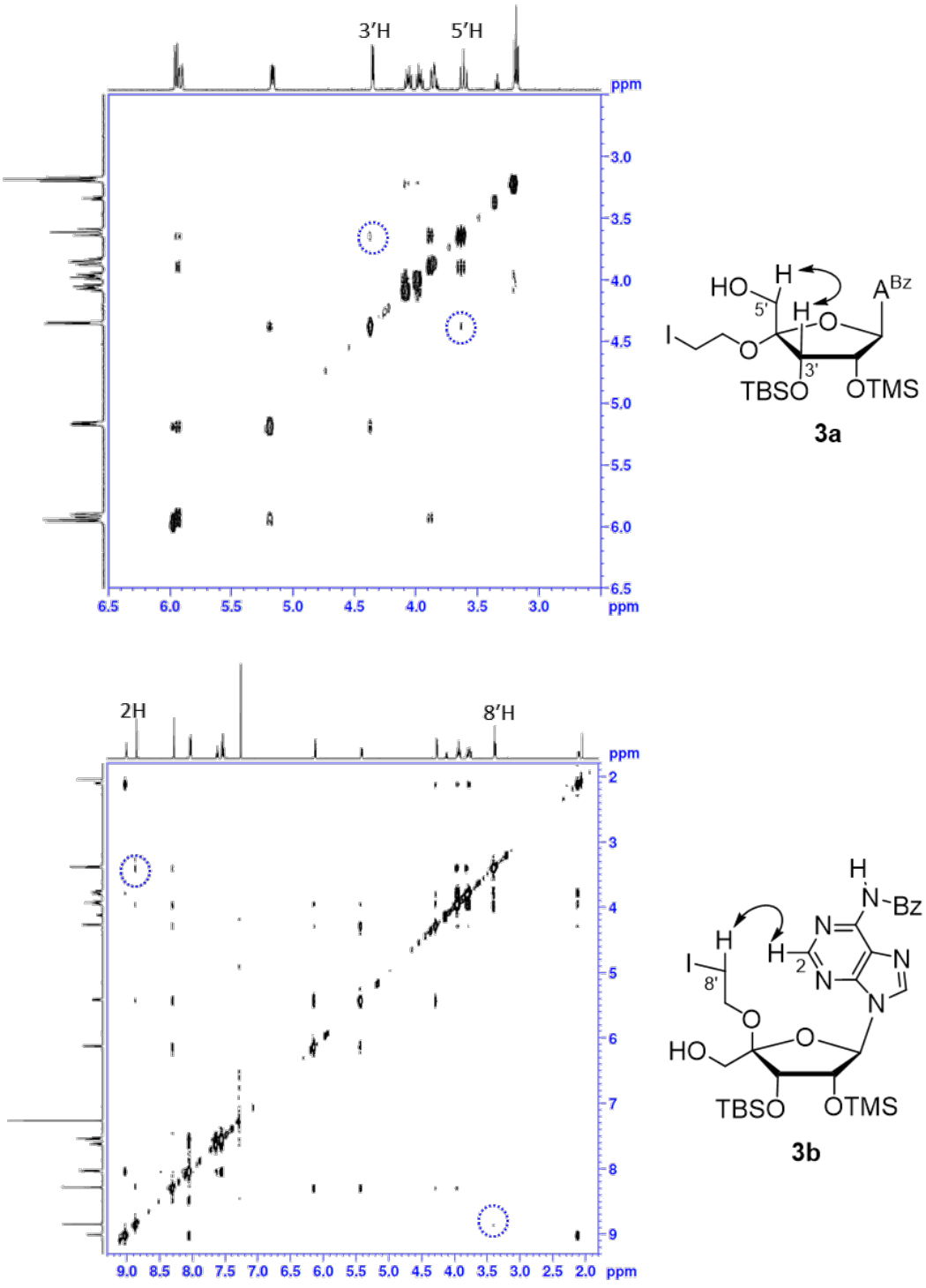
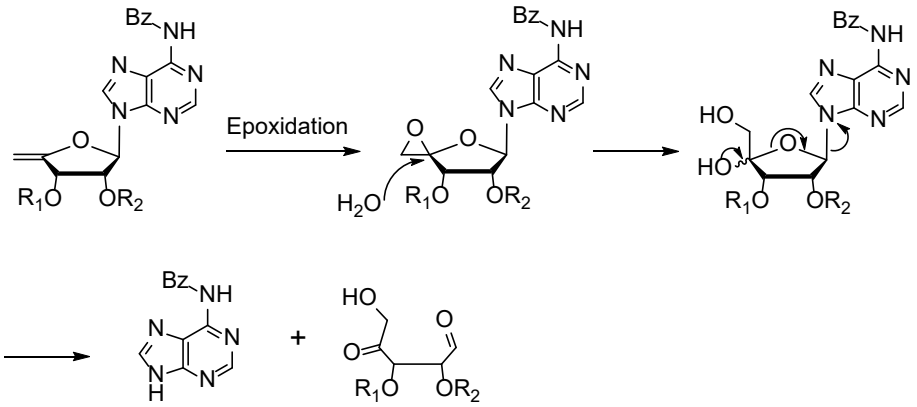


Figure S1. NOESY spectrum of compound **3a** (upper) and **3b** (lower). Measurement was performed in $CDCl_3$.



Scheme S1. Plausible depurination mechanism of epoxidized adenosine derivative.

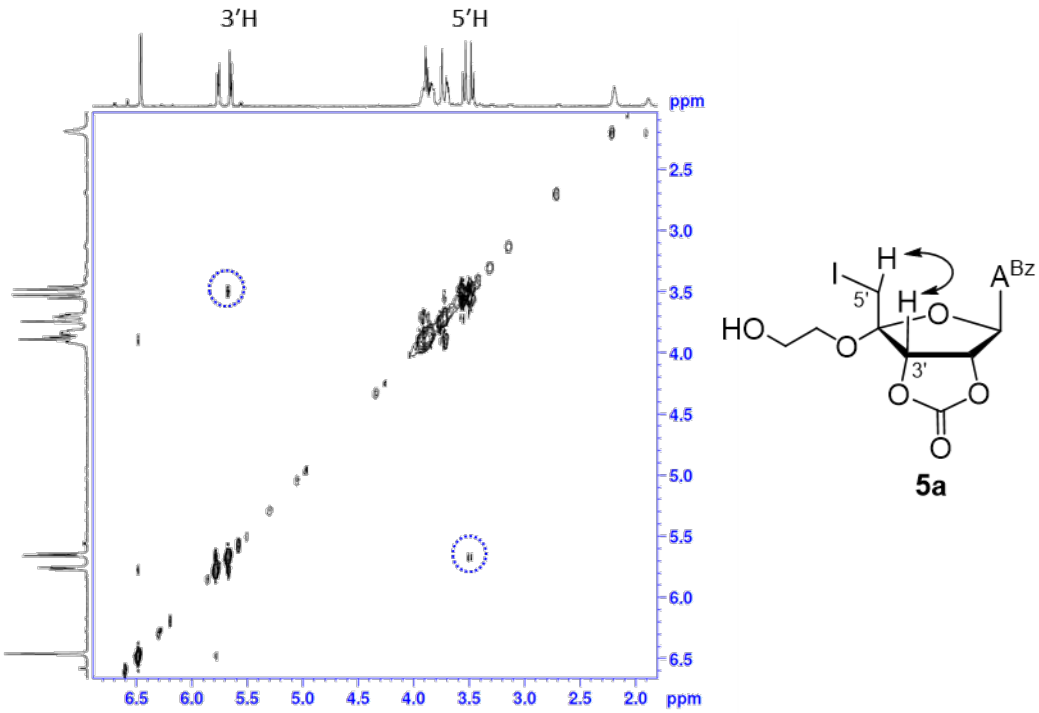


Figure S2. NOESY spectrum of compound **5a**. Measurement was performed in $CDCl_3$.

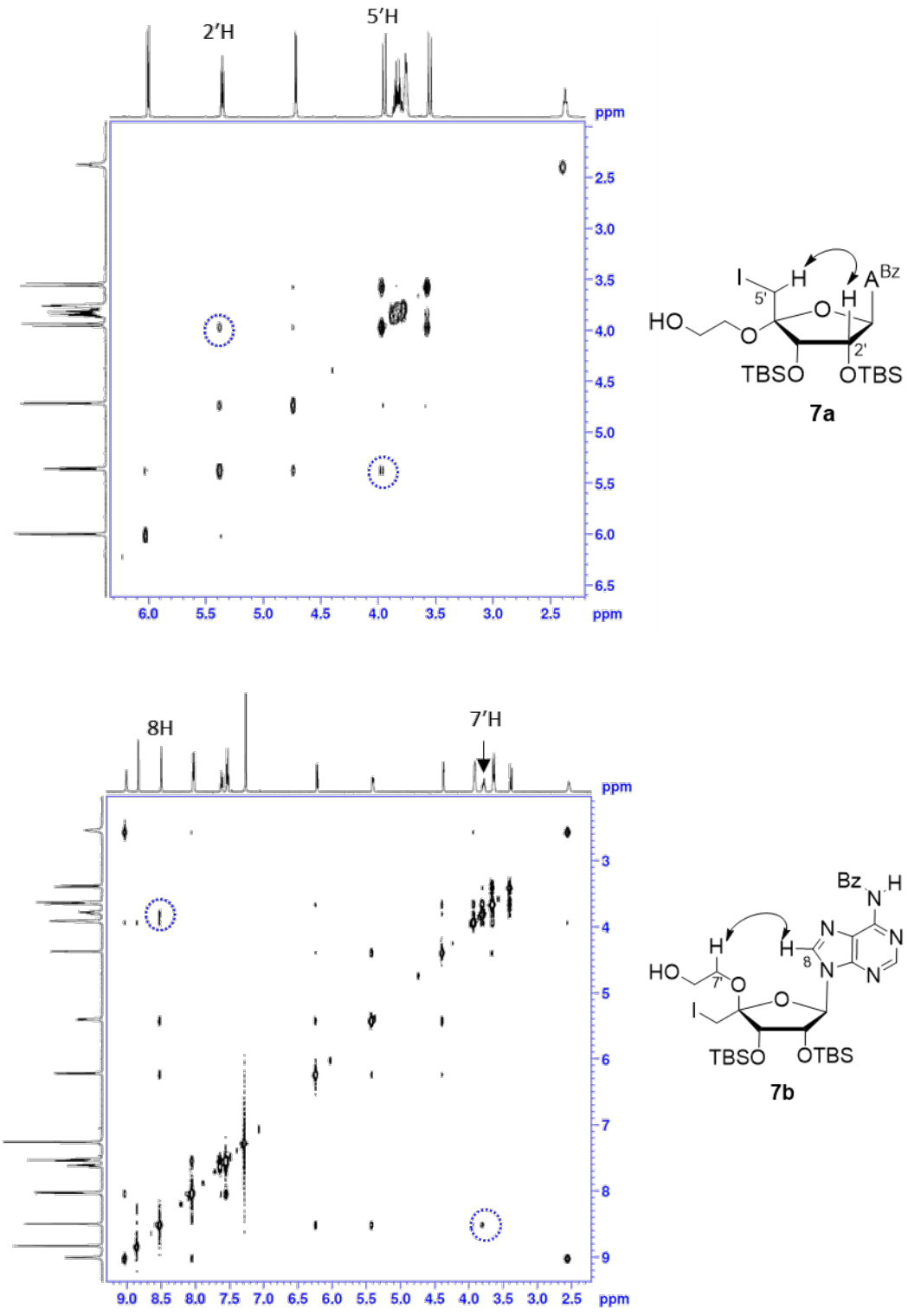


Figure S3. NOESY spectrum of compound **7a** (upper) and **7b** (lower). Measurement was performed in $CDCl_3$.

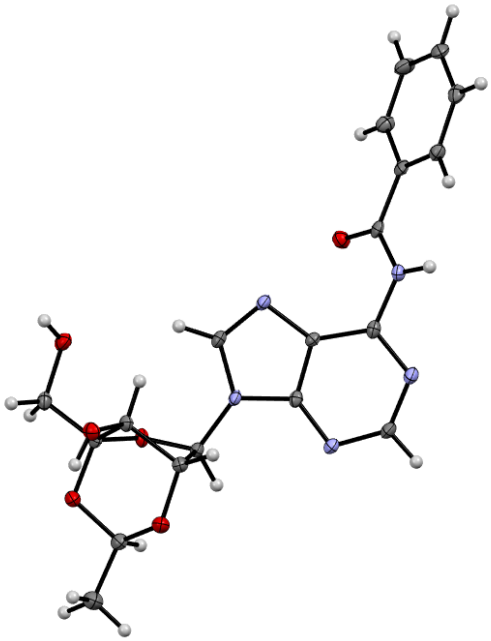


Figure S4. ORTEP drawings of compound **12** with 50% ellipsoid probability. The phase angles of pseudorotation was 13.8º.

Table S1. Crystallographic and Refinement Parameters of compound **12**.

	12
Empirical formula	$C_{19}H_{19}N_5O_6$
Formula weight	413.39
Crystal dimensions	0.2 x 0.4 x 0.4 mm
Crystal system	Monoclinic
Space group	*C2* (#5)
a / Å	23.660(2)
b / Å	6.1601(6)
c / Å	14.0178(13)
α / deg	90
β / deg	112.573(2)
γ / deg	90
Volume / Å^3	1886.5(3)
Z	4
Density (calc.) / g/cm^3	1.455
F_{000}	864
Reflections collected	4765
Unique reflections	3282
R_{int}	0.0269
Absorption coefficient / cm^{-1}	1.11
R_1 [I>2.00σ(I)]	0.0365
wR_2 (All reflections)	0.0974
Goodness-of-fit on F^2	1.029
Largest diff. peak/hole / e Å^{-3}	0.176/-0.256
CCDC No.	2253791

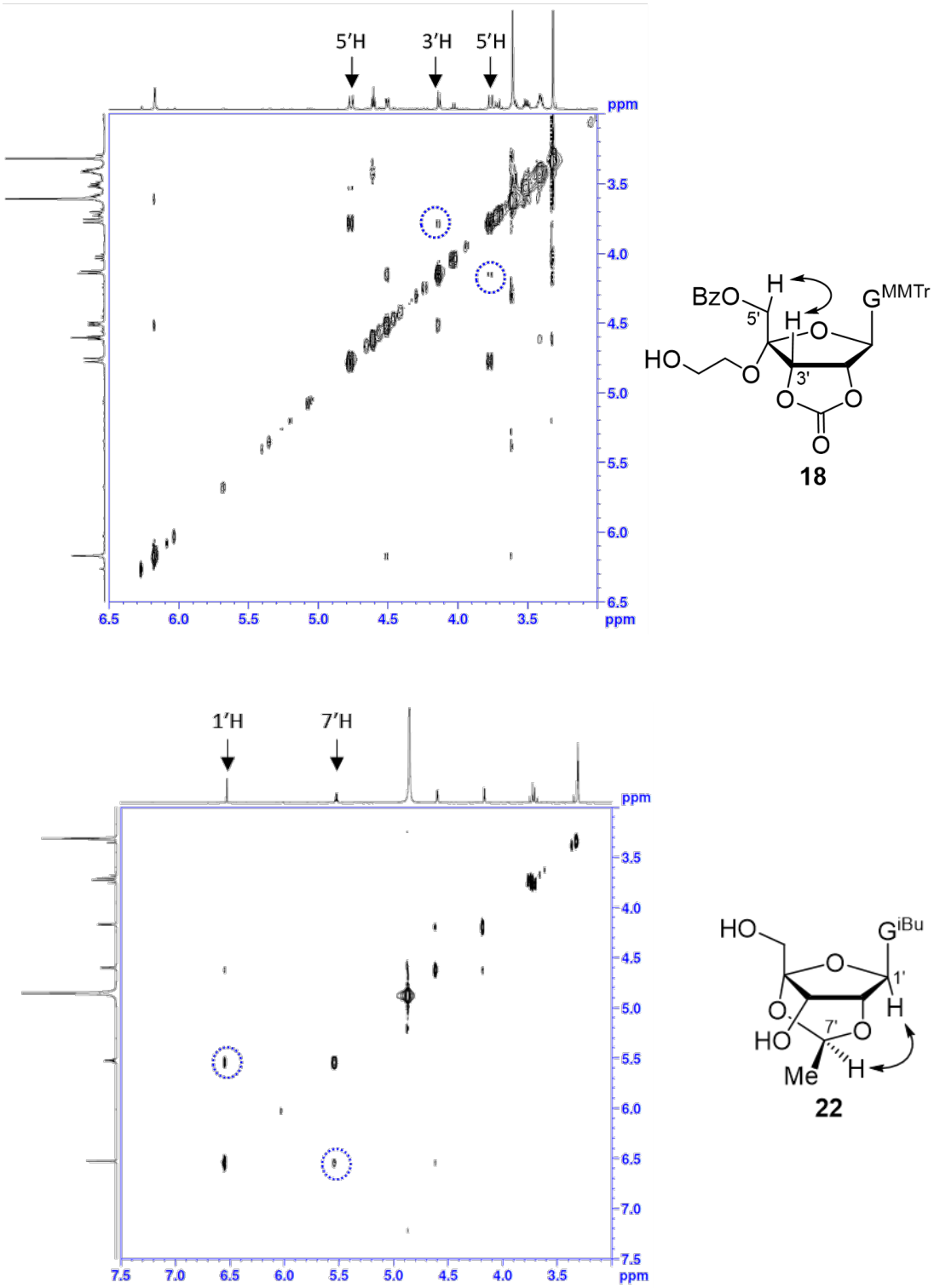
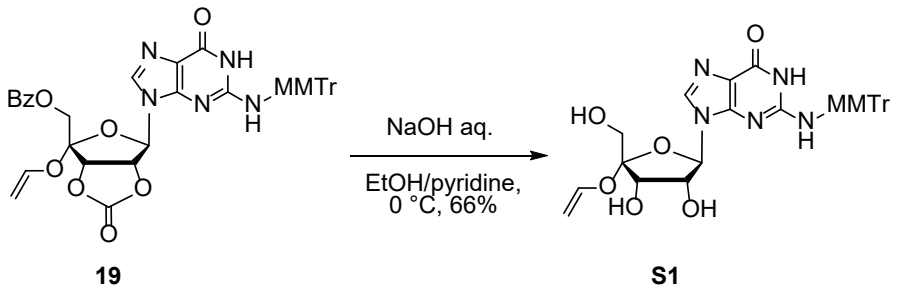


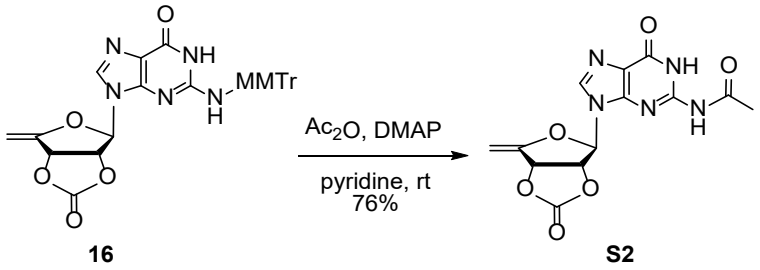
Figure S5. NOESY spectrum of compound **18** (upper) and compound **22** (lower). Measurement was performed in DMSO-d_6 and CD_3OD for compound **18** and **22**, respectively.



19 **S1**

Scheme S2. Synthesis of compound **S1**.

N^2-(4-Methoxytrityl)-4′-C-(vinyloxy)guanosine (**S1**). To a solution of compound **9** (240 mg, 0.33 mmol) in EtOH/pyridine (1:1, 3 mL), 2 M NaOH aq. (0.83 mL, 1.66 mmol) was added at 0 °C, and the reaction mixture was stirred at same temperature for 1 h. The reaction mixture was neutralized with DOWEX 50Wx8, and then filtered for removing the resins. The resulting filtrate was concentrated in vacuo. The obtained residue was purified by silica gel column chromatography ($CHCl_3$ / MeOH = 20:1) to give compound **S1** as a white solid (131 mg, 66%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 (s, 1H), 7.35–7.27 (m, 9H), 7.26–7.22 (m, 3H), 6.86 (d, 2H, *J* = 9.0 Hz), 6.53 (dd, 1H, *J* = 14.0, 6.0 Hz), 5.46–5.44 (m, 1H), 4.51 (d, 1H, *J* = 14.0 Hz), 4.12–4.09 (m, 3H), 3.78 (s, 3H), 3.48 3.43 (ABq, 2H, *J* = 12.0 Hz). ^{13}C{^{1}H} NMR (125 MHz, CD_3OD) δ 160.0, 159.3, 152.8, 151.7, 146.0, 146.2, 145.8, 138.4, 138.0, 131.3, 130.0, 130.0, 128.9, 128.9, 128.0, 128.0, 118.2, 114.2, 107.7, 93.0, 90.2, 73.1, 72.0, 72.0, 63.4, 55.7. IR (ATR) cm^{-1}: 3324, 1676, 1562, 1508. HRMS (ESI-TOF): calcd for $C_{32}H_{31}N_5O_7Na$ $[M+Na]^+$ 620.2121, found 620.2124.

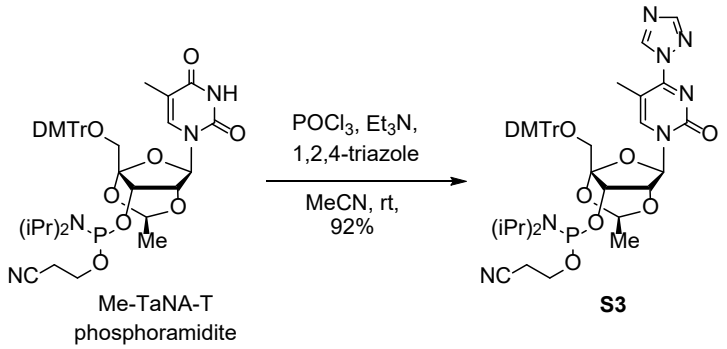


16 **S2**

Scheme S3. Substitution reaction of MMTr-protection (compound **16**) to acetyl protection (compound **S2**).

N^2-Acetyl-9-(2,3-O-carbonyl-5-deoxy-b-D-erythro-pento-4-enofuranosyl)guanine. To a solution of compound **16** (100 mg, 0.18 mmol) in anhydrous pyridine (2 mL), acetic anhydride (36 μL, 0.36 mmol) and DMAP (2.4 mg, 0.02 mmol) were added under an argon atmosphere. The reaction mixture was stirred at room temperature for 23 h and evaporated in vacuo. The obtained residue was purified by column chromatography (silica gel, $CHCl_3$/ MeOH = 20:1) to give compound **S2** as a white solid (47 mg, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.06 (s, 1H), 11.35 (s, 1H), 8.12 (s, 1H), 6.77 (s, 1H), 6.52 (d, 1H, *J* = 7.0 Hz), 5.88 (d, 1H, *J* = 7.0 Hz), 4.73 (d, 1H, *J* = 2.5 Hz), 4.59 (d, 1H, *J* = 2.5 Hz),

2.23 (s, 3H). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-d_6) δ 173.4, 158.4, 154.5, 153.3, 147.9, 147.5, 139.1, 120.6, 90.6, 87.9, 81.3, 78.3, 24.1. IR (ATR) cm^{-1}: 3147, 1794, 1669, 1609, 1559. HRMS (ESI-TOF): calcd for $C_{13}H_{10}N_5O_6$ $[M-H]^-$ 332.0631, found 332.0631.



Scheme S4. Synthesis of triazolyl compound **S3** from Me-TaNA-T phosphoramidite.

1-[(1R,3R,5S,7R,8S)-8-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-5-(4,4'-dimethoxytrityloxy)methyl-3-methyl-2,4,6-trioxabicyclo[3.2.1]octan-7-yl]-4-(1,2,4-triazolyl)thymine (**S3**). Under an argon atmosphere, $POCl_3$ (113 μL, 1.25 mmol) and Et_3N (1.0 mL, 7.47 mmol) were added dropwise to a solution of 1,2,4-triazole (430 mg, 6.23 mmol) in MeCN (15 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC, and then Me-TaNA-T phosphoramidite (200 mg, 0.25 mmol) in MeCN (5 mL) was added to the mixture. The reaction mixture was further stirred at room temperature for 2 h before being quenched by sat. $NaHCO_3$ aq., and then diluted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/2 to 1/3) to obtain compound **S3** (195 mg, 92%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.26 (s, 0.5H), 9.25 (s, 0.5H), 8.40 (s, 1H), 8.07 (s, 0.5H), 8.06 (s, 0.5H), 7.46–7.40 (m, 2H), 7.35–7.27 (m, 7H), 6.89–6.82 (m, 4H), 6.33 (s×2, 1H), 5.47–5.42 (m, 1H), 4.79–4.77 (m, 1H), 4.37–4.32 (m, 1H), 3.87–3.79 (m, 7H), 3.67–3.50 (m, 5H), 3.44–3.41 (m, 1H), 2.64–2.48 (m, 1H), 2.45–2.34 (m, 1H), 1.70 (s, 1.5H), 1.66 (s, 1.5H), 1.38–1.36 (m, 3H), 1.19–1.08 (m, 12H) $^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$) δ: 150.2, 149.3. IR (ATR) ν: 2966, 2933, 2877, 2837, 1675, 1630, 1607, 1502 cm^{-1}. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{44}H_{52}N_7O_9NaP$ 876.3462, Found 876.3469.

Table S2. ESI-MS analysis data and the overall yields of new ONs produced in this study.

ONs	Formula	Calcd. [M]	Found [M]	Overall yield (%)
ON1	$C_{118}H_{148}N_{49}O_{68}P_{11}$	3681.47	3682.00	16%
ON2	$C_{118}H_{148}N_{49}O_{68}P_{11}$	3681.47	3681.90	17%
ON3	$C_{120}H_{150}N_{49}O_{70}P_{11}$	3739.50	3740.00	15%
ON4	$C_{120}H_{150}N_{49}O_{70}P_{11}$	3739.50	3740.00	14%
ON5	$C_{144}H_{178}N_{49}O_{90}P_{11}$	4375.97	4376.00	5%

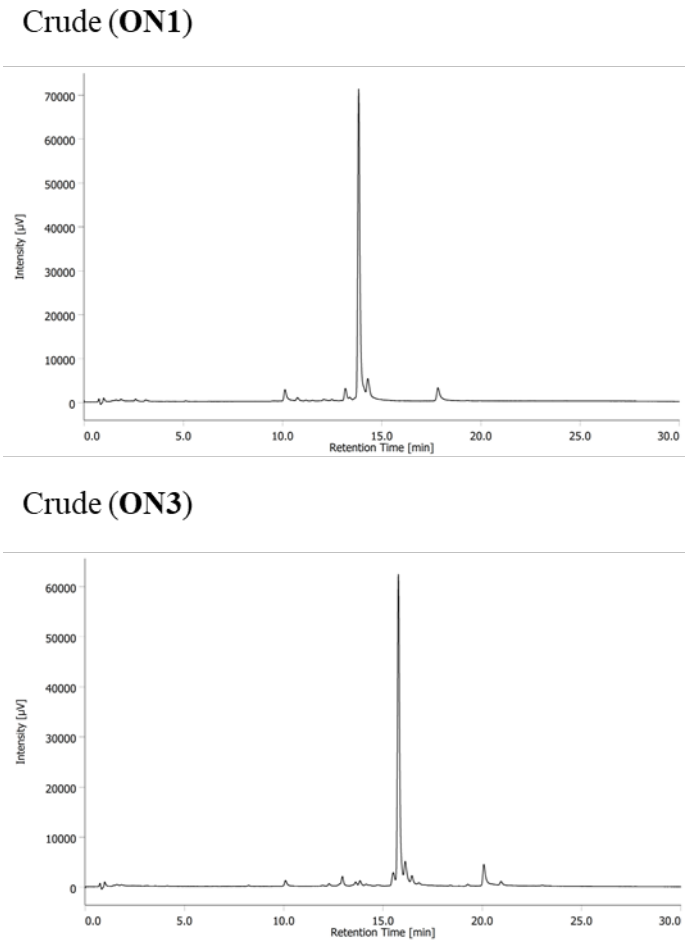


Figure S6. HPLC chromatograms of the crude products (**ON1** and **ON3**) before HPLC purification. HPLC conditions: Waters XBridge® MS C_{18} 5.0 μm, 4.6 × 50 mm; gradient, 5-15% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min; flow rate, 1.0 mL/min; column temp., 40 ˚C.

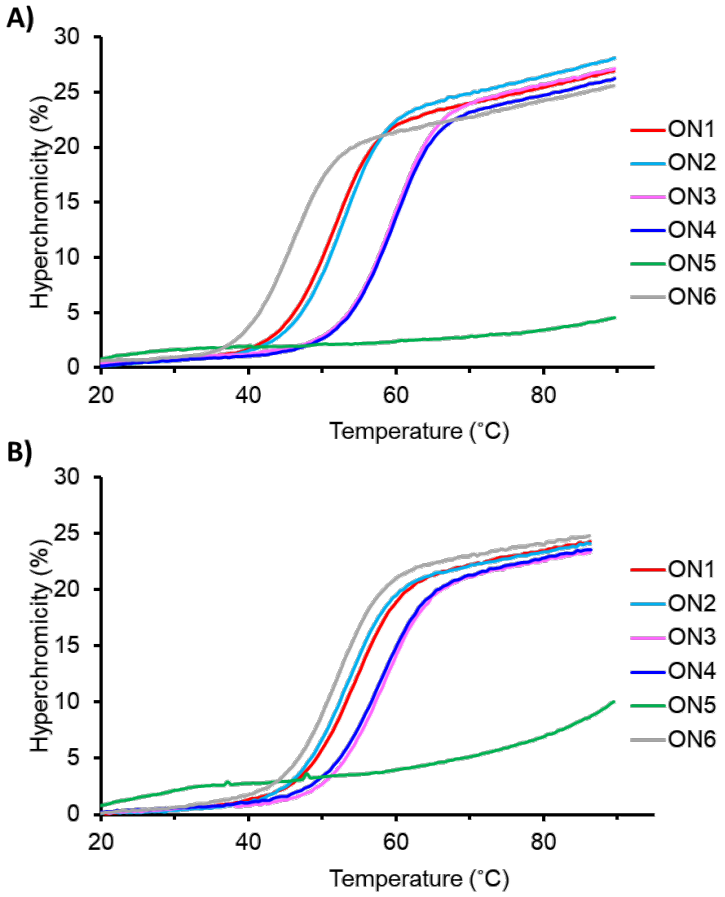


Figure S7. UV melting curves of DNA-RNA duplex and DNA-DNA duplex. A) For target ssRNA and B) for target ssDNA

Table S3. Duplex stability of ONs modified by 2′,4′-BNA/LNA with ssRNA and ssDNA [a]

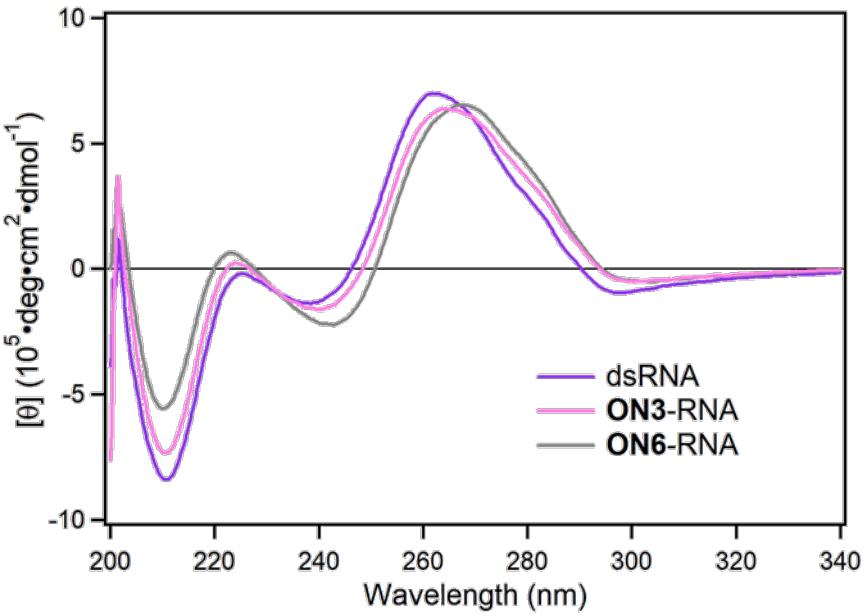
ON (5′ to 3′)	ssRNA	ssDNA
	T_m (ΔT_m/mod.) (°C)	T_m (ΔT_m/mod.) (°C)
ACGAGAACATCC	50 (+5.0)	54 (+3.0)
ACGAGAACATCC	52 (+7.0)	54 (+3.0)
ACGAGAACATCC	58 (+6.5)	57 (+3.0)
ACGAGAACATCC	58 (+6.5)	57 (+3.0)
ACGAGAACATCC (**ON6**)	45	51

[a] Conditions: 10 mM sodium phosphate buffer (pH 7.0) containing 200 mM NaCl and 2.5 μM of each ON. Underlined letters in the sequences denotes the 2′,4′-BNA/LNA derivatives. The ssRNA and ssDNA sequences were 5′-r(GGAUGUUCUCGU)-3′ and 5′-d(GGATGTTCTCGT)-3′, respectively. ΔT_m /mod.: Change in T_m value per modification relative to natural DNA (ON6).

Table S4. T_m and ΔT_m values (°C) of full- and mis-match duplexes with ssRNA and ssDNA[a]

	ssRNA: 5′-r(GGAUG**Y**UCUCGU)-3′			
5′-ACGAGA**X**CATCC-3′	**Y** = U	**Y** = A	**Y** = G	**Y** = C
X = Me-TaNA-A (**ON1**)	51	39 (−12)	42 (−9)	41 (−10)
X = A (**ON6**)	45	36 (−9)	38 (−7)	36 (−9)
	ssDNA: 5′-d(GGATG**Y**TCTCGT)-3′			
5′-ACGAGA**X**CATCC-3′	**Y** = T	**Y** = A	**Y** = G	**Y** = C
X = Me-TaNA-A (**ON1**)	54	41 (−13)	45 (−9)	42 (−12)
X = A (**ON6**)	51	39 (−12)	45 (−6)	40 (−11)

[a] Conditions: 10 mM sodium phosphate buffer (pH 7.0) containing 200 mM NaCl and 2.5 μM of each ON. The ΔT_m values, which are the changes in T_m values relative to those of the full-match duplex (Y = U or T), are shown in parentheses.



FigureS8. CD spectra of duplexes with RNA complements by DNA (**ON6**) and **ON3**. The sequence of RNA complement was 5′-r(GGAUGUUCUCGU)-3′. dsRNA denotes RNA duplex (5′-r(ACGAGAACAUCC)-3′/3′-r(UGCUCUUGUAGG)-5′). Conditions: 10 mM sodium phosphate buffer (pH 7.0) containing 200 mM NaCl and 2.5 μM of each ON.

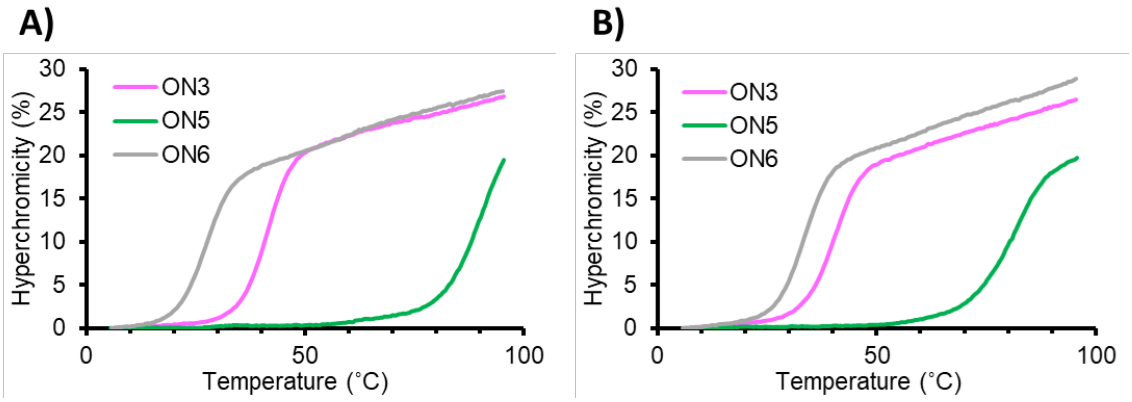


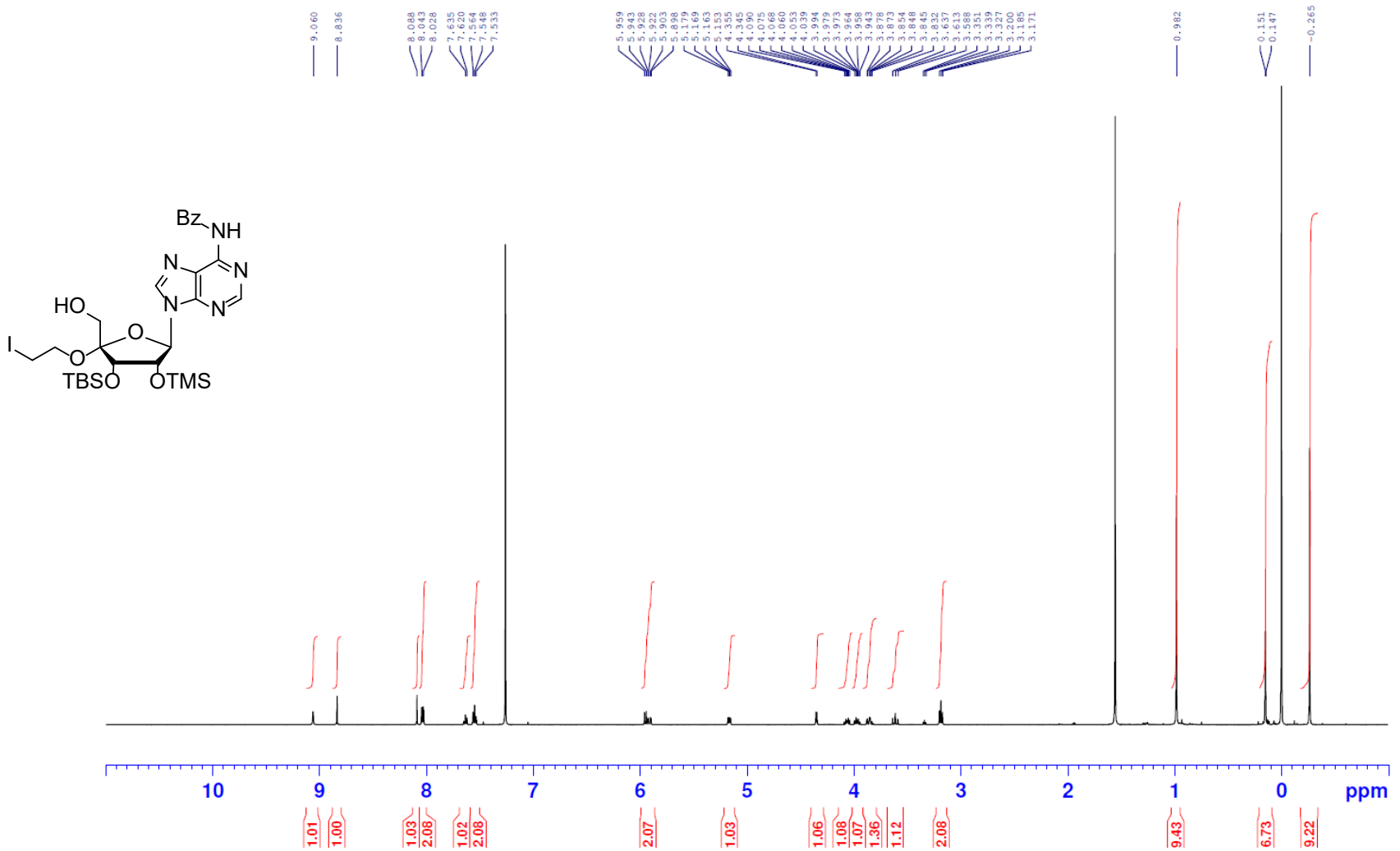
Figure S9. UV melting curves of DNA-RNA duplex (**A**) and DNA-DNA duplex (**B**). Conditions: 10 mM sodium phosphate buffer (pH 7.0) containing 2.5 μM of each ON. The ssRNA and ssDNA sequences are 5′-r(GGAUGUUCUCGU)-3′ and 5′-d(GGATGTTCTCGT)-3′, respectively. T_m values; **ON3**: 41 and 40 ºC, **ON5**: > 90 and 82 ºC, **ON6**: 27 and 33 ºC for RNA and DNA, respectively.

Table S5. Duplex stability of modified ONs.[a]

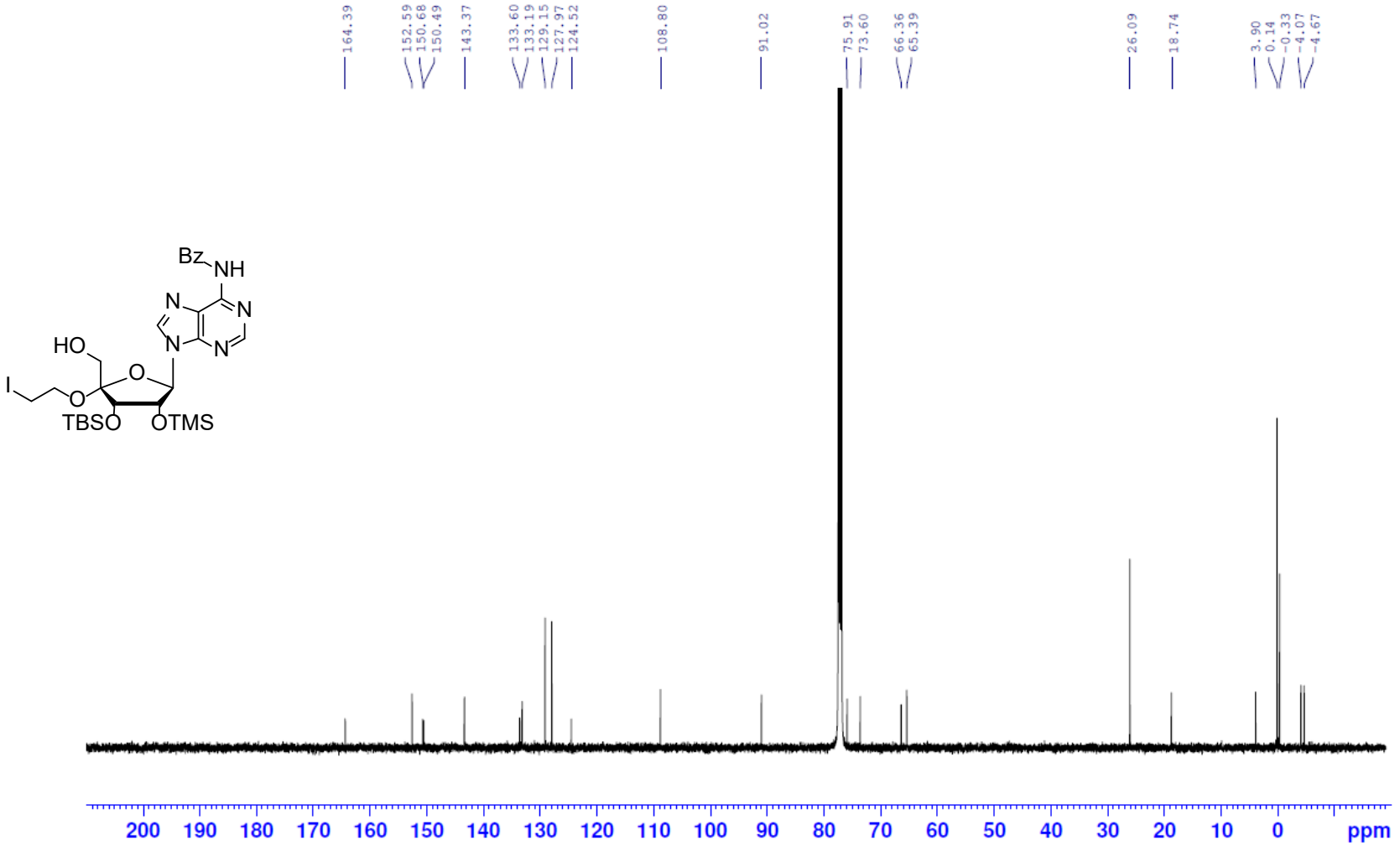
Duplexes	T_m ΔT_m/mod. (°C)
5′-d(ACGAGAA**A**CATCC)-3′ 3′-d(TGCTCT**T**GTAGG)-5′	56 (+2.5)
5′-d(ACGA**G**AACATCC)-3′ 3′-d(TGCT$^{\mathbf{m}}$**C**TTGTAGG)-5′	55 (+2.0)
5′-d(ACGA**GA**ACATCC)-3′ 3′-d(TGCT$^{\mathbf{m}}$**CT**TGTAGG)-5′	67 (+4.0)
5′-d(ACGA**G**AA**A**CATCC)-3′ 3′-d(TGCT$^{\mathbf{m}}$**C**TT**T**GTAGG)-5′	68 (+4.3)
5′-d(ACGAGAACATCC)-3′ 3′-d(TGCTCTTGTAGG)-5′	51

[a]Conditions: 10 mM sodium phosphate buffer (pH 7.0) containing 200 mM NaCl and 2.5 μM of each ON. Bold letters in the sequences denotes Me-TaNA derivatives.

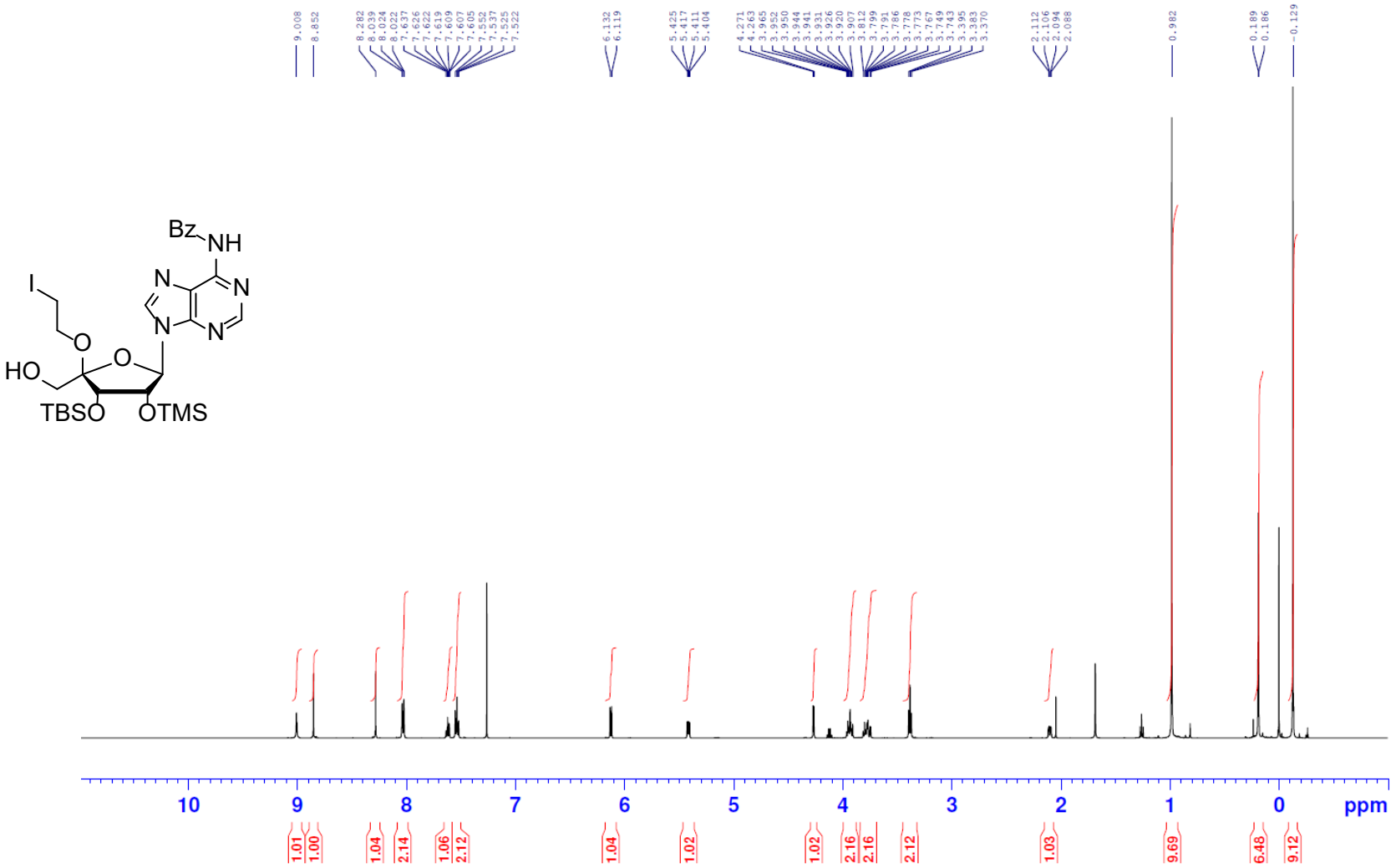
Compound **3a** (500 MHz ^{1}H NMR in $CDCl_3$)



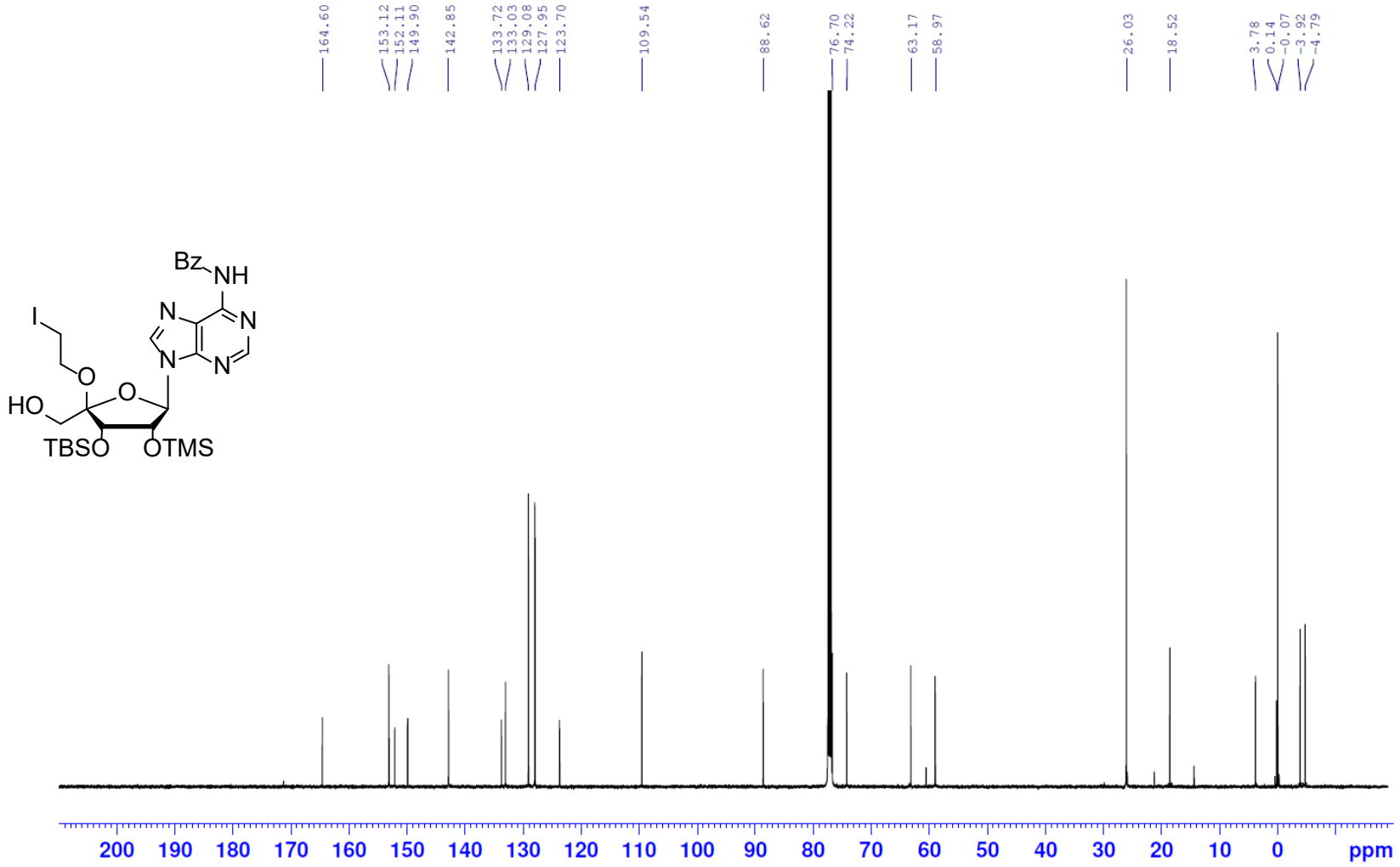
Compound **3a** (125 MHz ^{13}C NMR in $CDCl_3$)



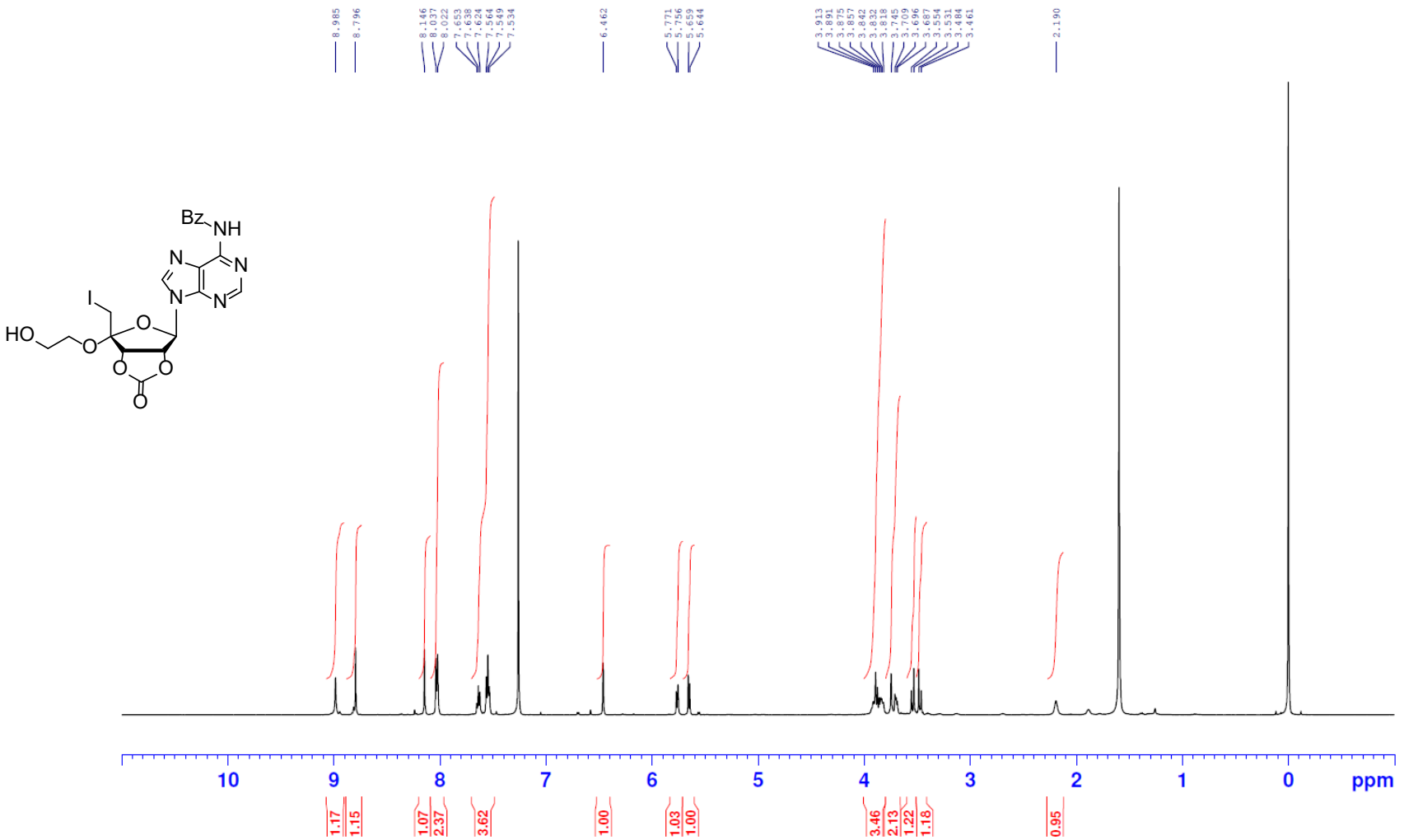
Compound **3b** (500 MHz ^{1}H NMR in $CDCl_3$)



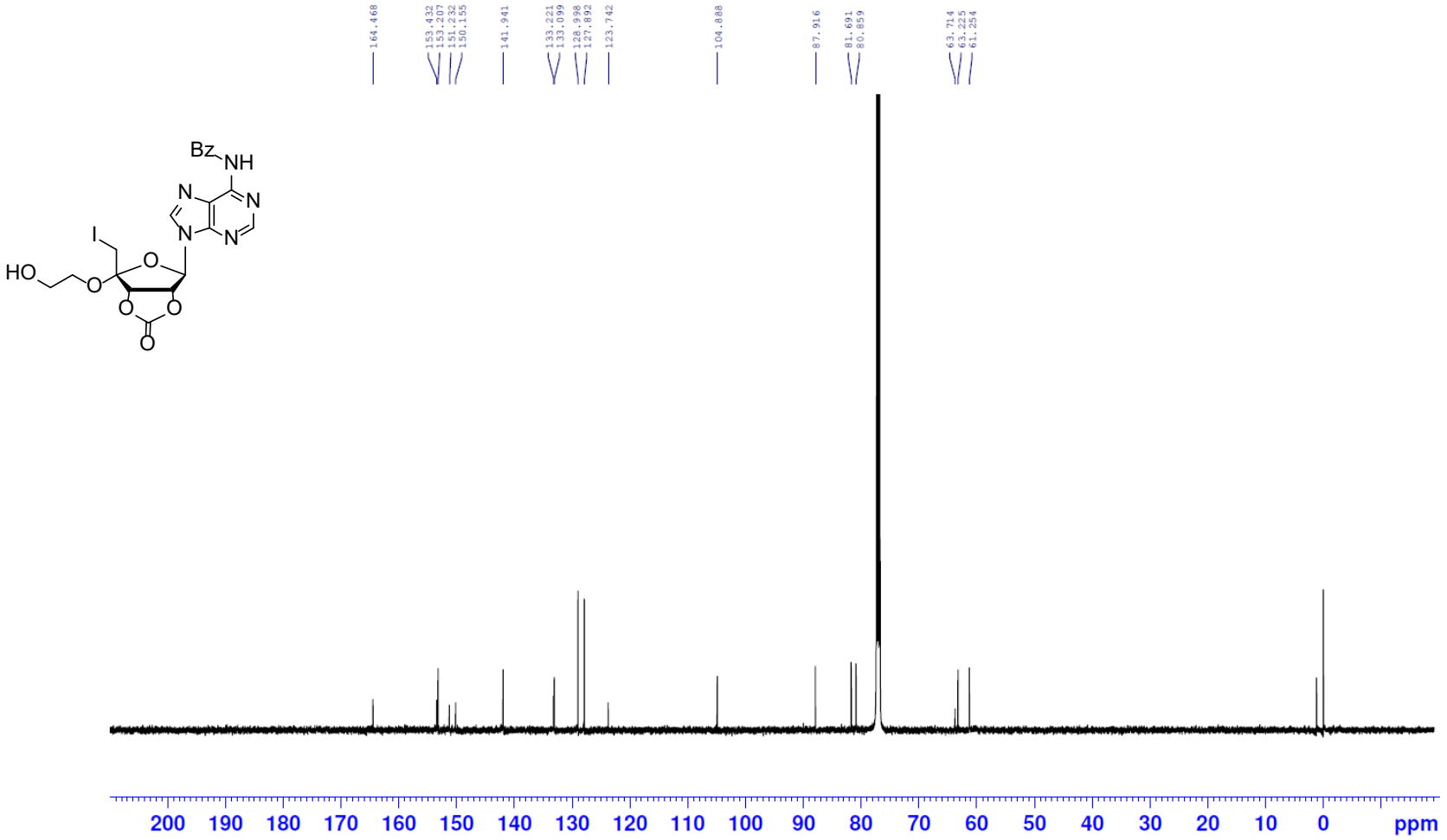
Compound **3b** (125 MHz ^{13}C NMR in $CDCl_3$)



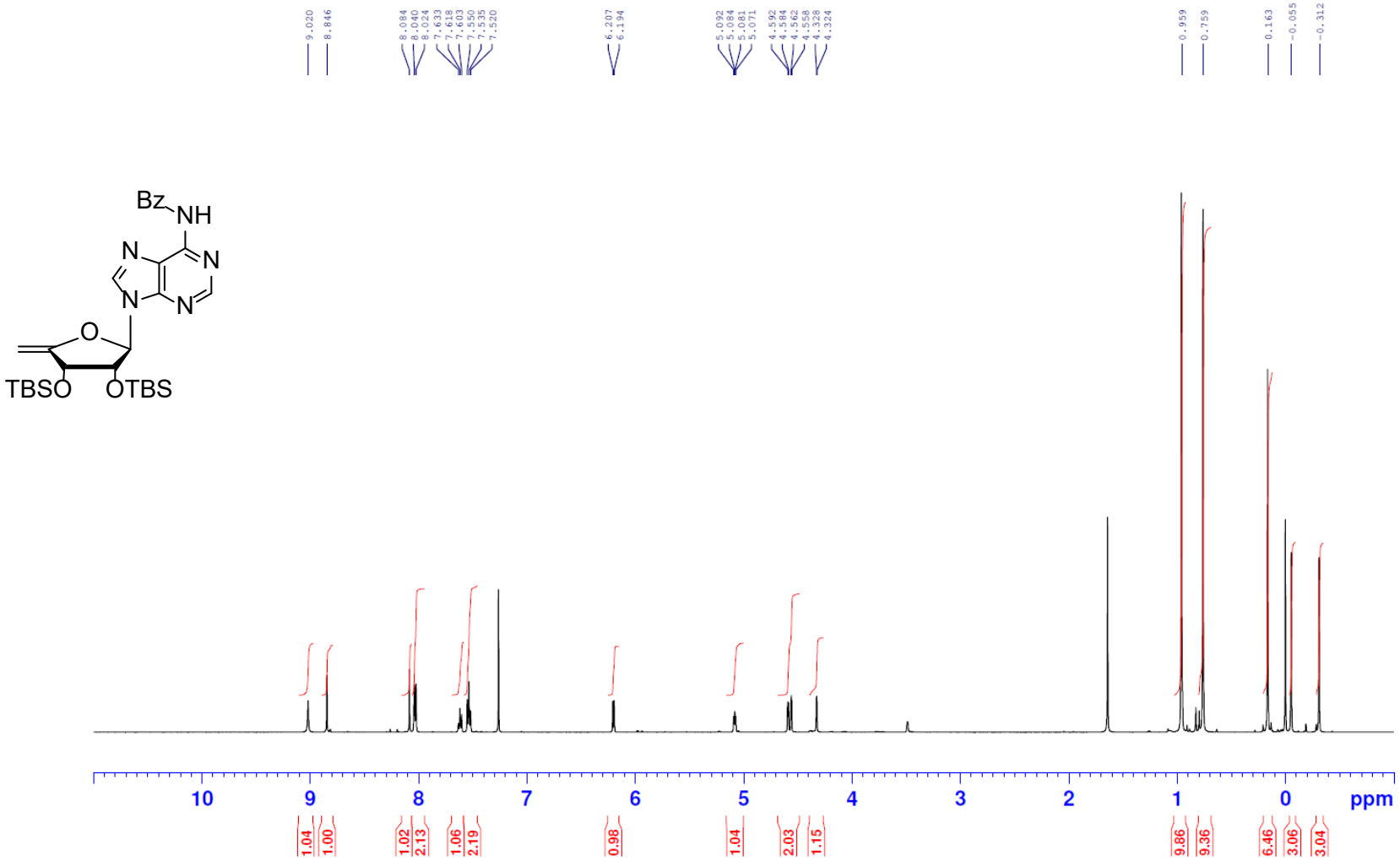
Compound **5a** (500 MHz ^{1}H NMR in $CDCl_3$)



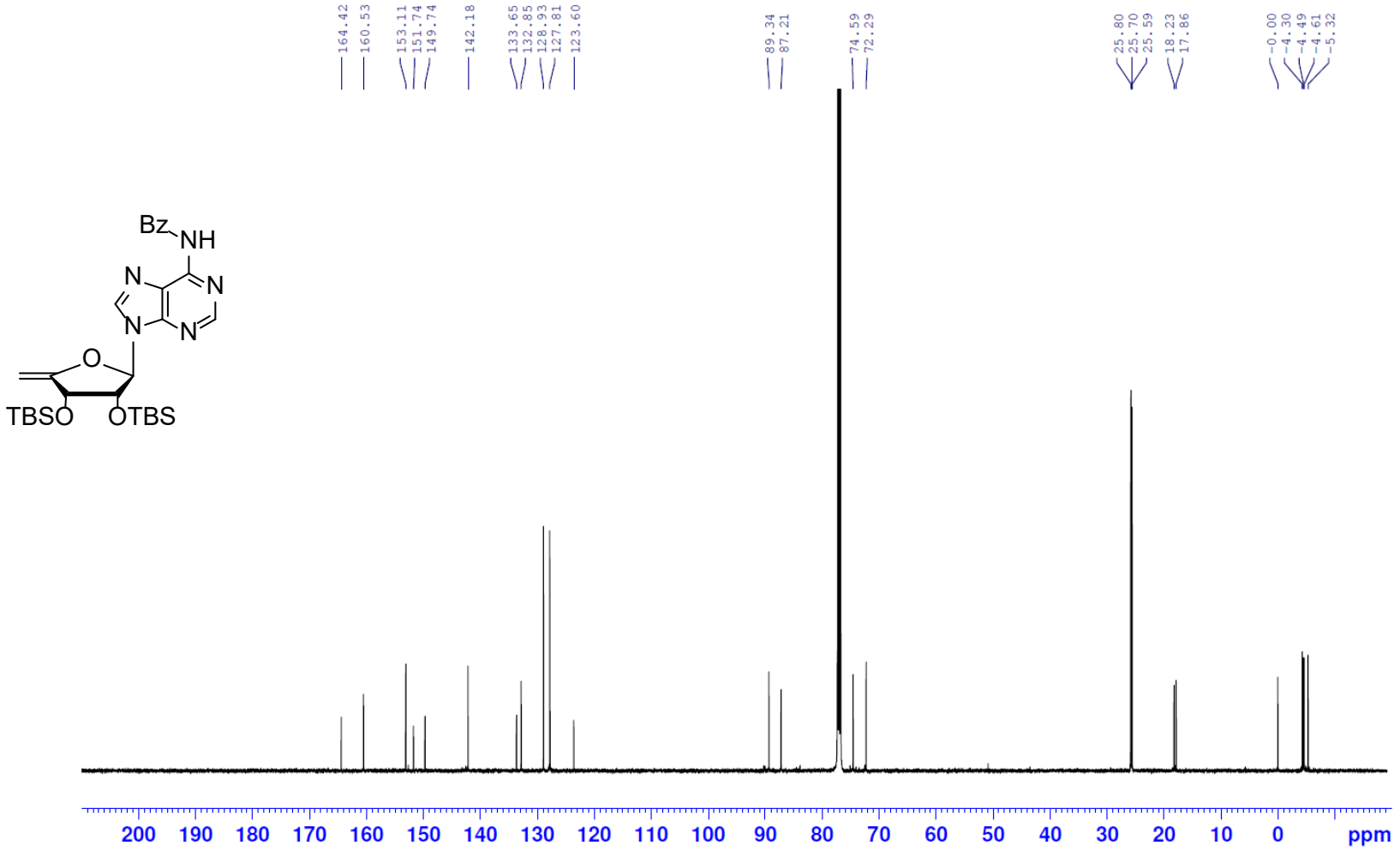
Compound **5a** (125 MHz ^{13}C NMR in $CDCl_3$)



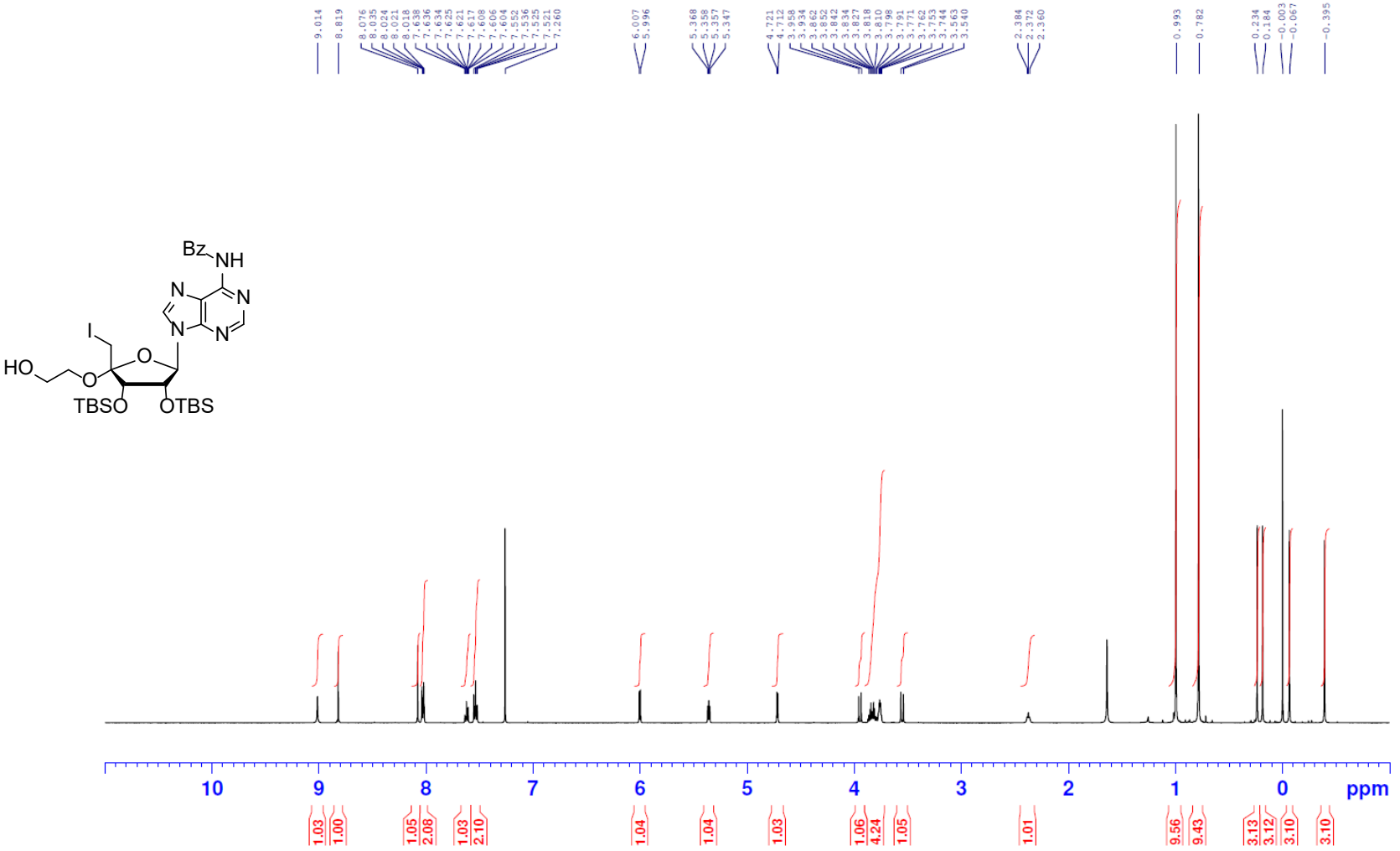
Compound **6** (500 MHz ^{1}H NMR in $CDCl_3$)



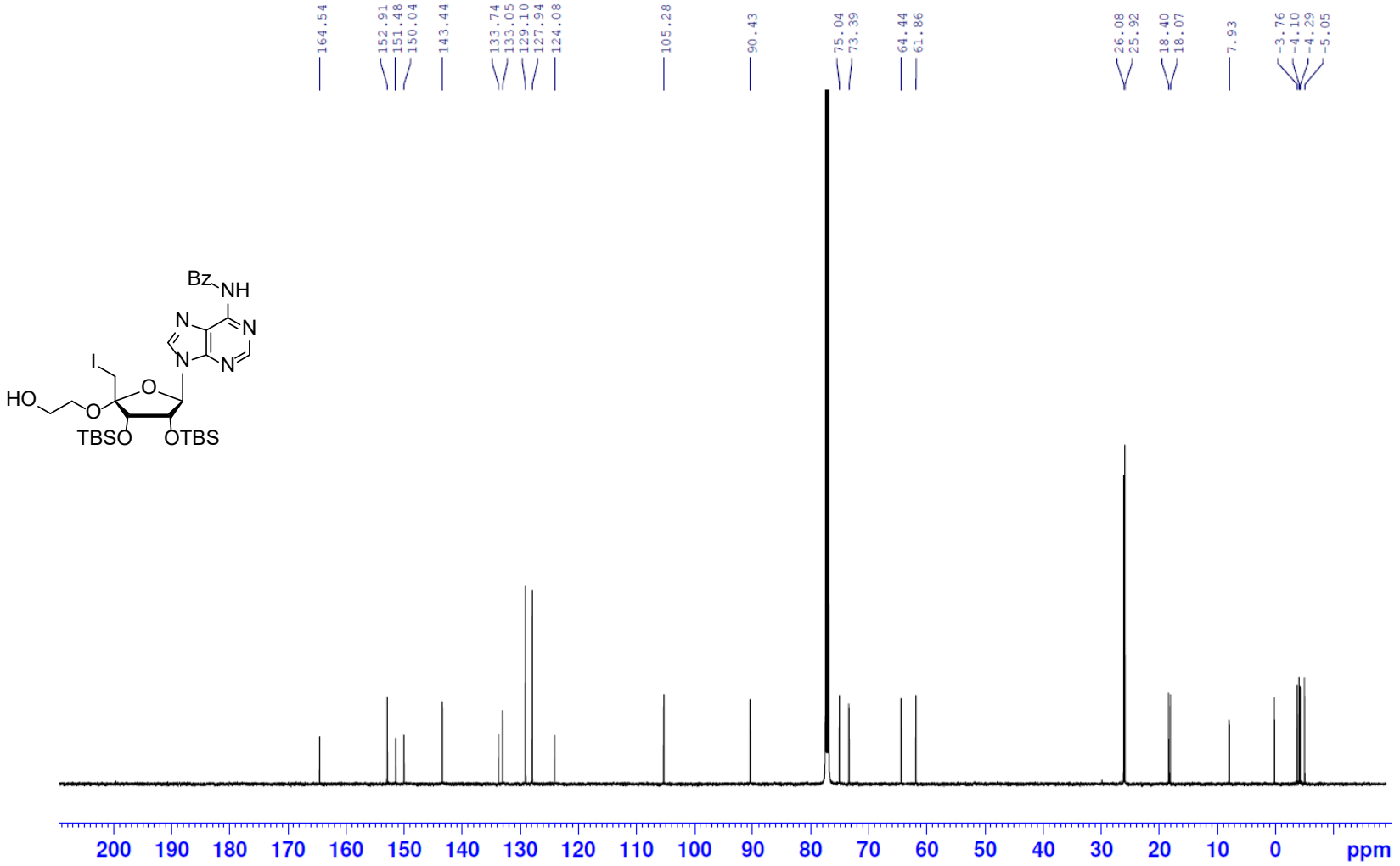
Compound **6** (125 MHz ^{13}C NMR in $CDCl_3$)



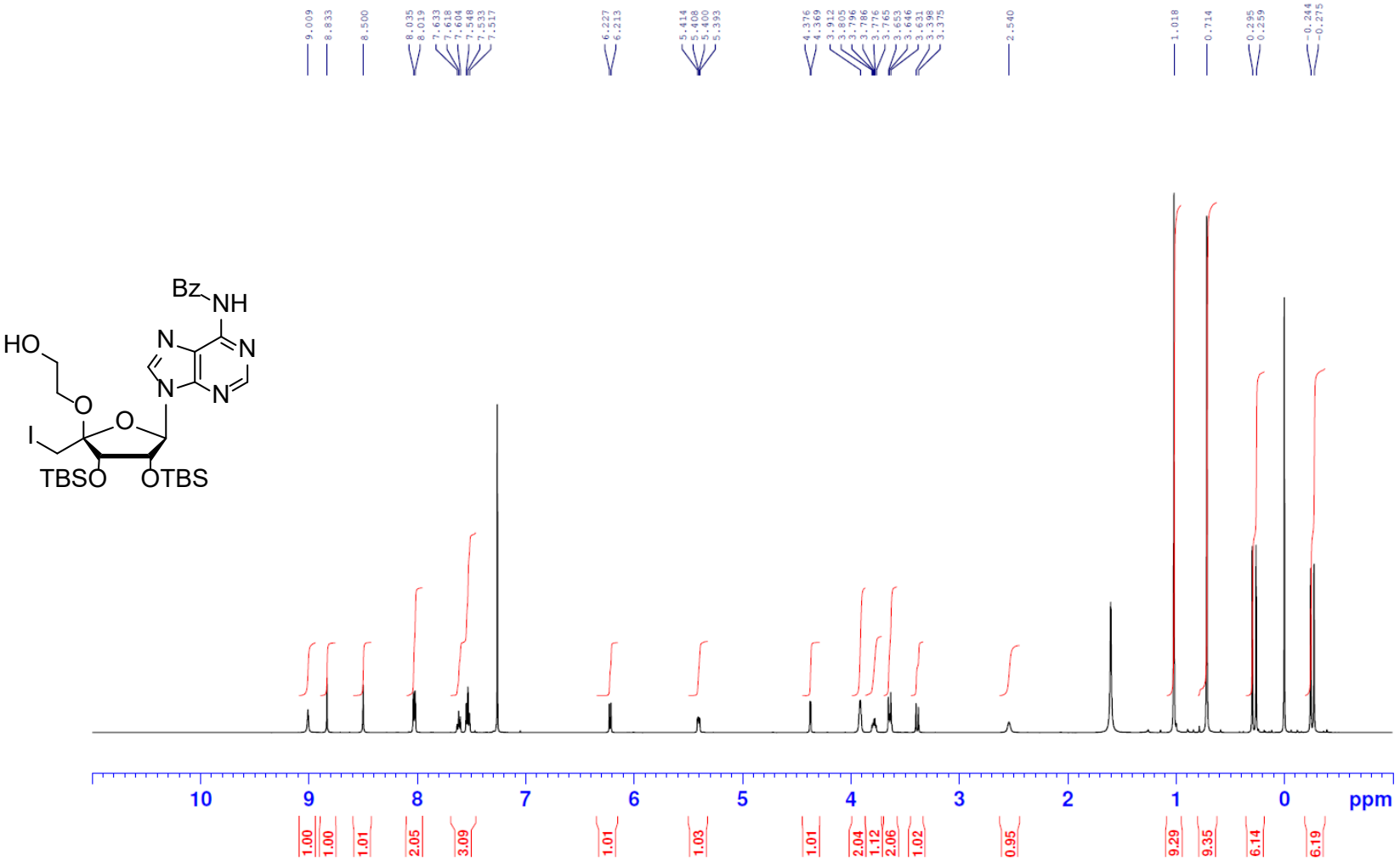
Compound **7a** (500 MHz ^{1}H NMR in $CDCl_3$)



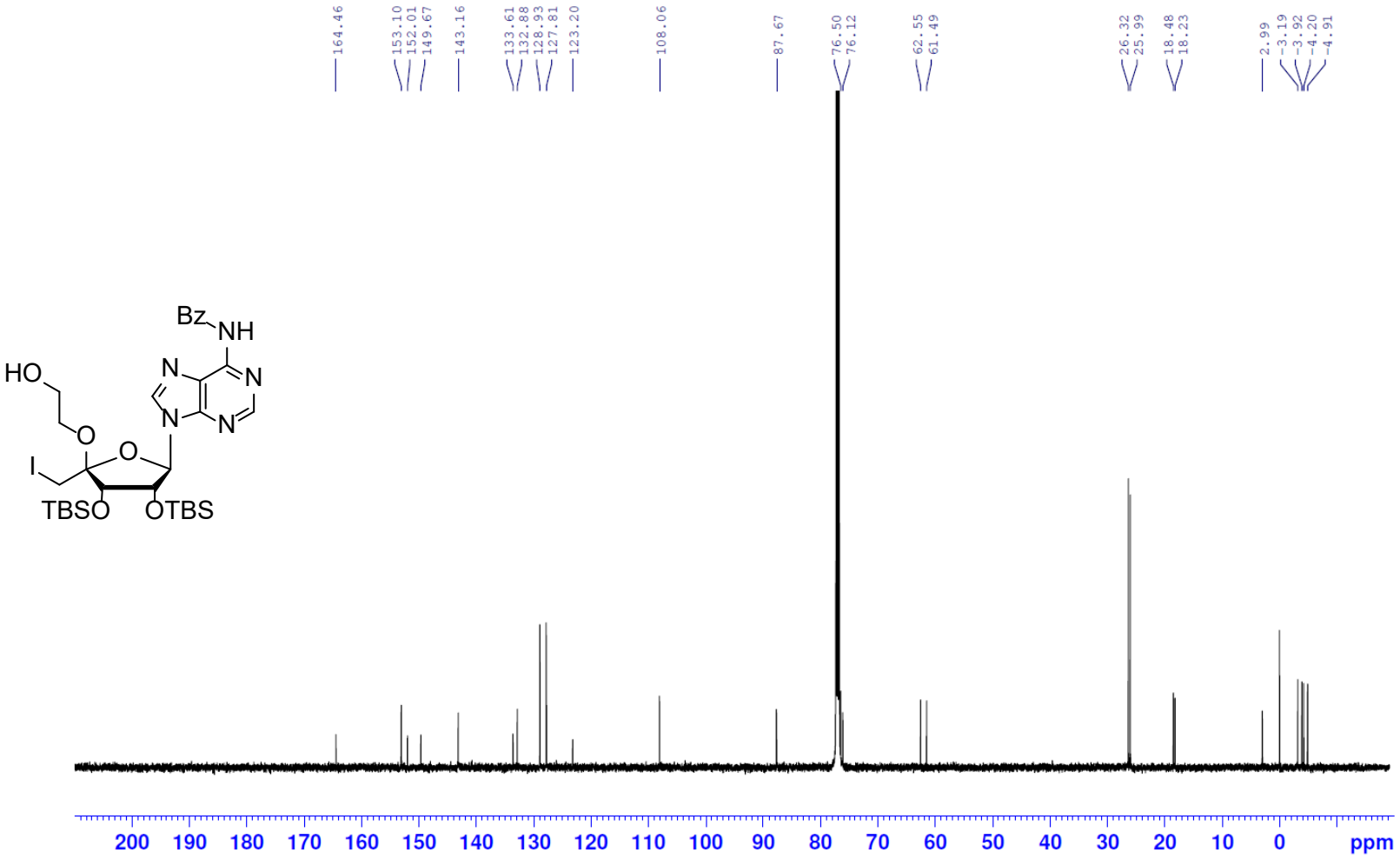
Compound **7a** (125 MHz ^{13}C NMR in $CDCl_3$)



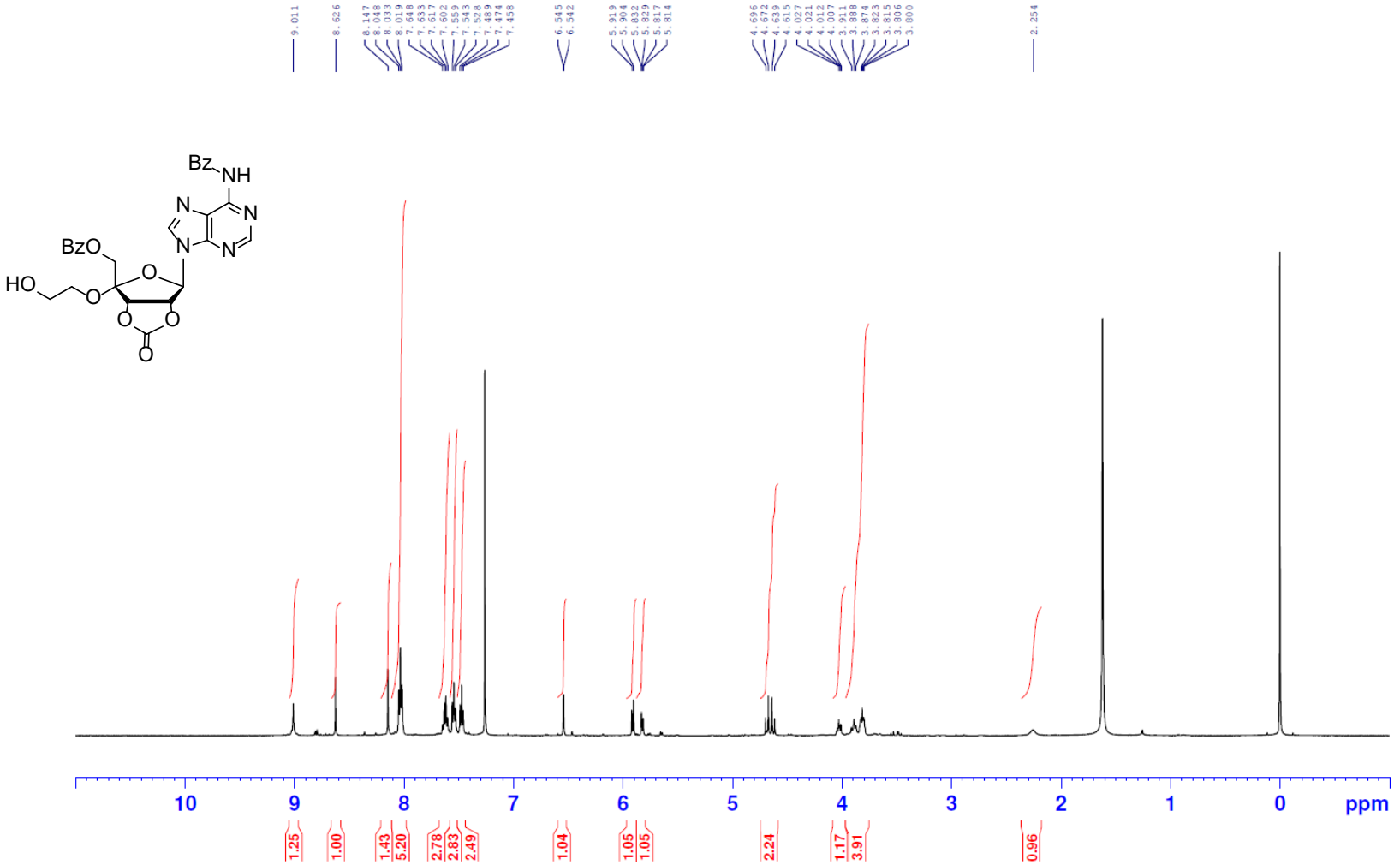
Compound **7b** (500 MHz ^{1}H NMR in $CDCl_3$)



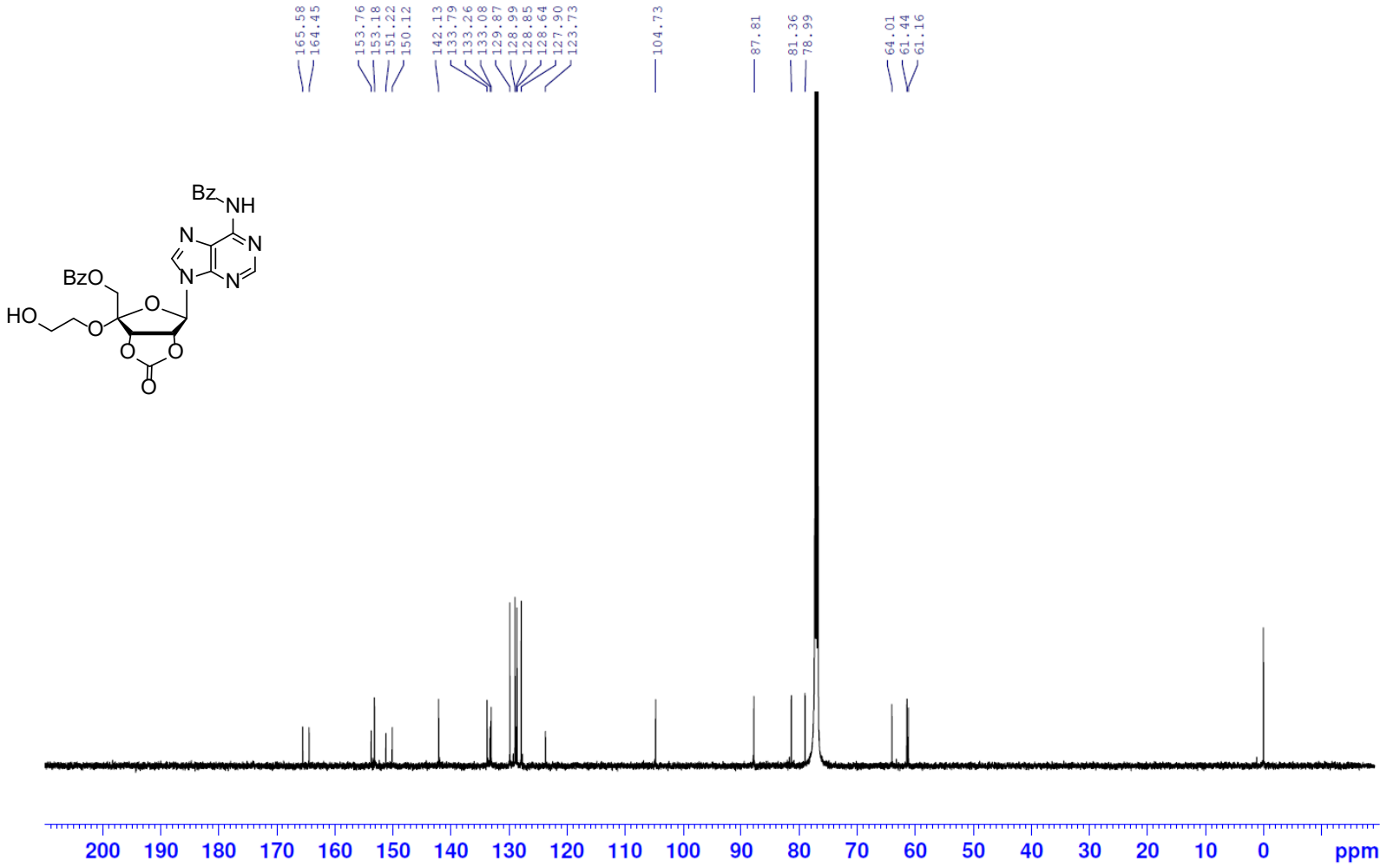
Compound **7b** (125 MHz ^{13}C NMR in $CDCl_3$)



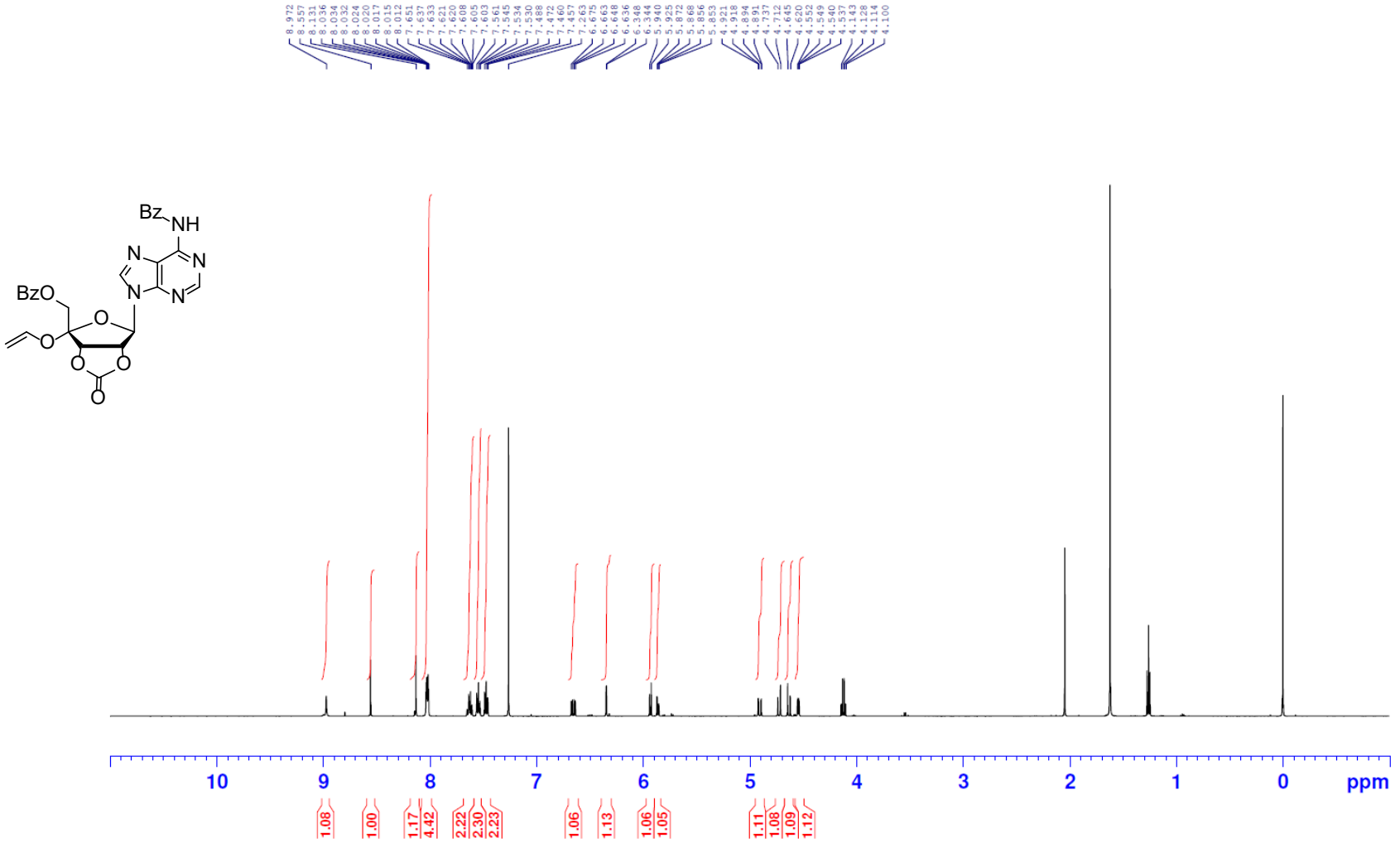
Compound **8** (500 MHz ^{1}H NMR in $CDCl_3$)



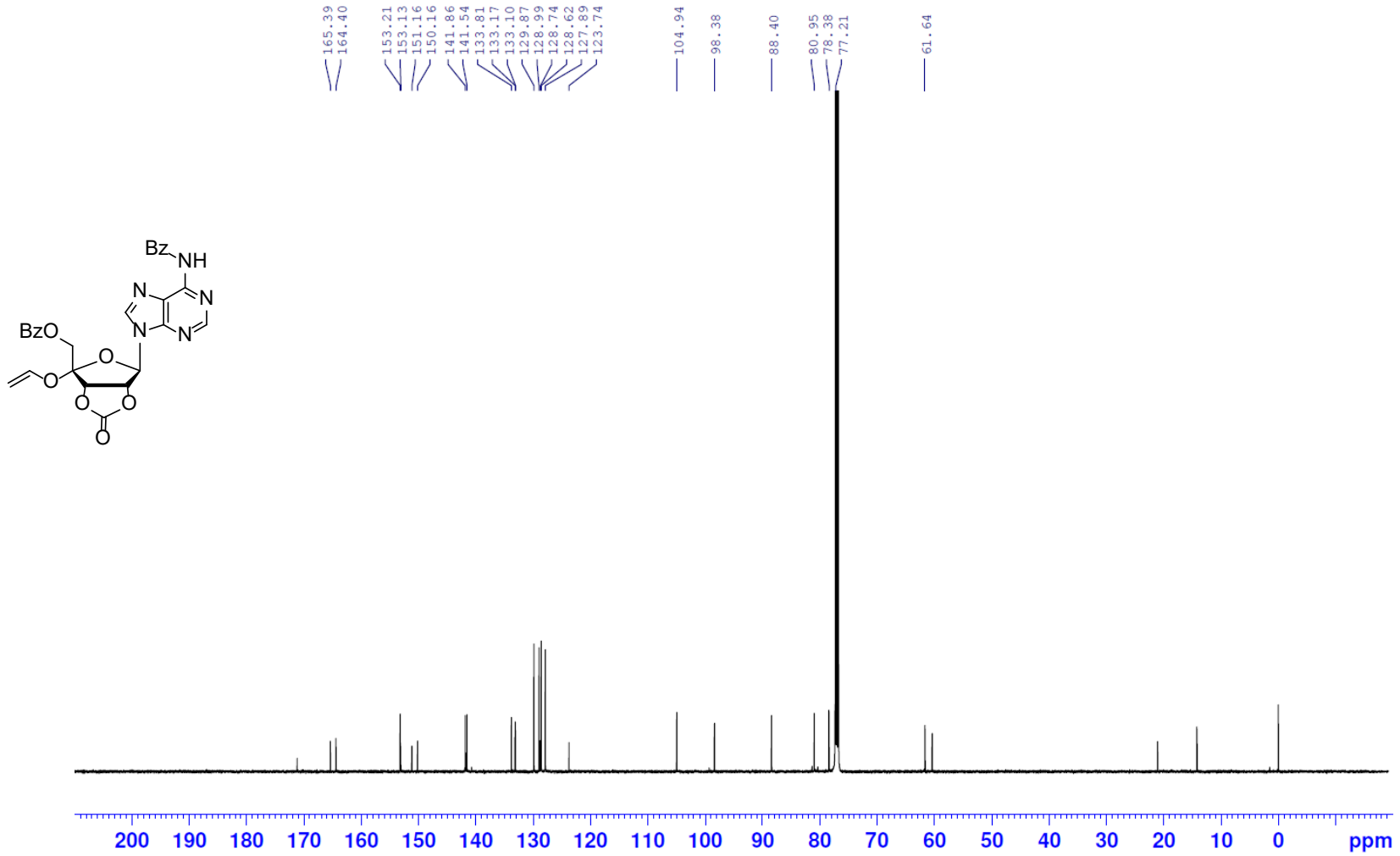
Compound **8** (125 MHz ^{13}C NMR in $CDCl_3$)



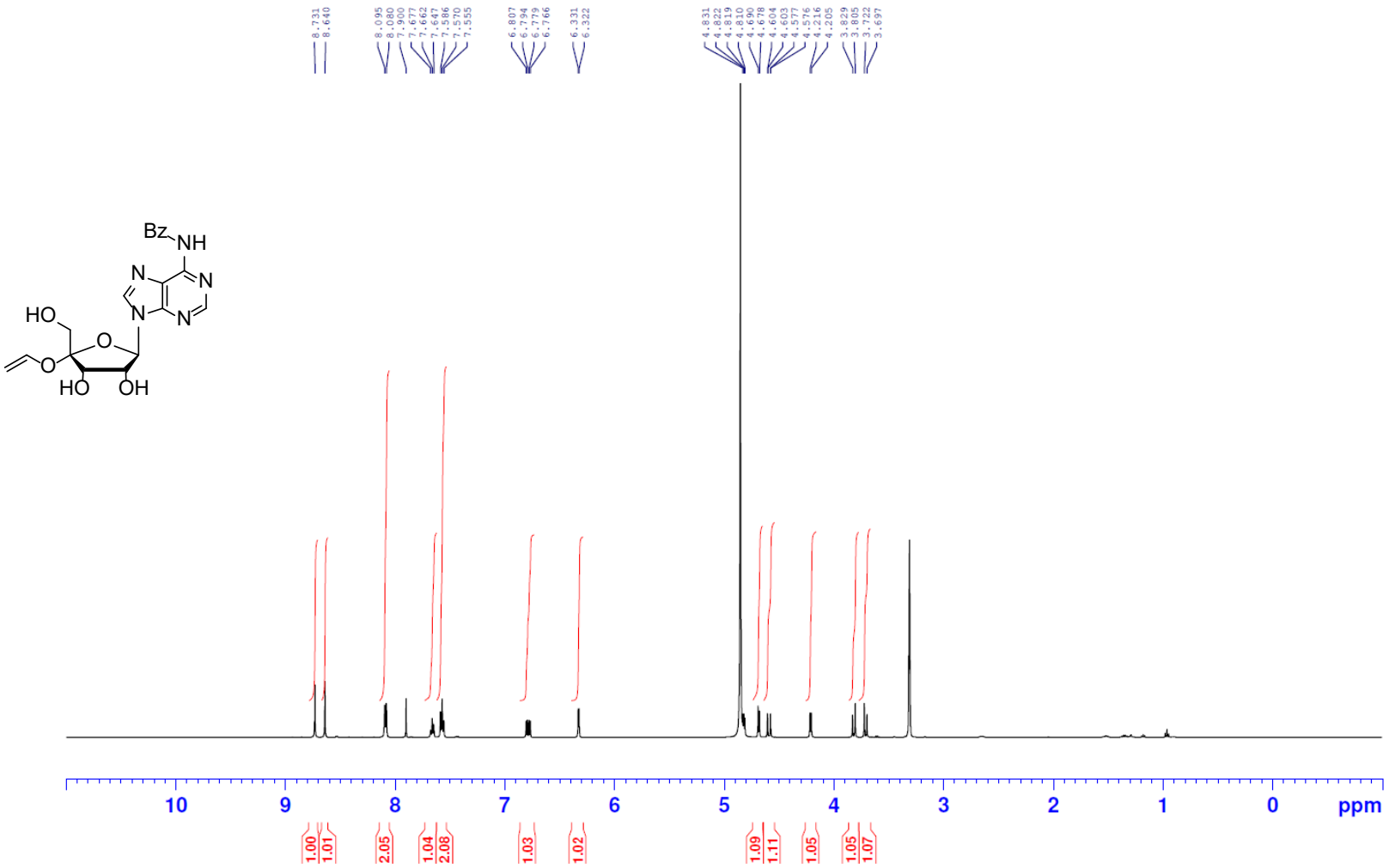
Compound **9** (500 MHz ^{1}H NMR in $CDCl_3$)



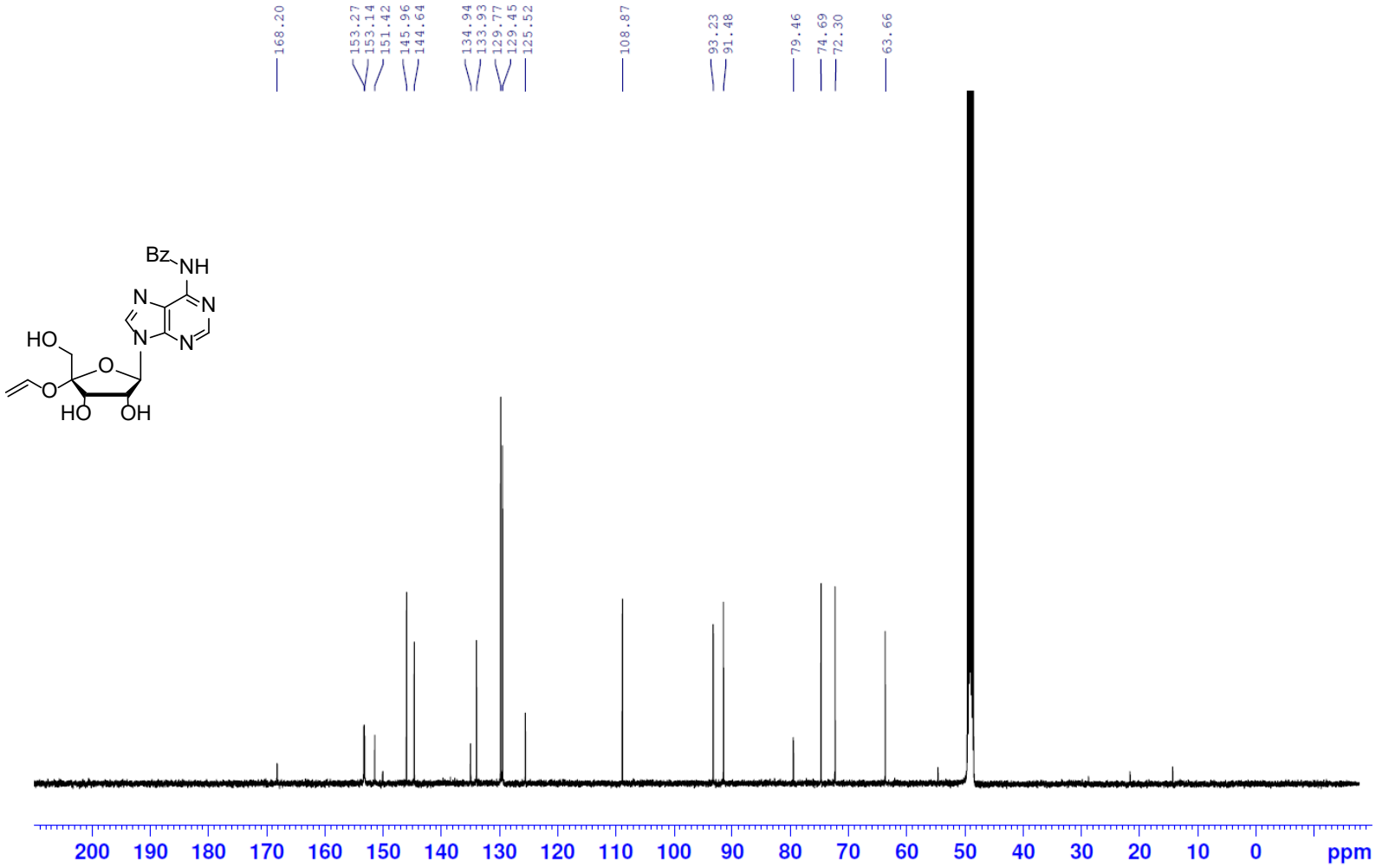
Compound **9** (125 MHz ^{13}C NMR in $CDCl_3$)



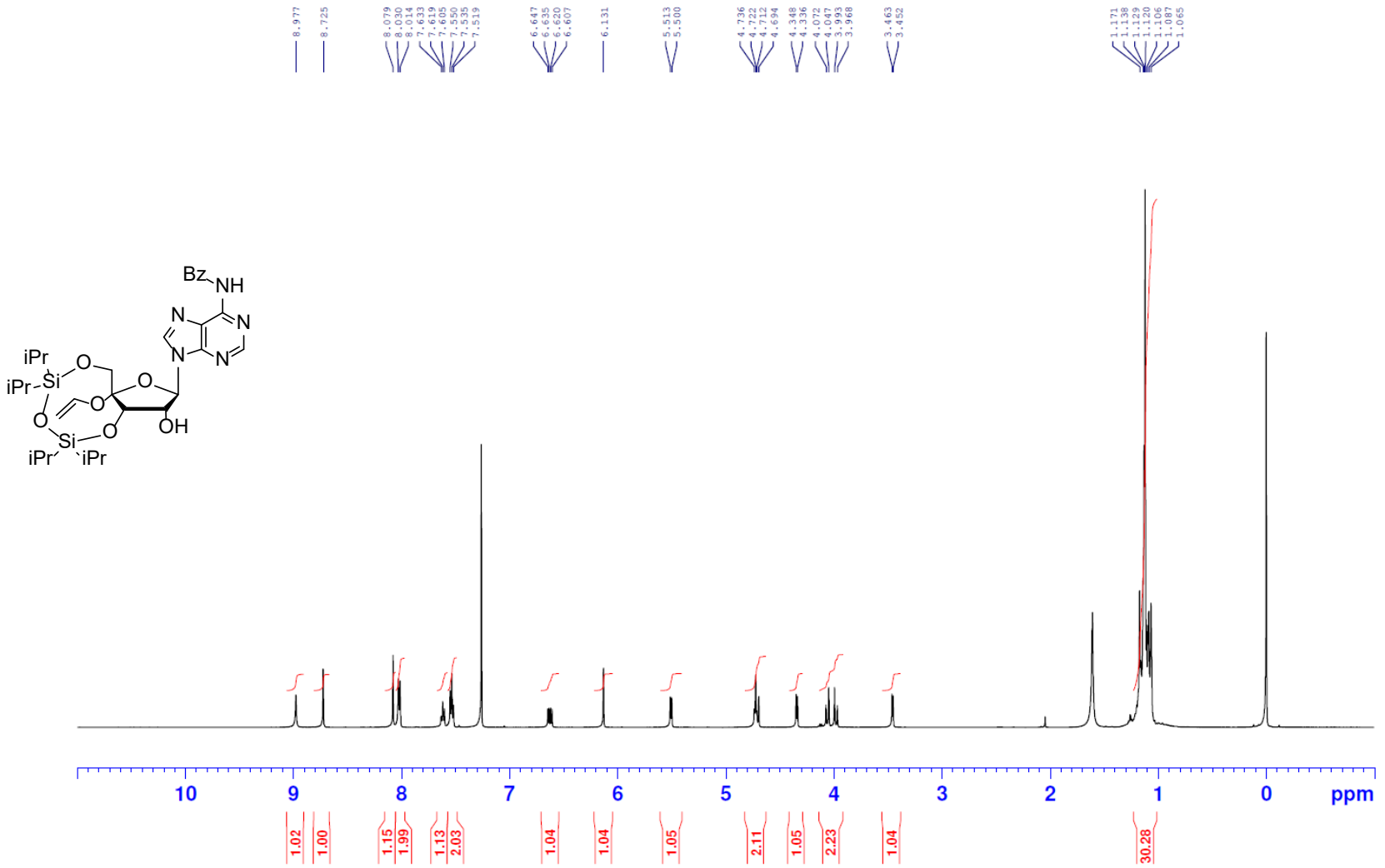
Compound **10** (500 MHz ^{1}H NMR in CD_3OD)



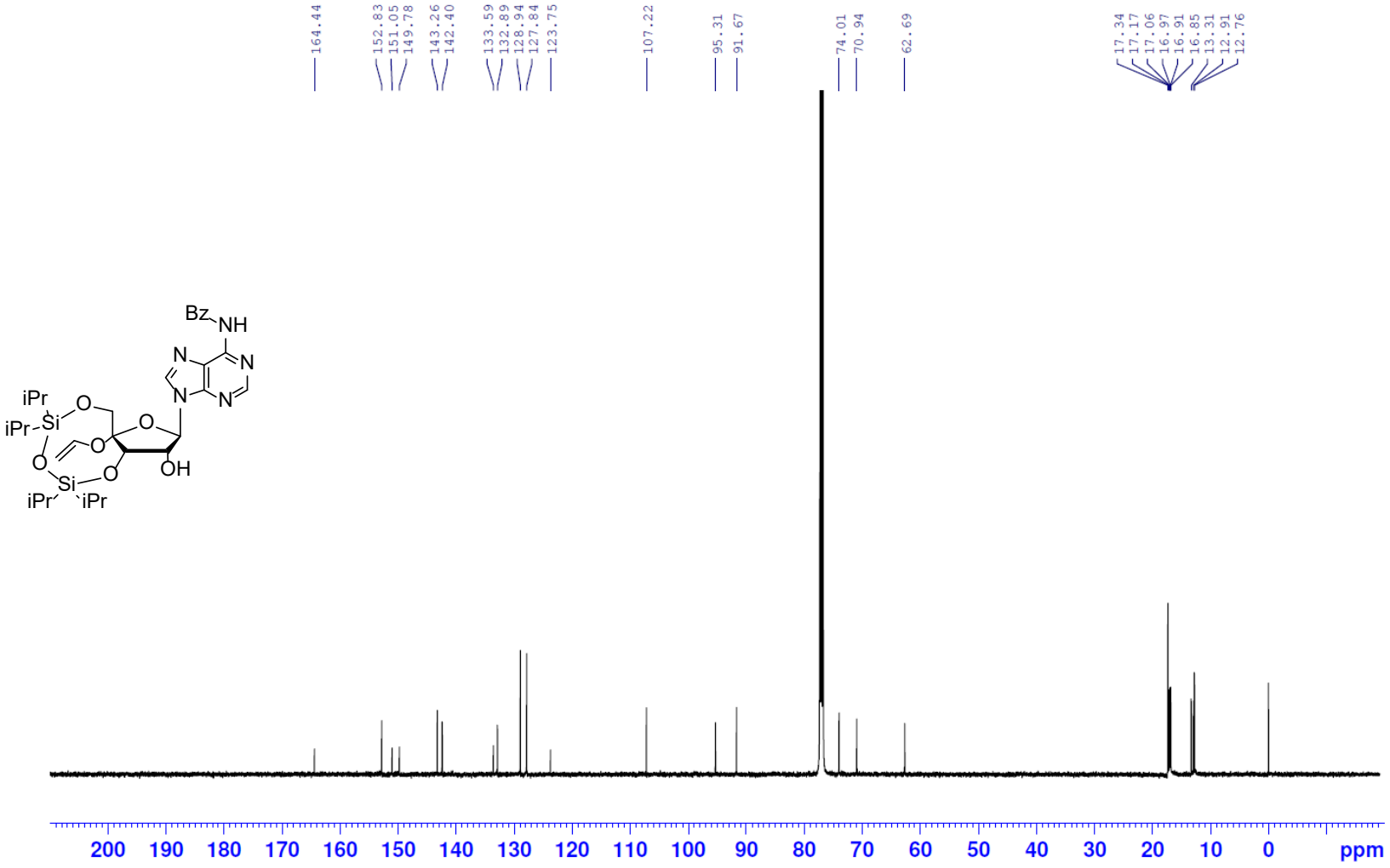
Compound **10** (125 MHz ^{13}C NMR in CD_3OD)



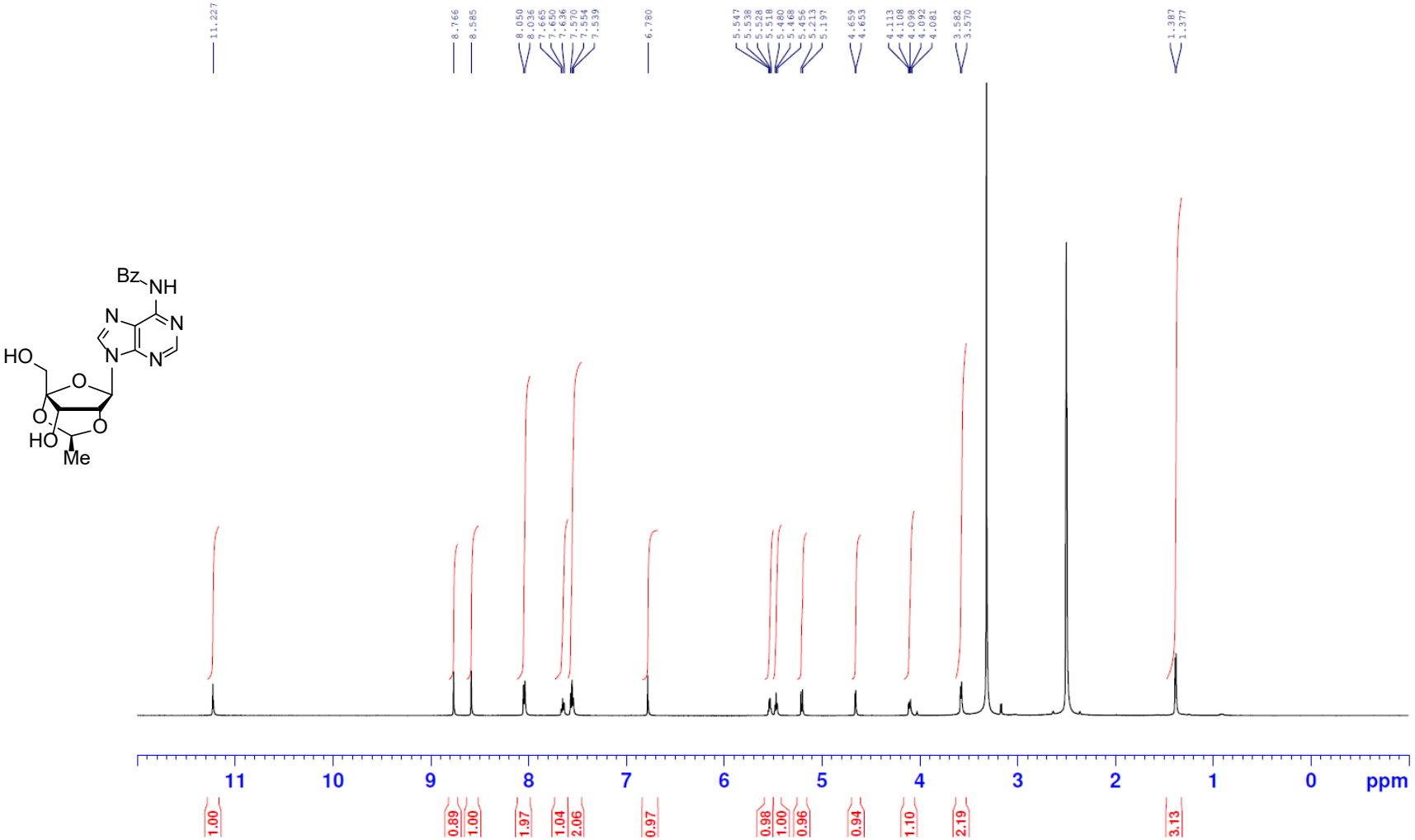
Compound **11** (500 MHz ^{1}H NMR in $CDCl_3$)



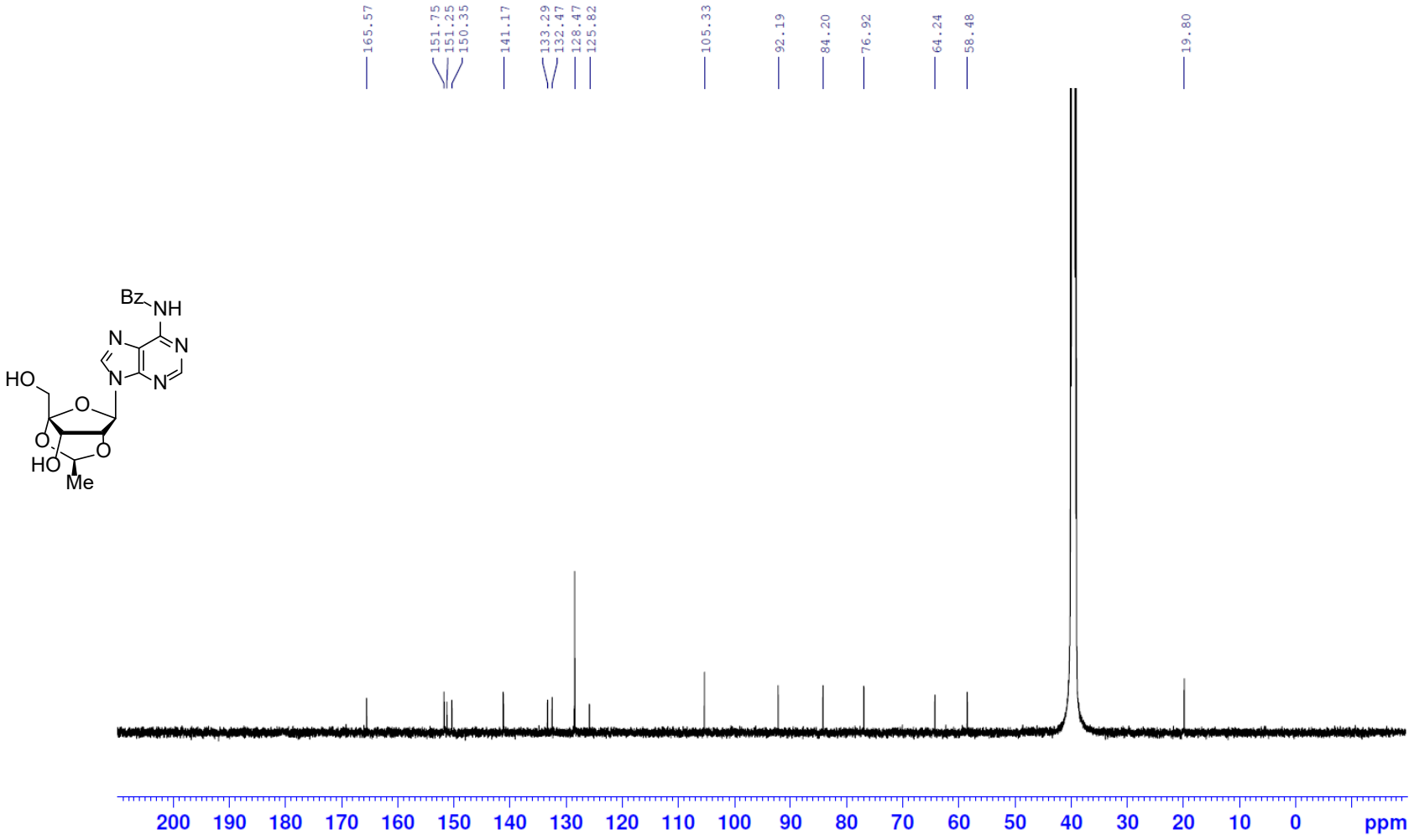
Compound **11** (125 MHz ^{13}C NMR in $CDCl_3$)



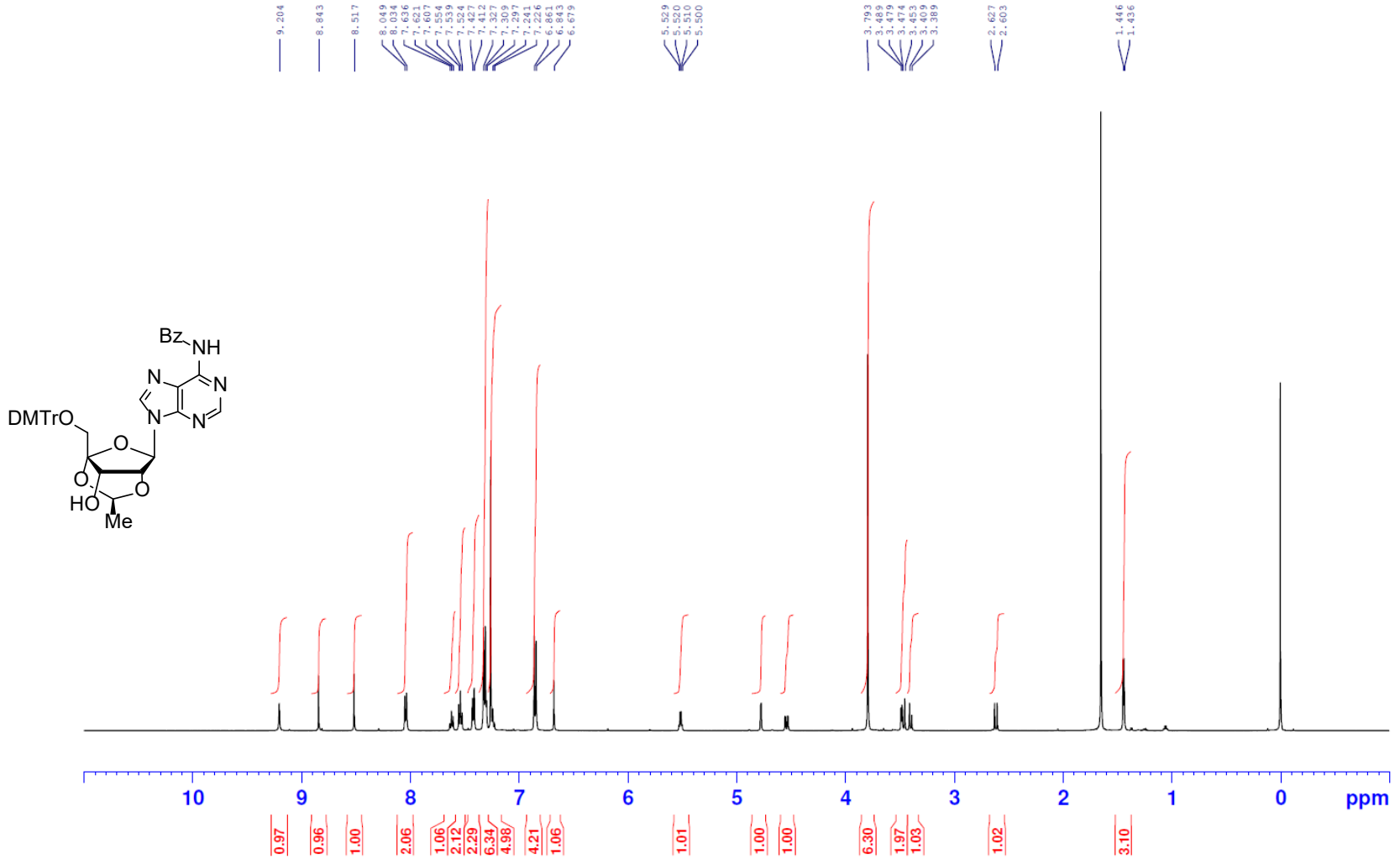
Compound **12** (500 MHz ^{1}H NMR in DMSO-d_6)



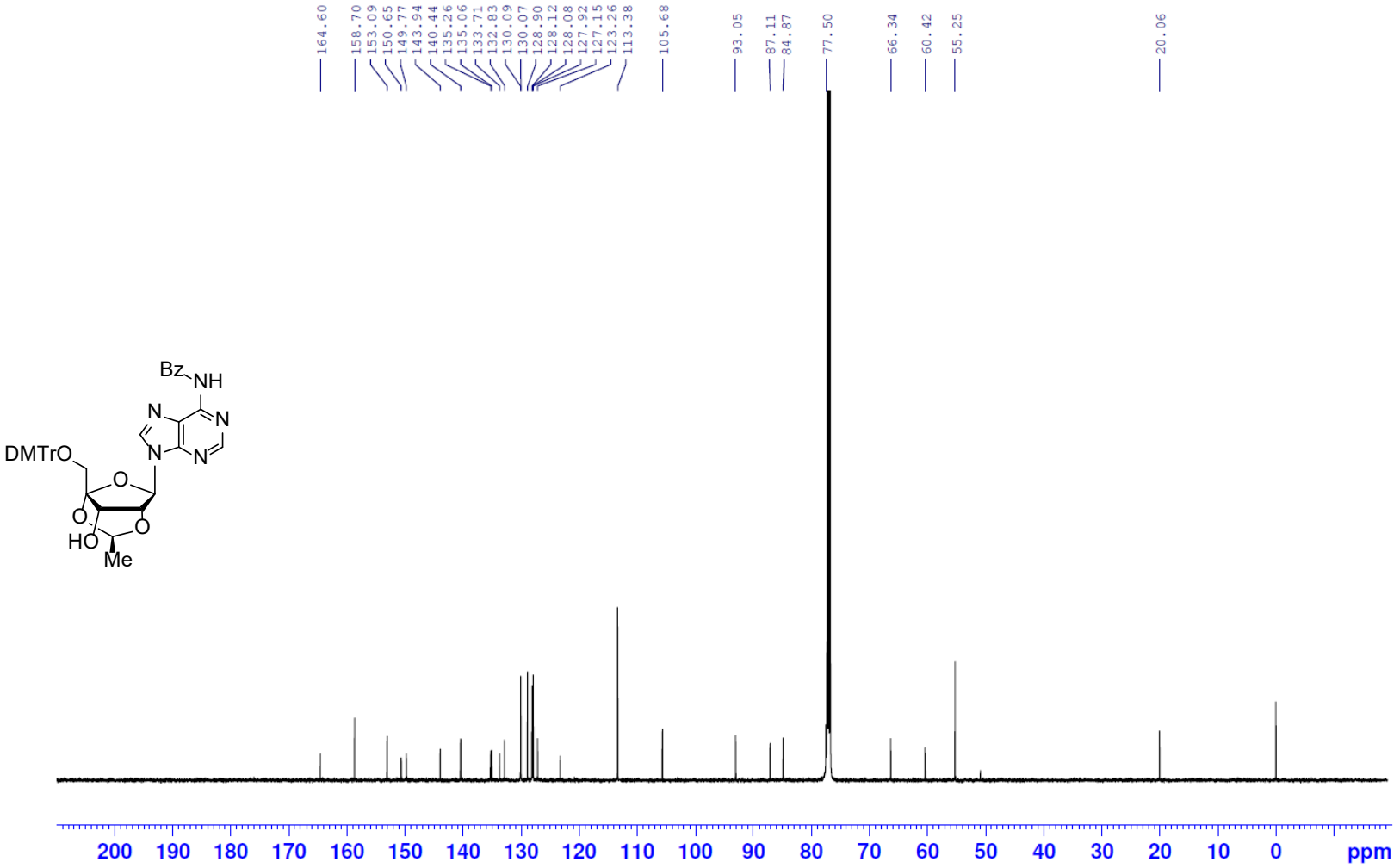
Compound **12** (125 MHz ^{13}C NMR in DMSO-d_6)



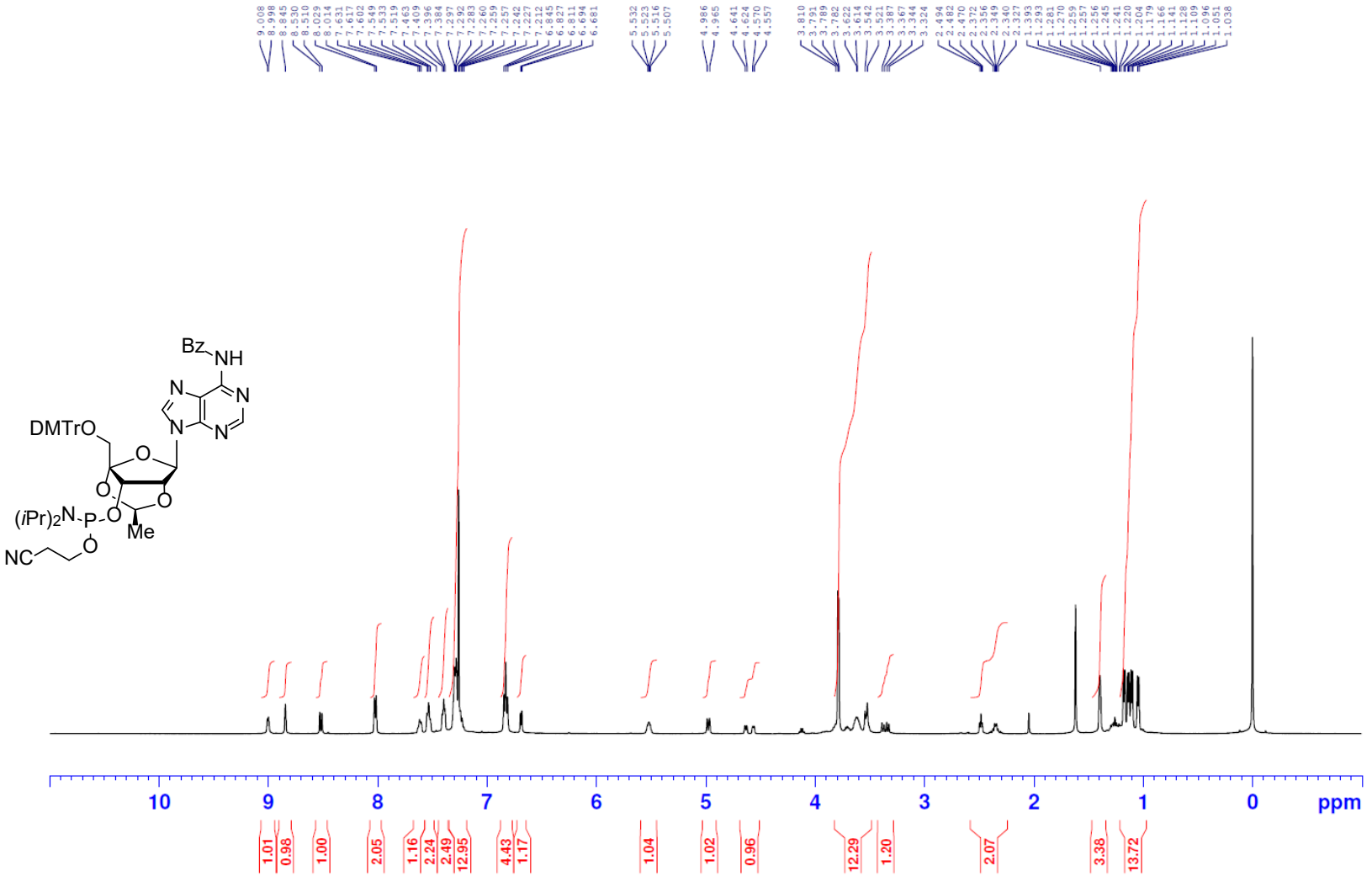
5′-*O*-DMTr-intermediate of adenosine compound (500 MHz ^{1}H NMR in $CDCl_3$)



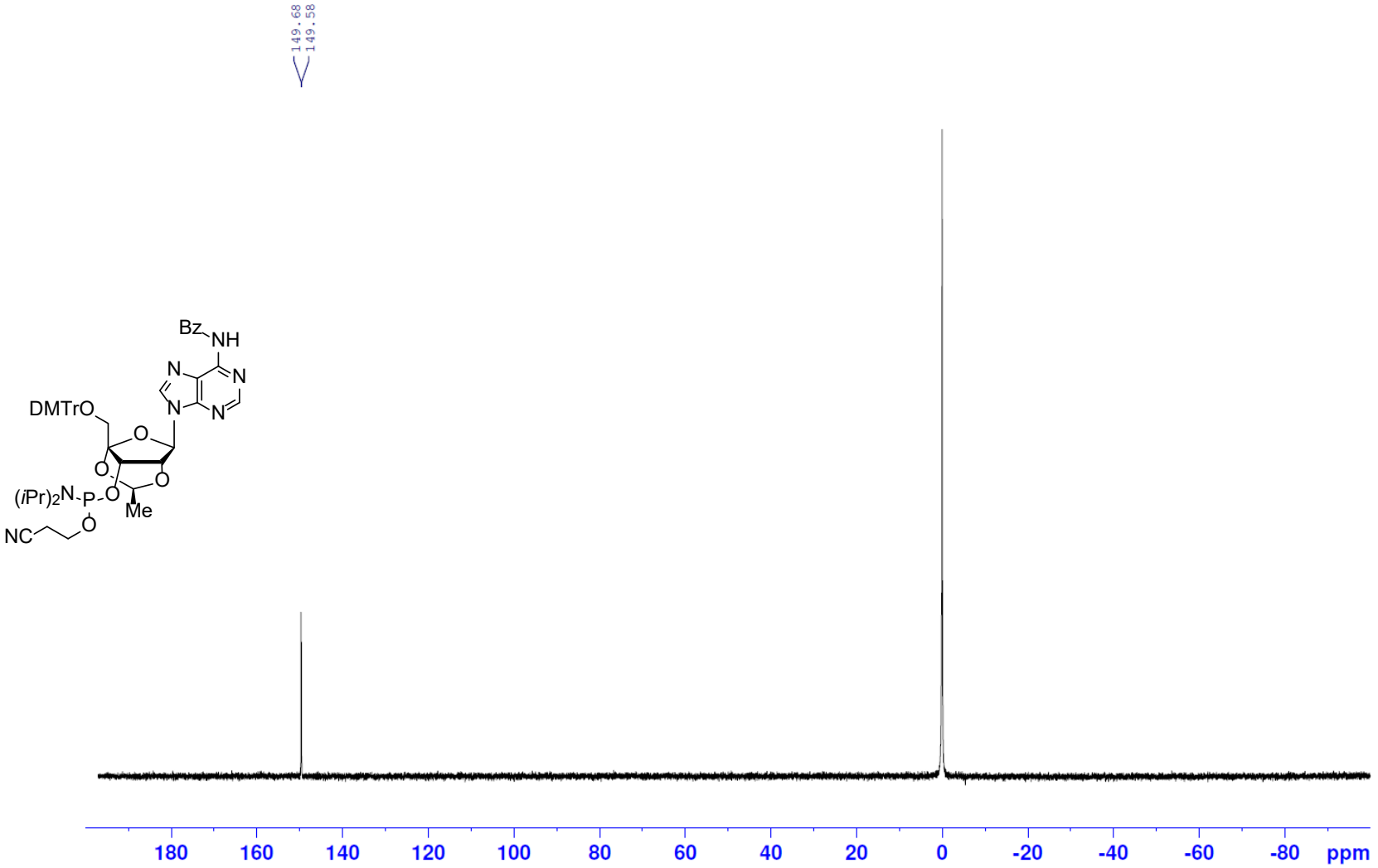
5′-*O*-DMTr-intermediate of adenosine compound (125 MHz ^{13}C NMR in $CDCl_3$)



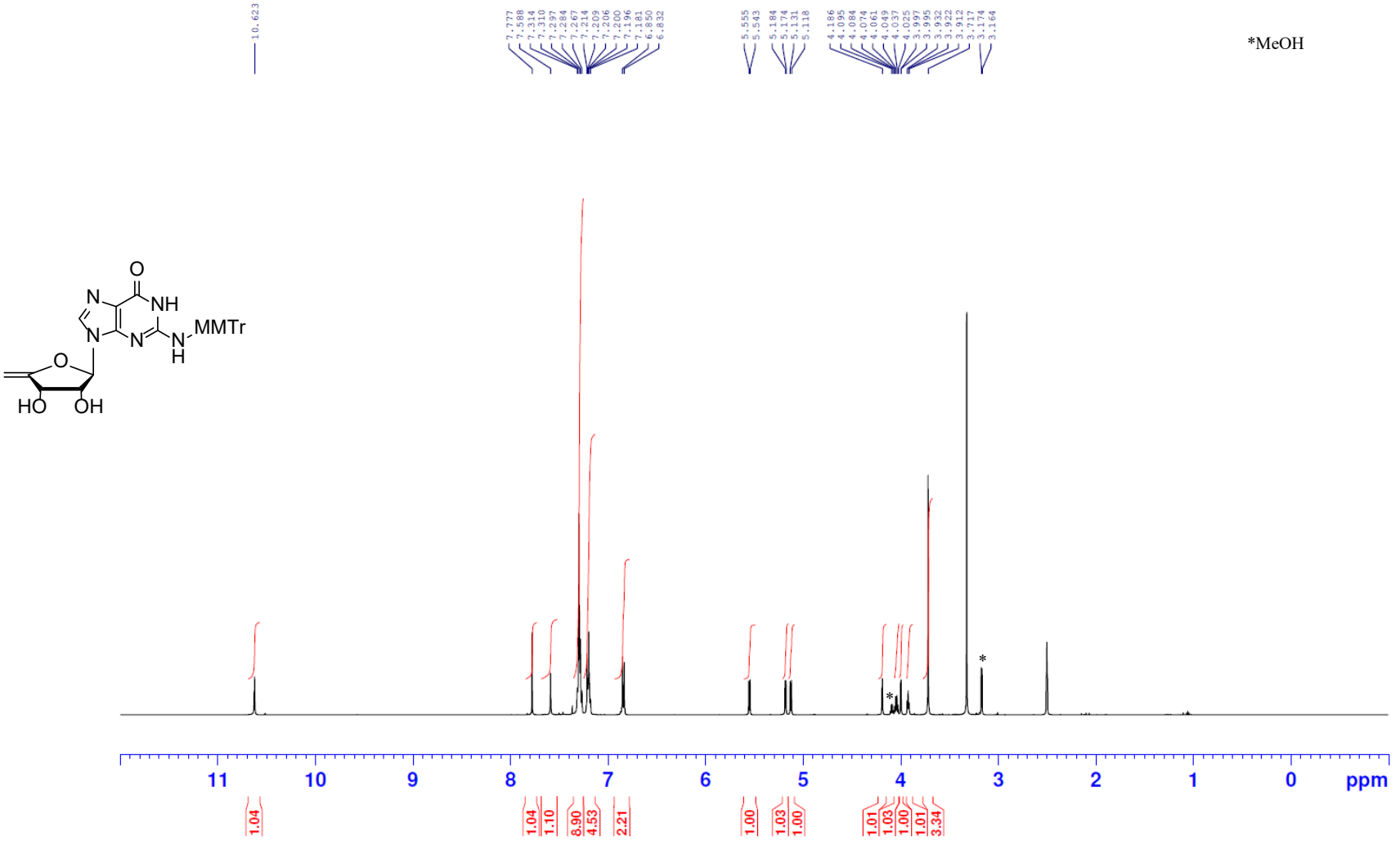
Compound **13** (500 MHz ^{1}H NMR in $CDCl_3$)



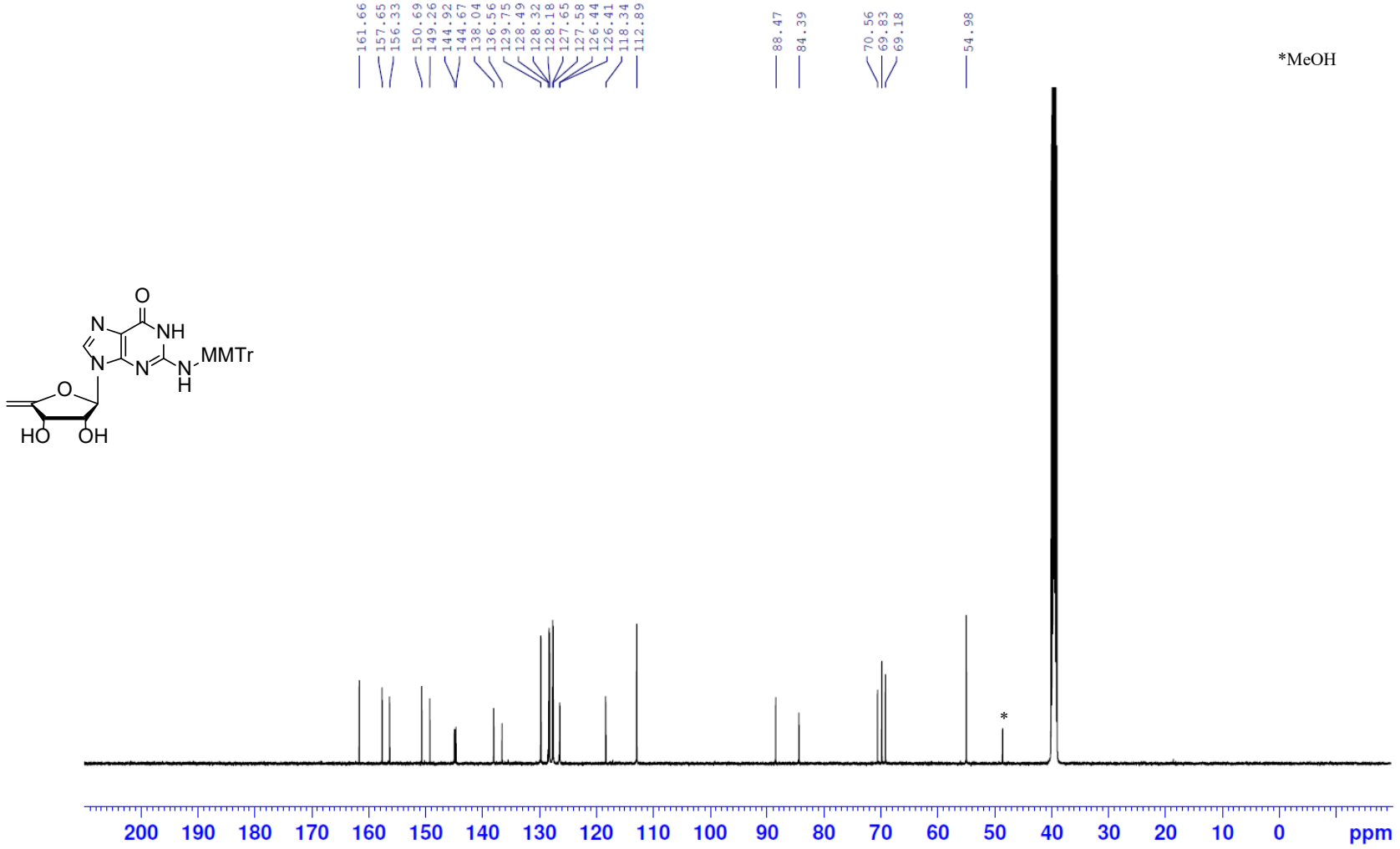
Compound **13** (202 MHz ^{31}P NMR in $CDCl_3$)



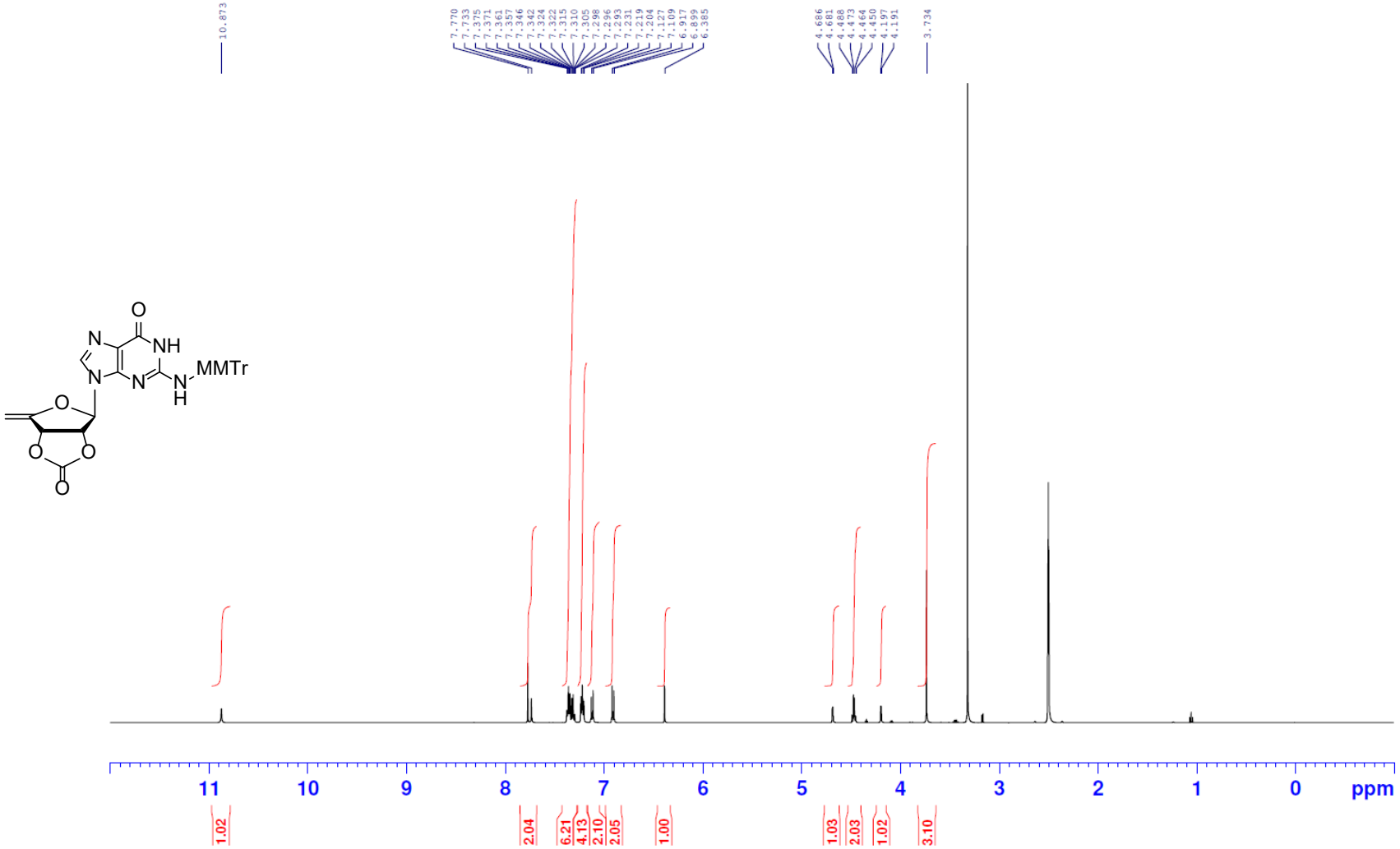
Compound **15** (500 MHz ^{1}H NMR in DMSO-d_6)



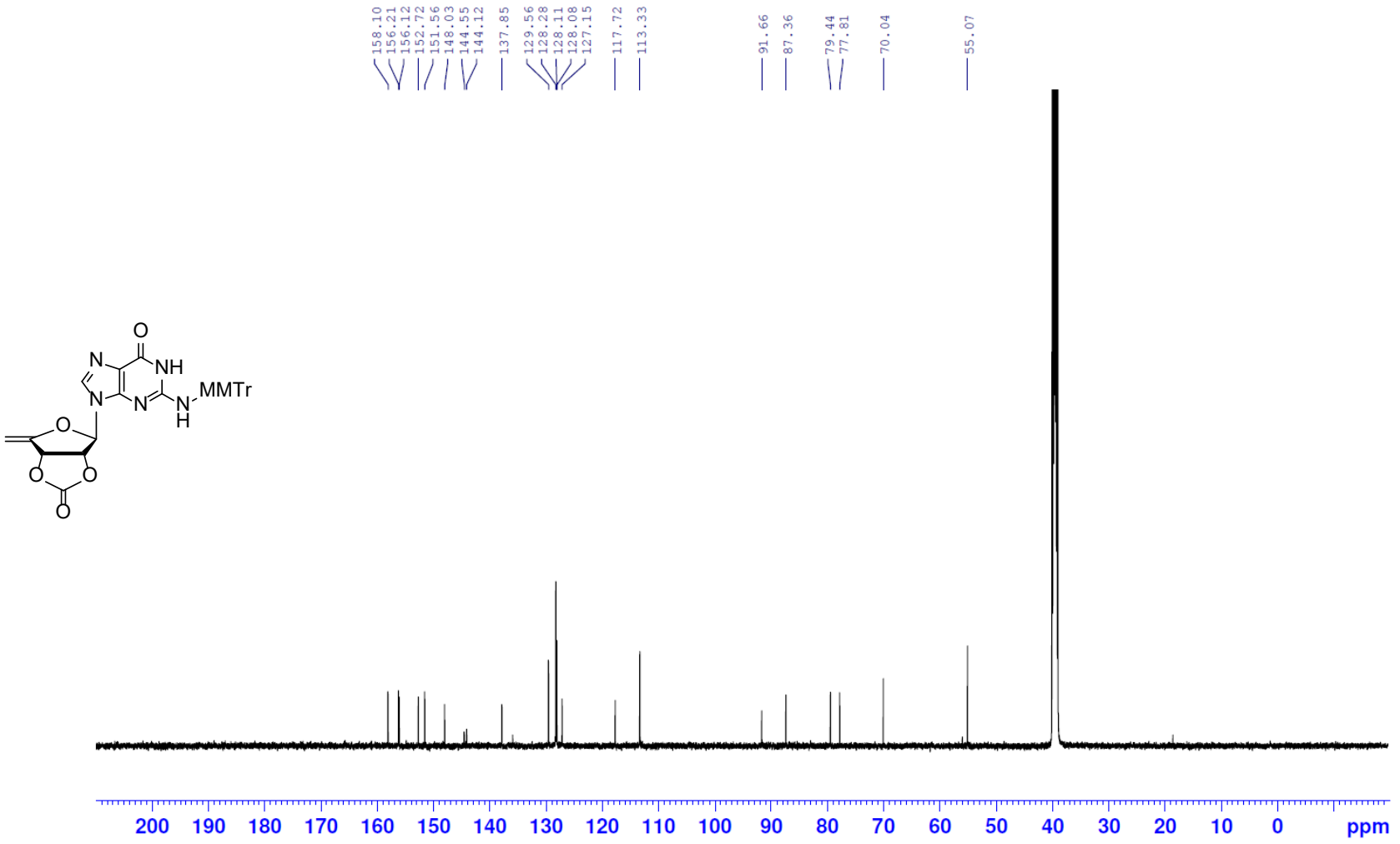
Compound **15** (125 MHz ^{13}C NMR in DMSO-d_6)



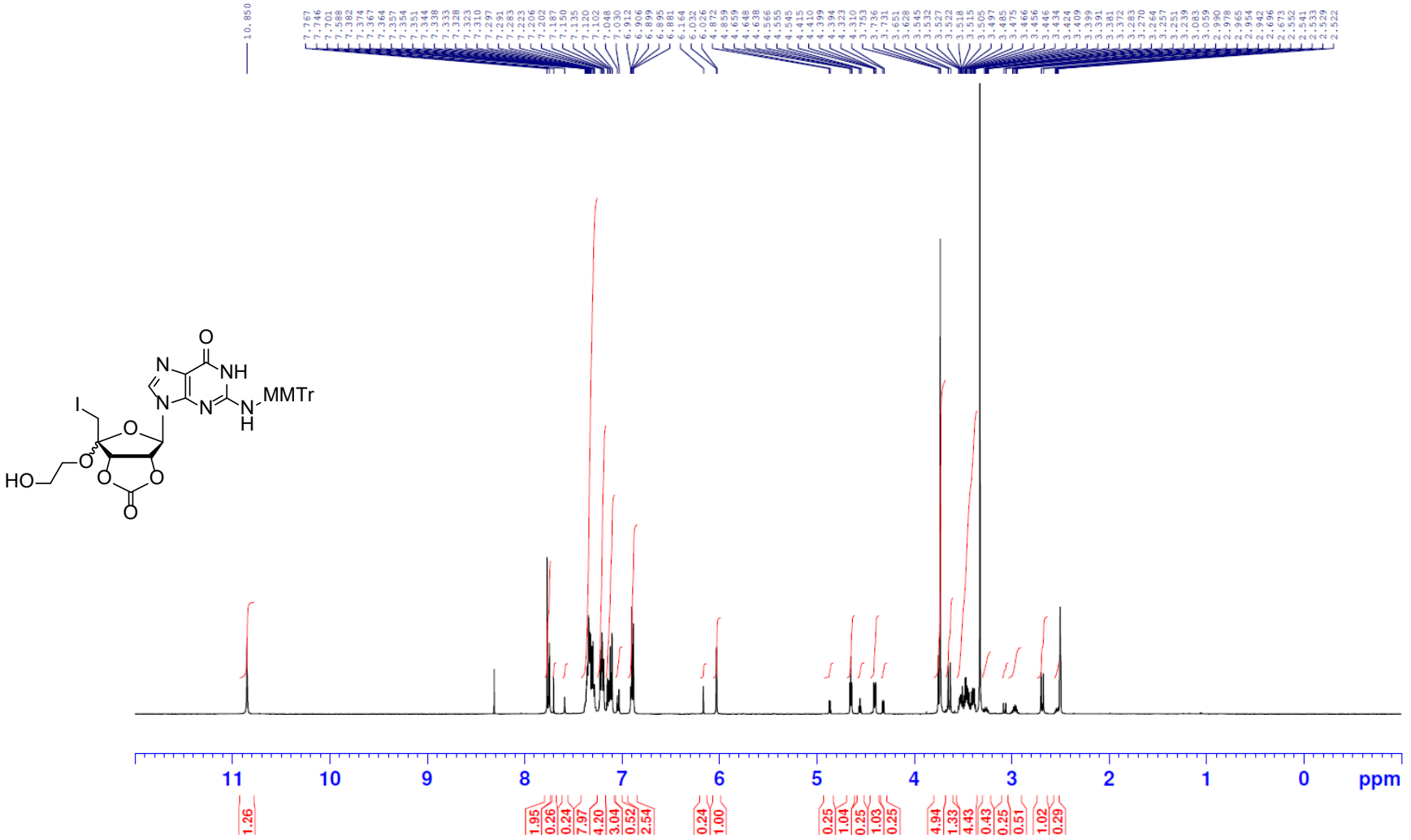
Compound **16** (500 MHz ^{1}H NMR in DMSO-d_6)



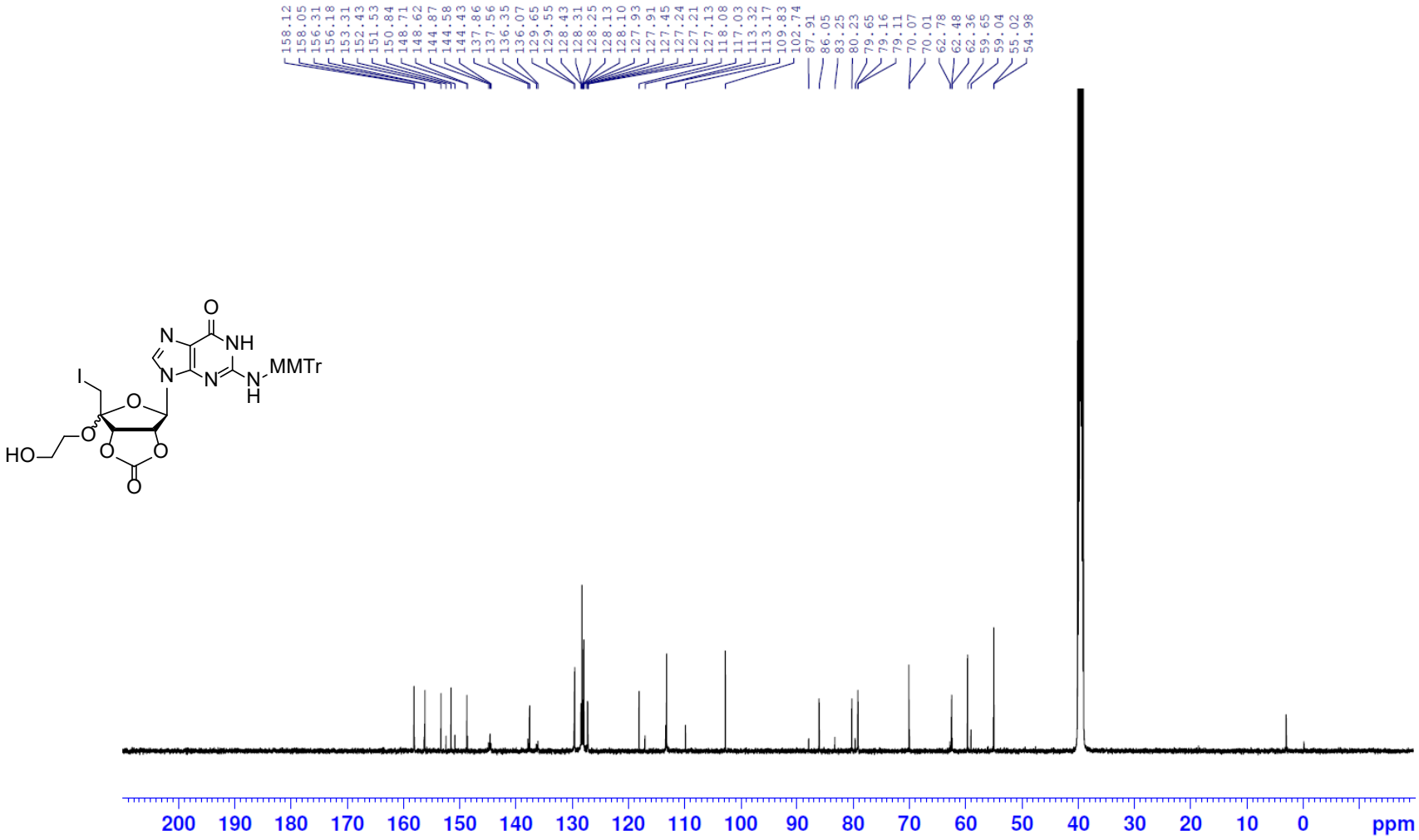
Compound **16** (125 MHz ^{13}C NMR in DMSO-d_6)



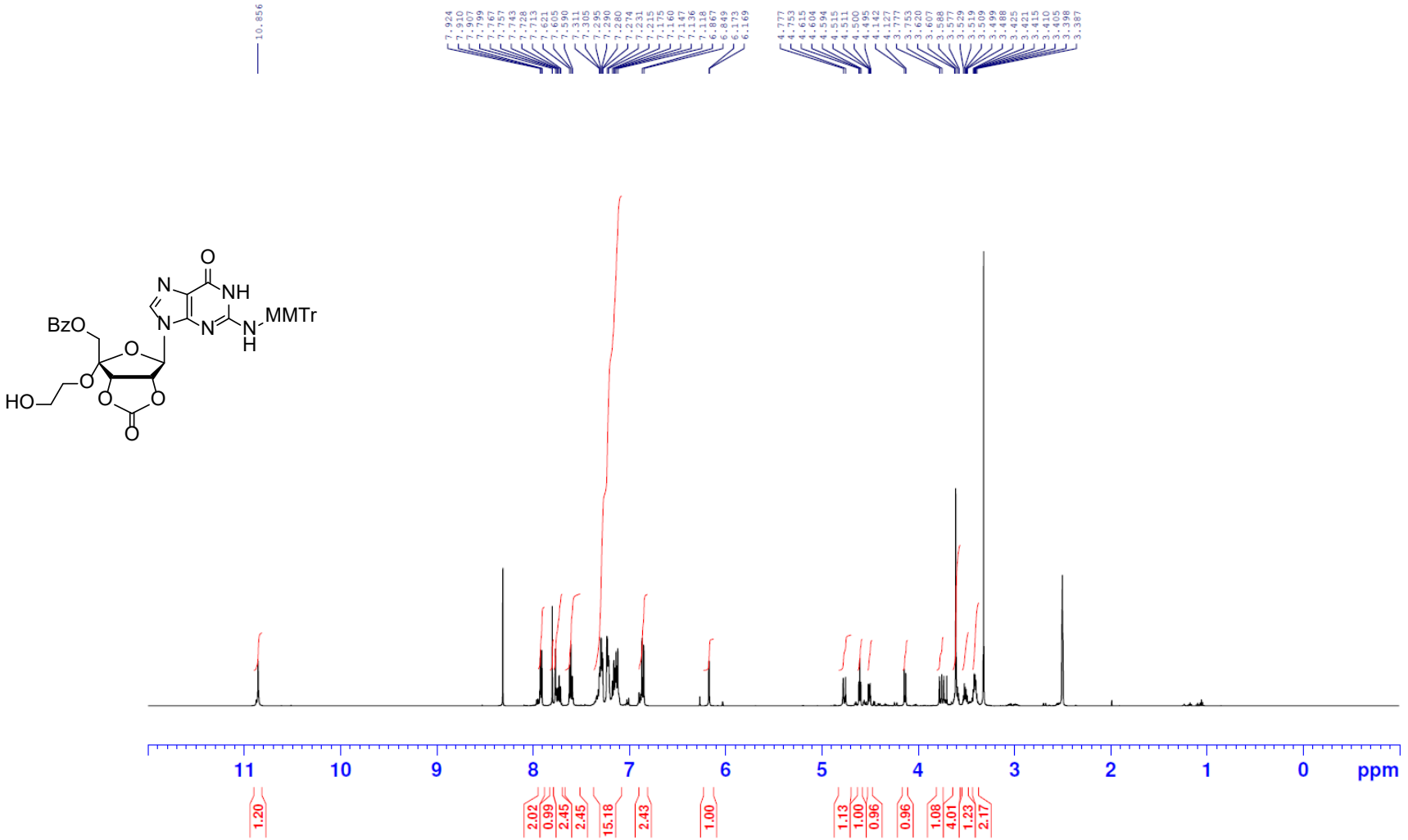
Compound **17** (500 MHz ^{1}H NMR in DMSO-d_6)



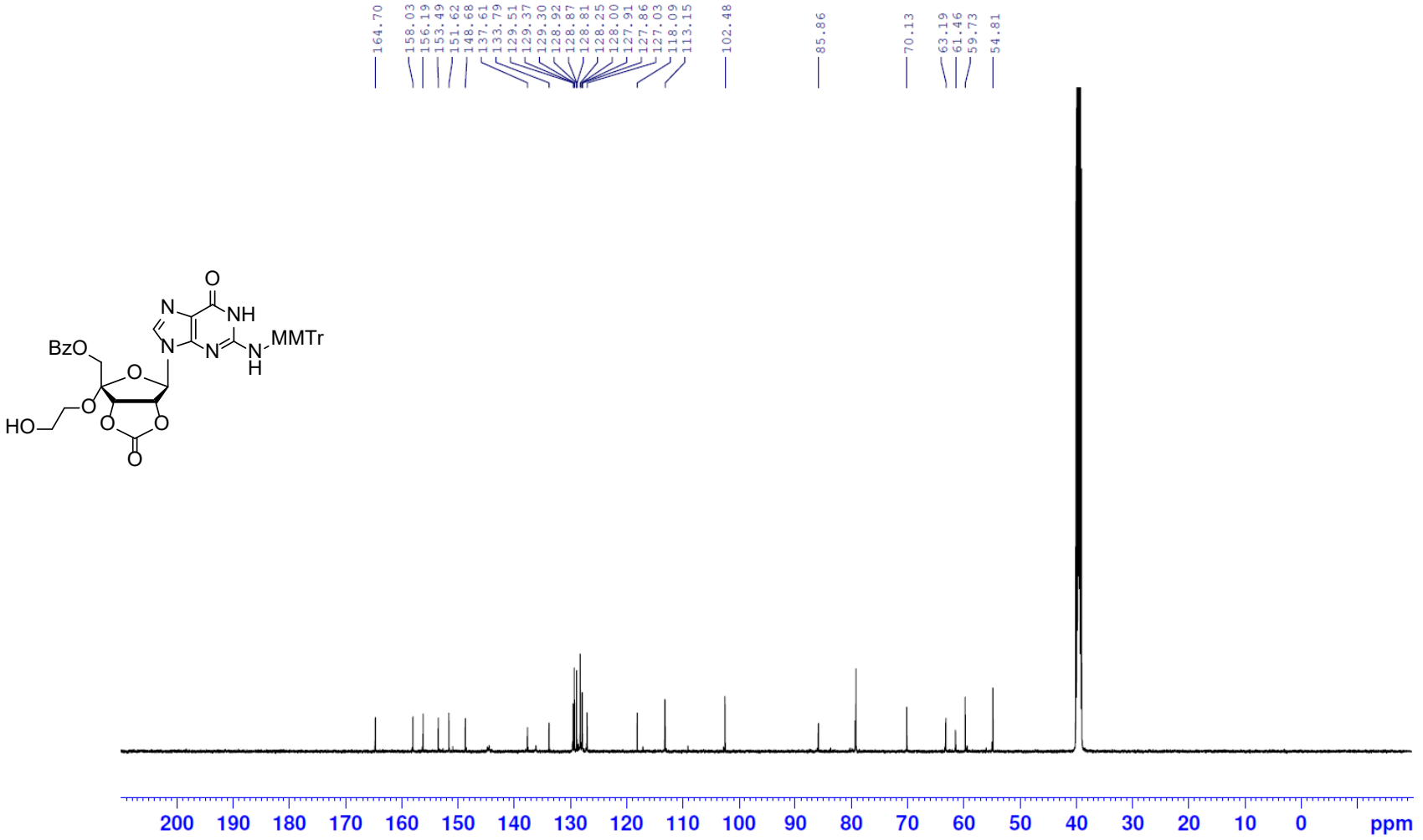
Compound **17** (125 MHz ^{13}C NMR in DMSO-d_6)



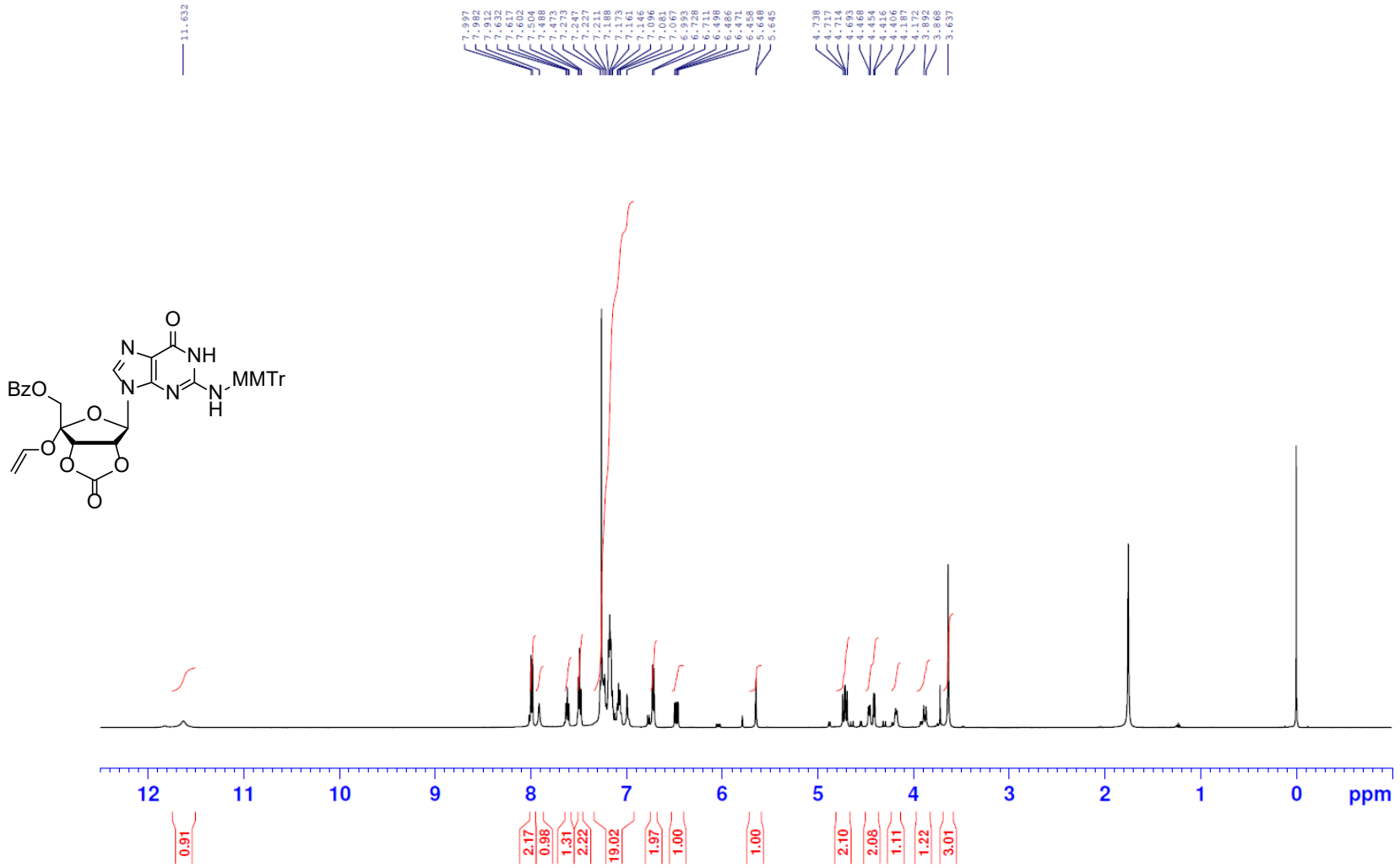
Compound **18** (500 MHz ^{1}H NMR in DMSO-d_6)



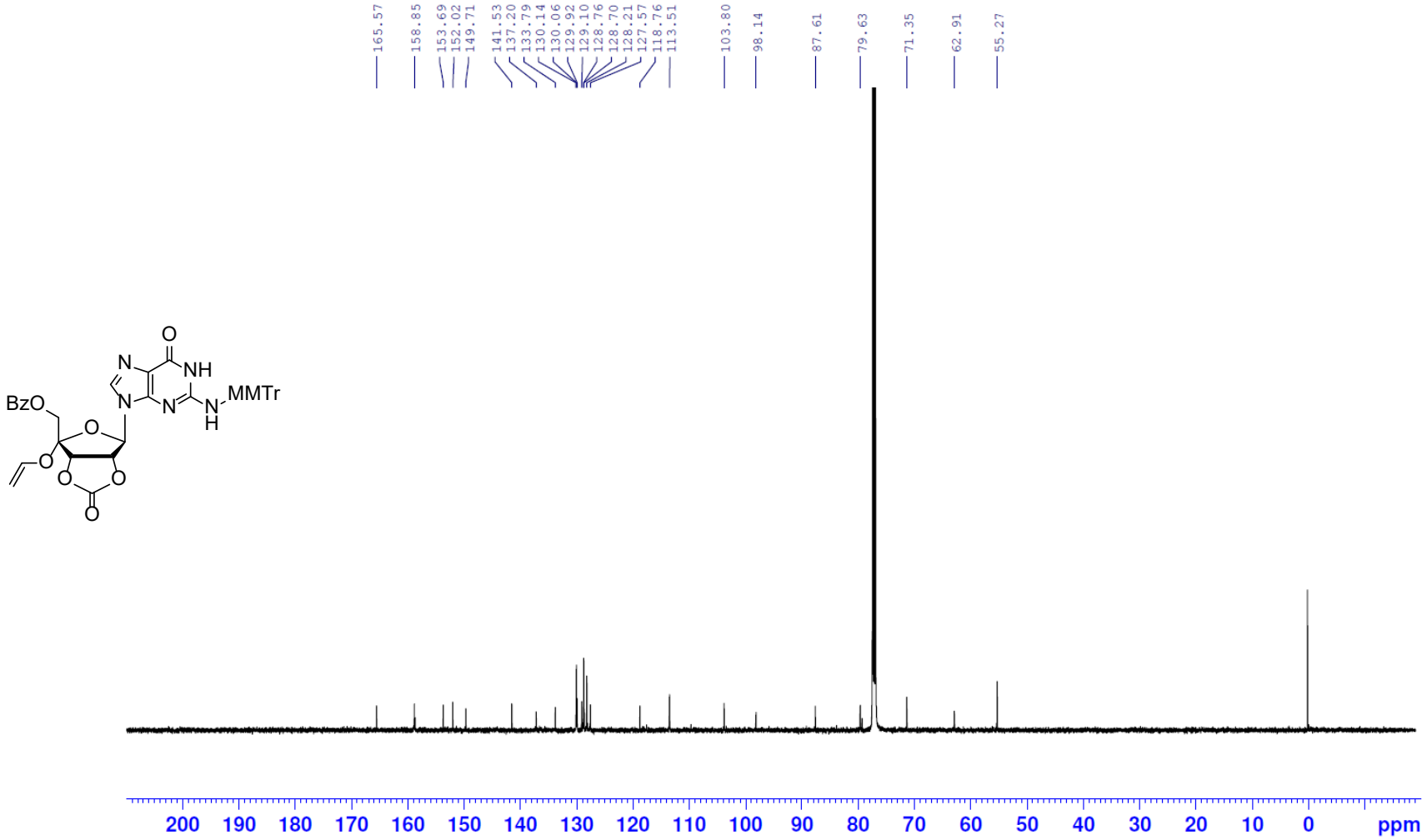
Compound **18** (125 MHz ^{13}C NMR in DMSO-d_6)



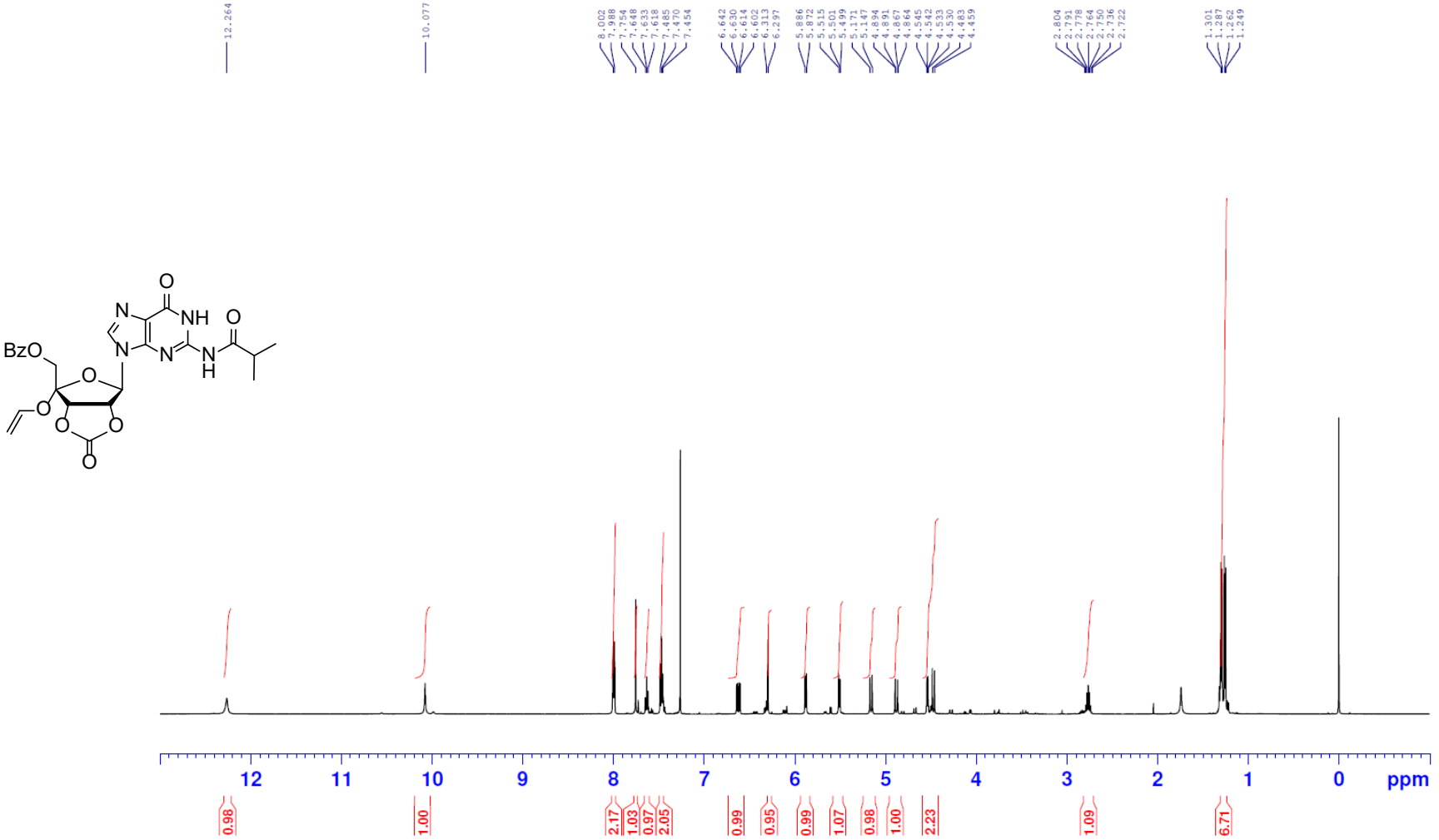
Compound **19** (500 MHz ^{1}H NMR in $CDCl_3$)



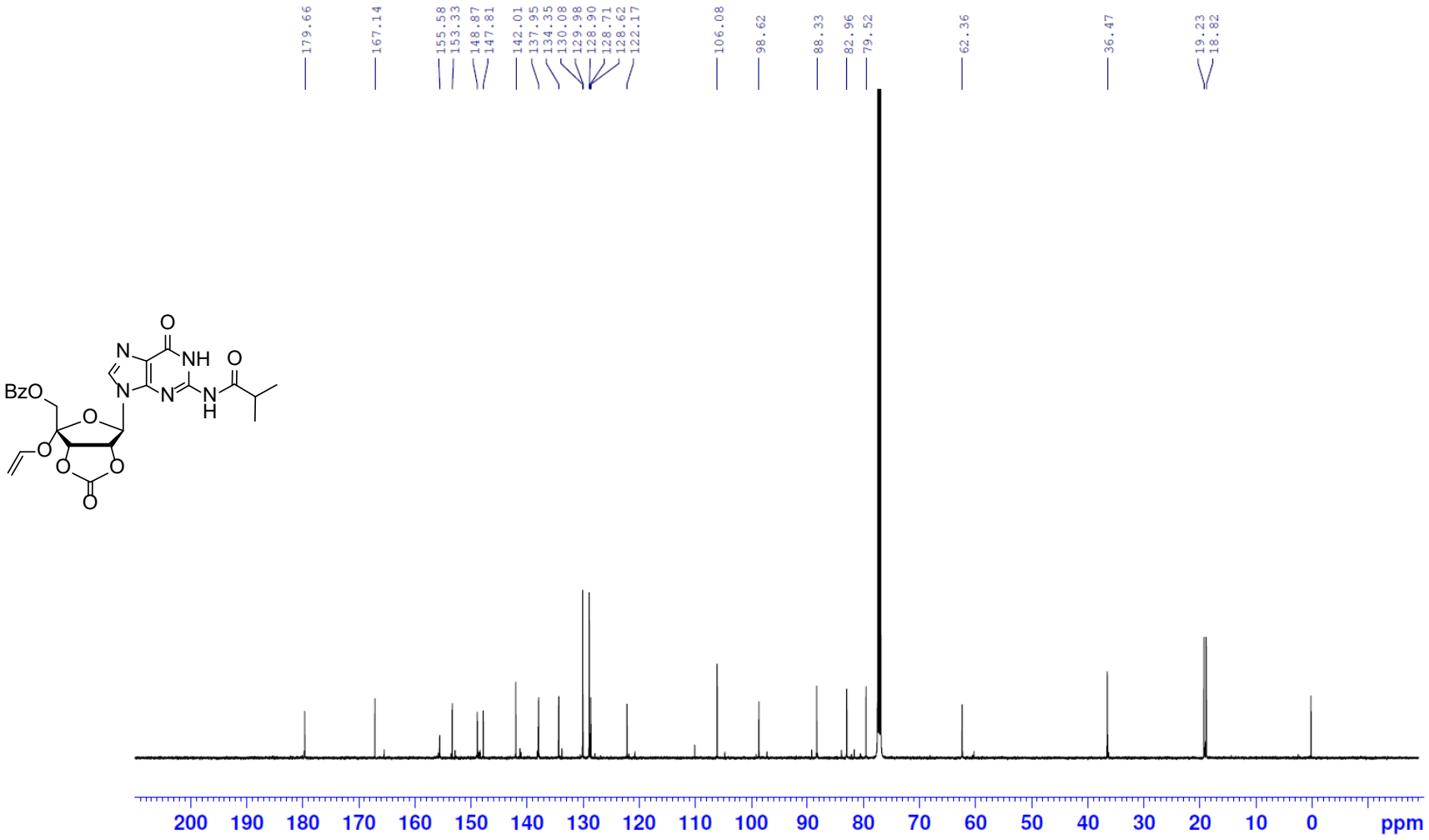
Compound **19** (125 MHz ^{13}C NMR in $CDCl_3$)



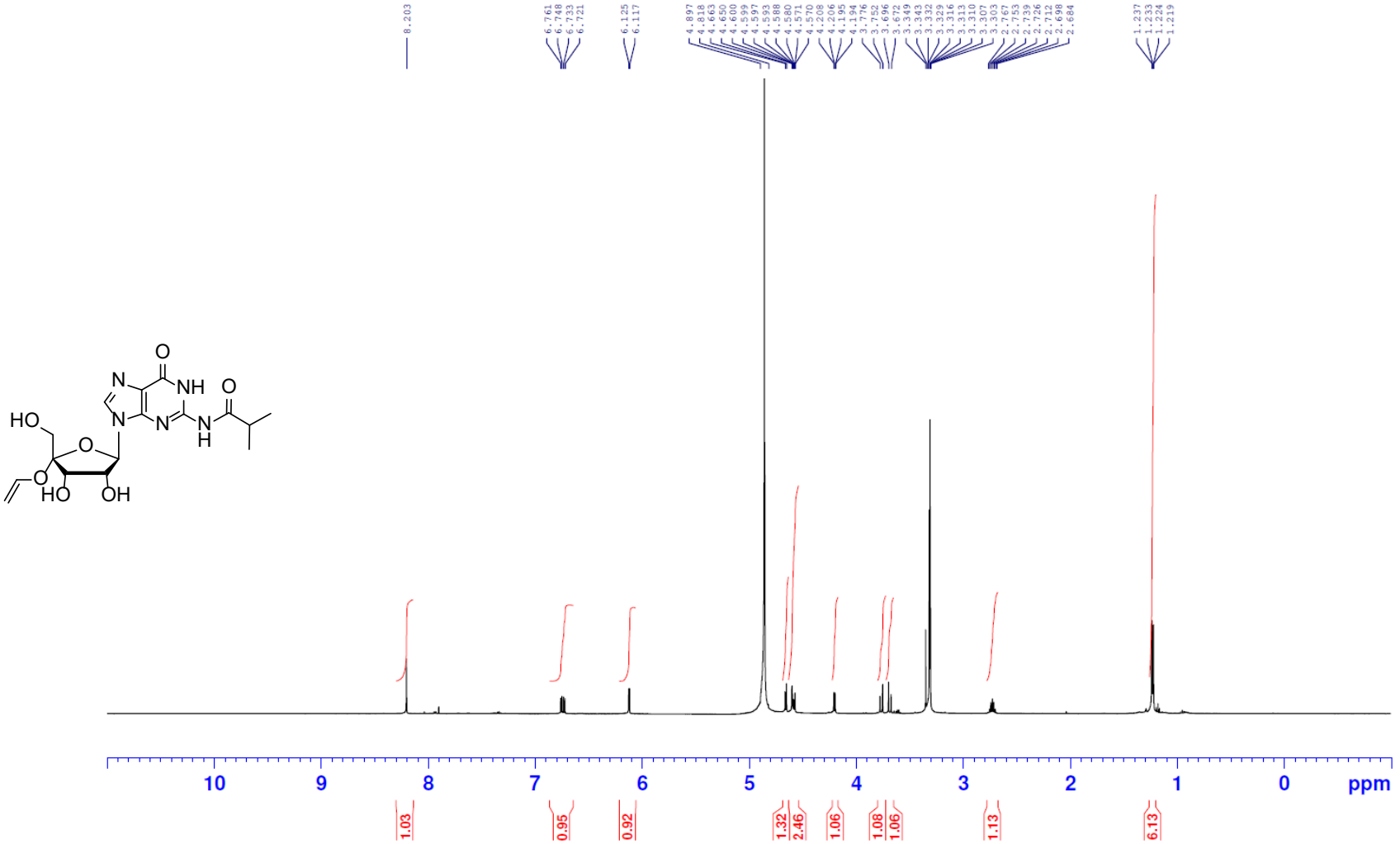
Compound **20** (500 MHz ^{1}H NMR in $CDCl_3$)



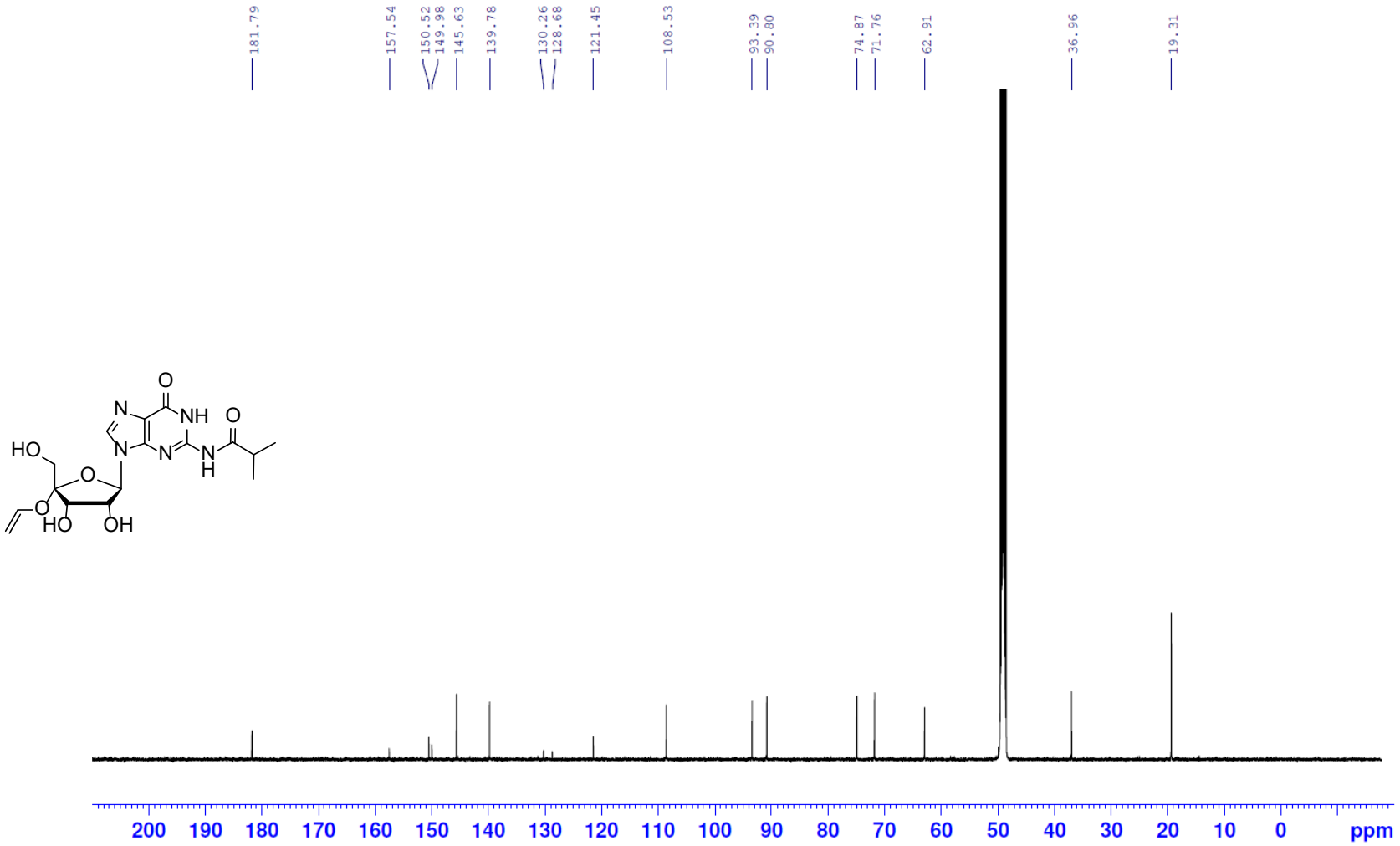
Compound **20** (125 MHz ^{13}C NMR in $CDCl_3$)



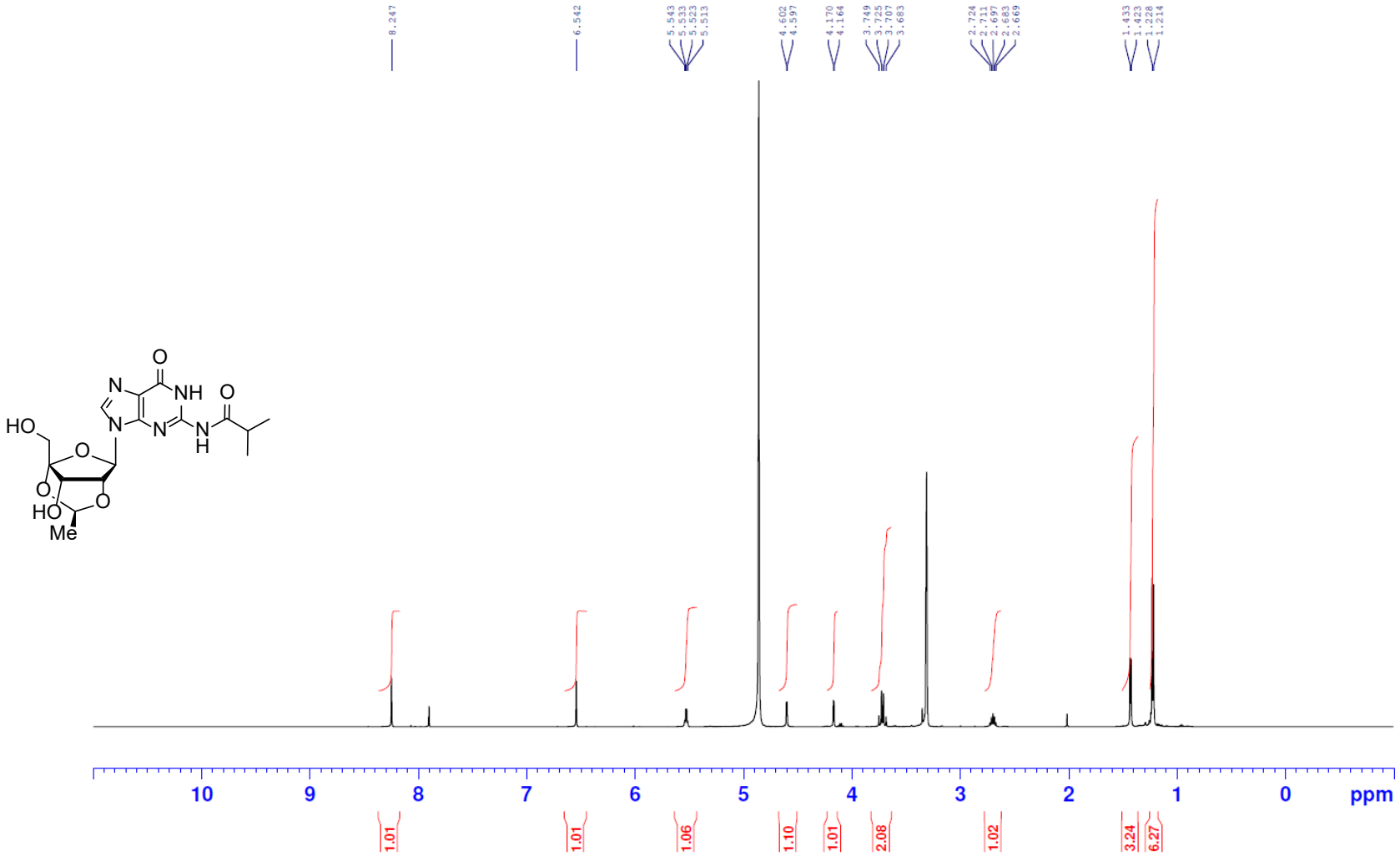
Compound **21** (500 MHz ^{1}H NMR in CD_3OD)



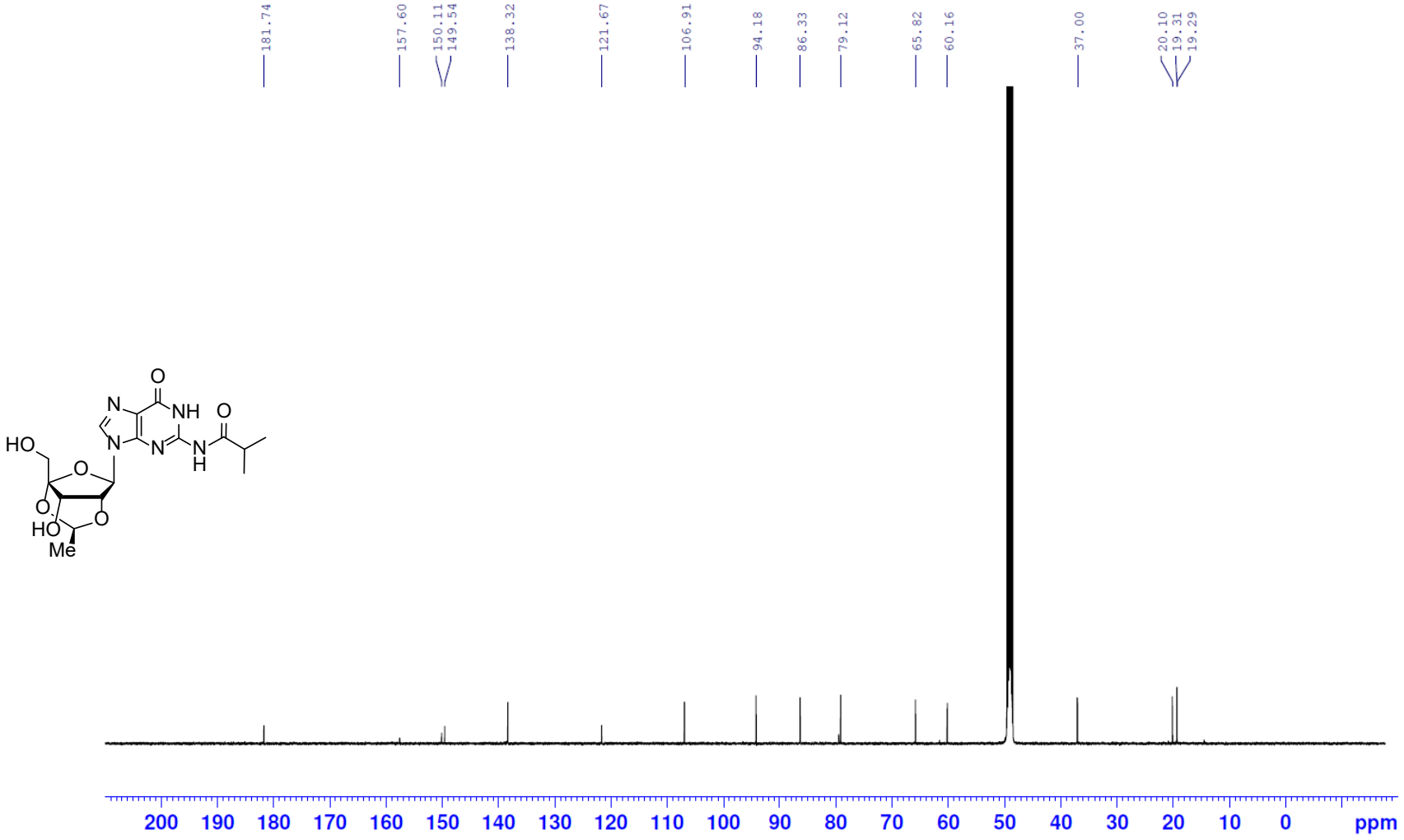
Compound **21** (125 MHz ^{13}C NMR in CD_3OD)



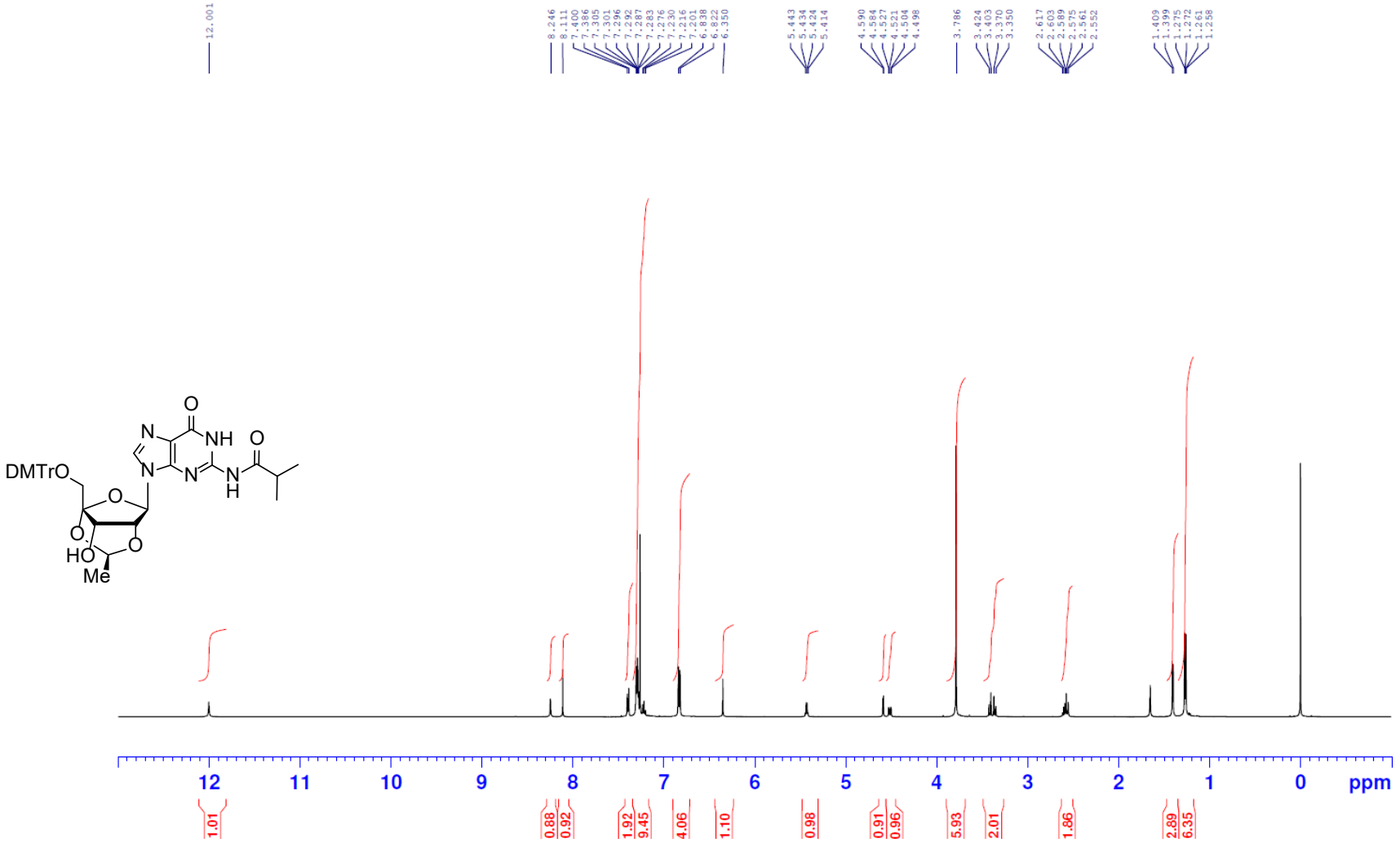
Compound **22** (500 MHz ^{1}H NMR in CD_3OD)



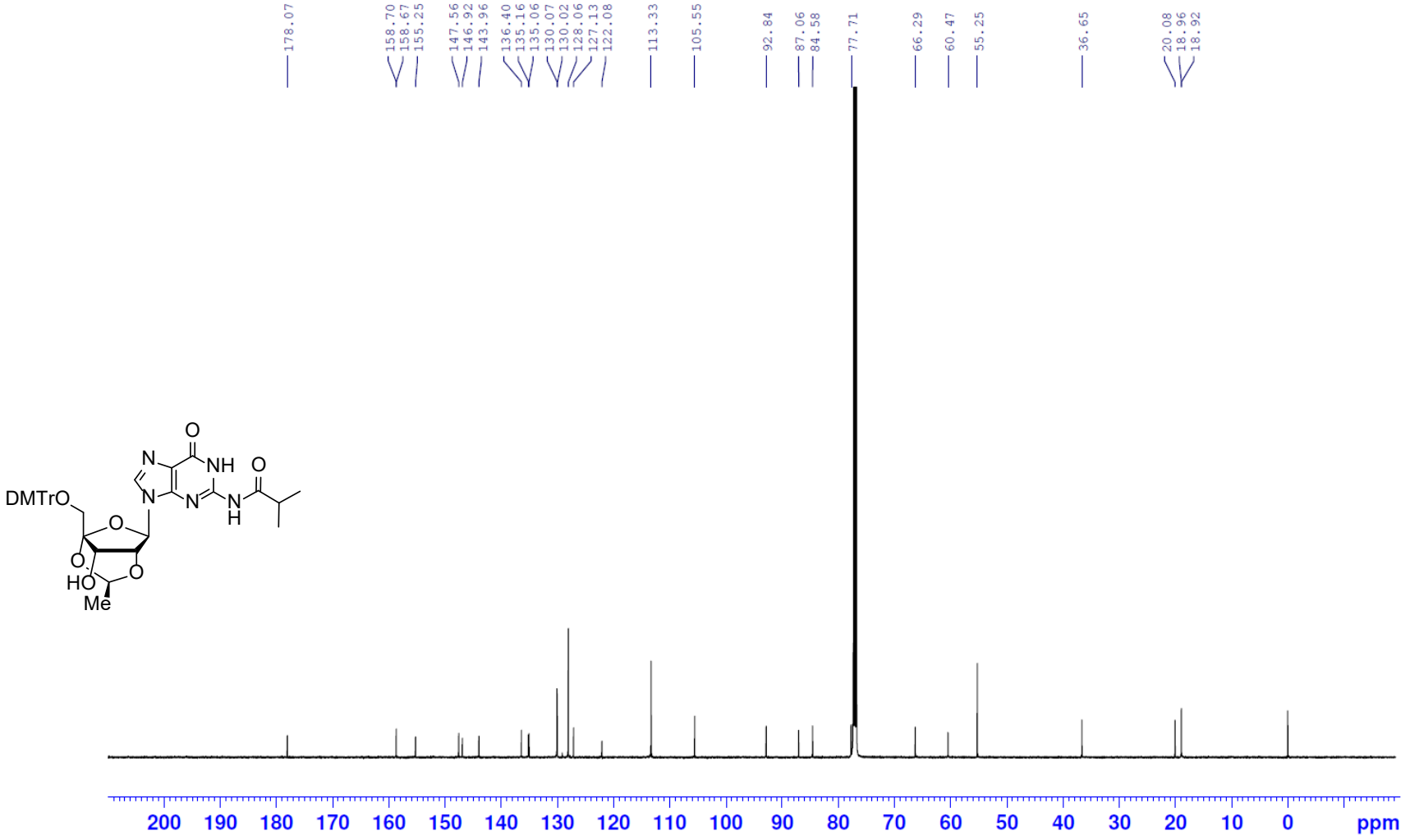
Compound **22** (125 MHz ^{13}C NMR in CD_3OD)



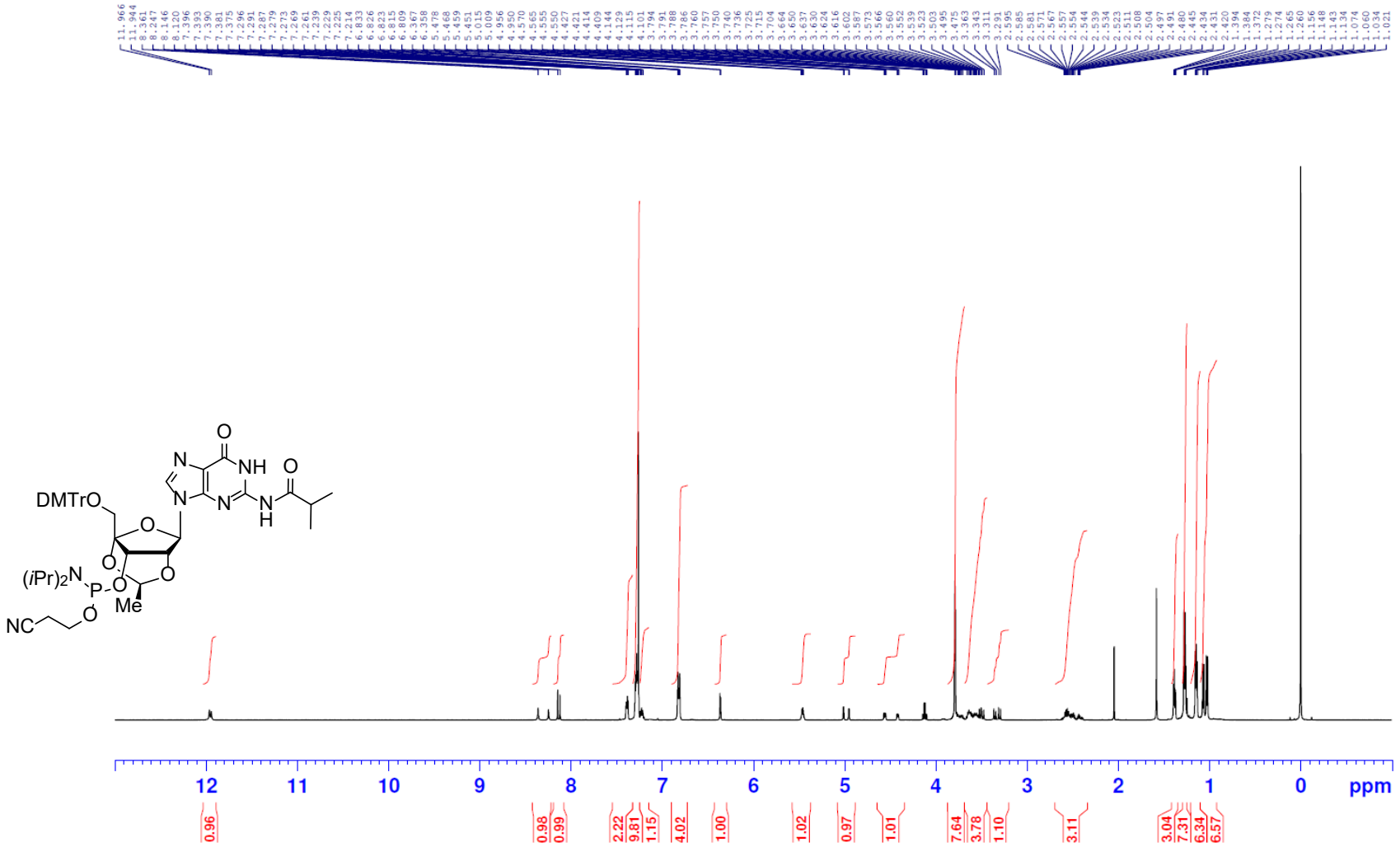
5′-*O*-DMTr-intermediate of guanosine compound (500 MHz ^{1}H NMR in $CDCl_3$)



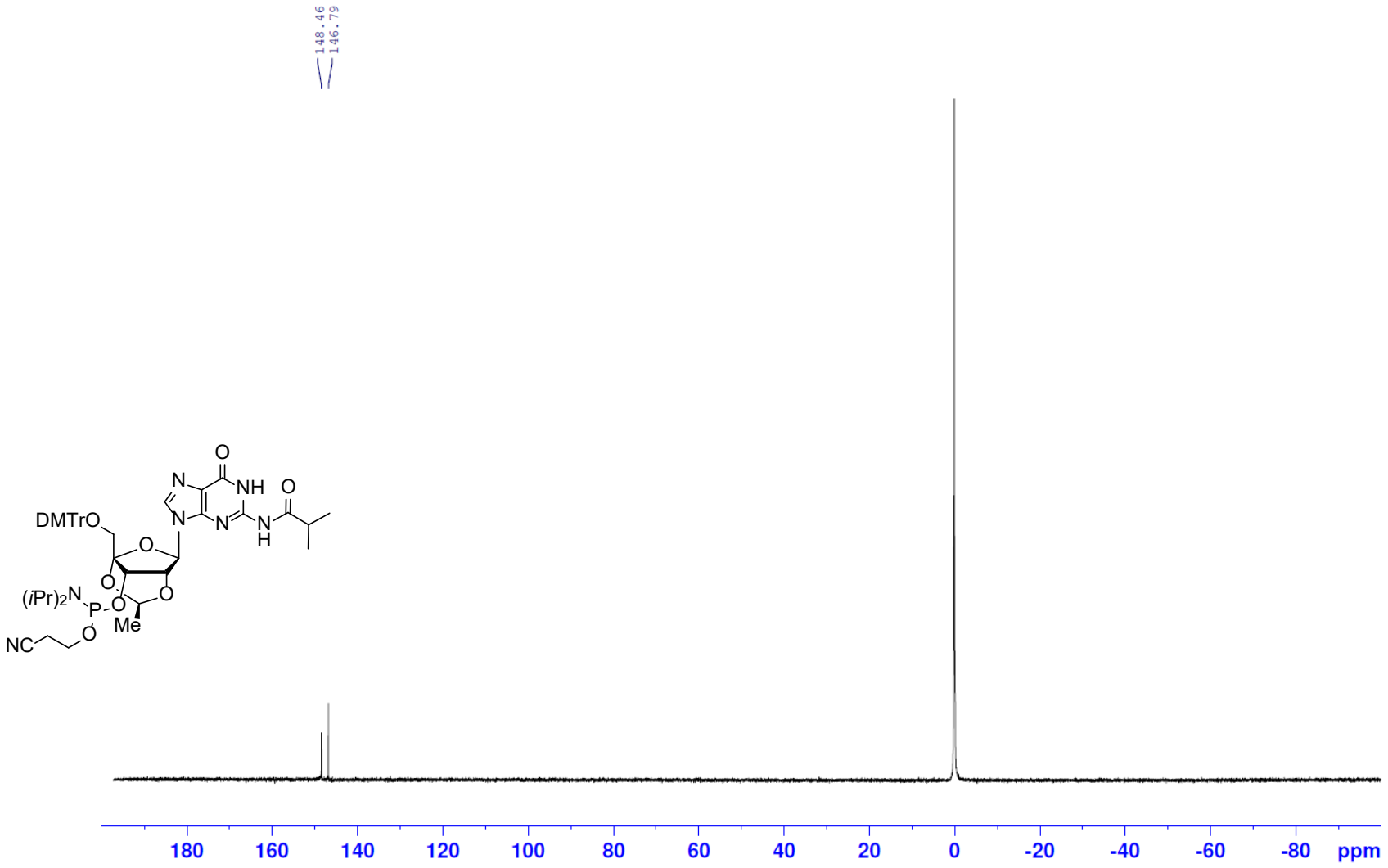
5′-*O*-DMTr-intermediate of guanosine compound (125 MHz ^{13}C NMR in $CDCl_3$)



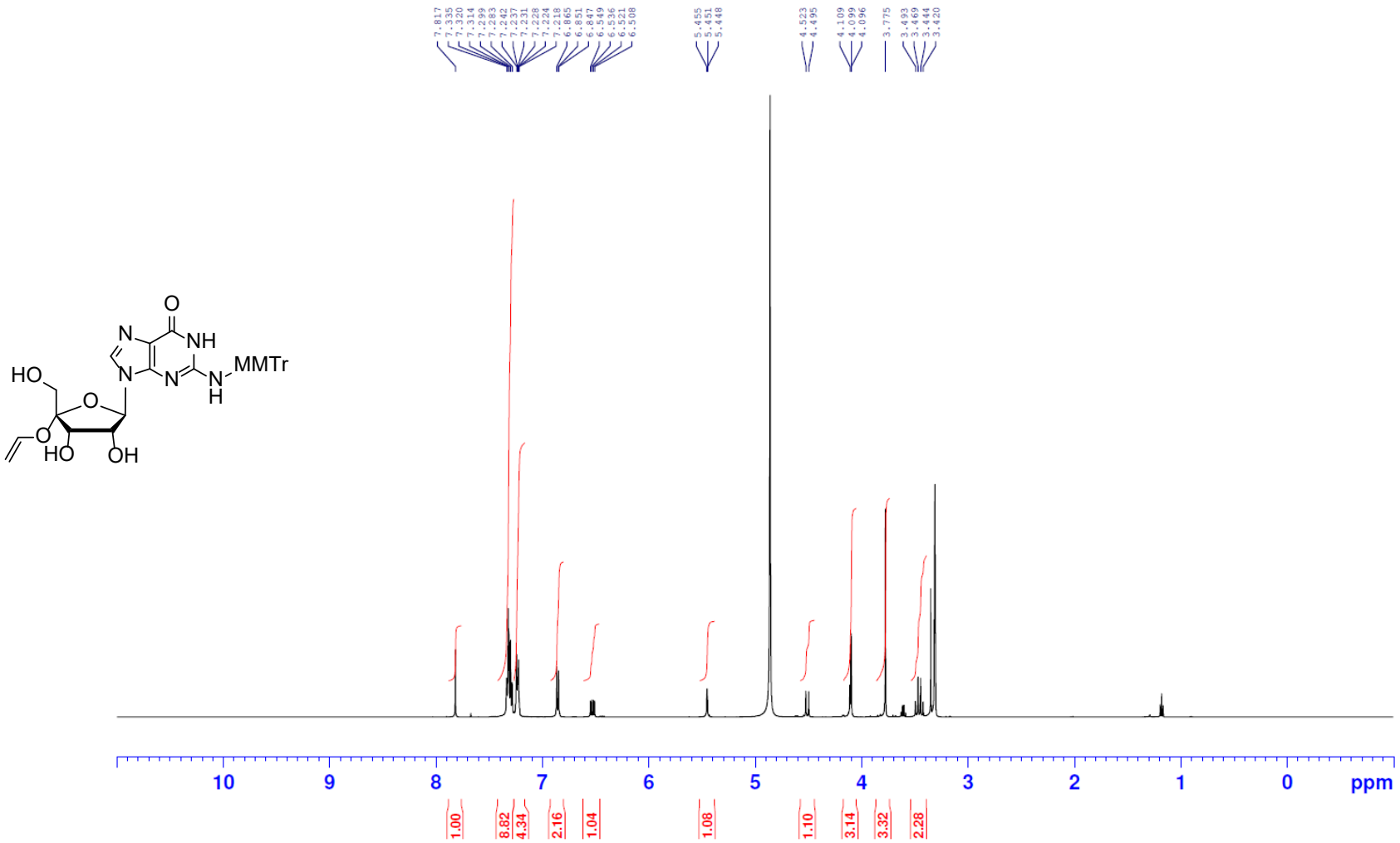
Compound **23** (500 MHz ^{1}H NMR in $CDCl_3$)



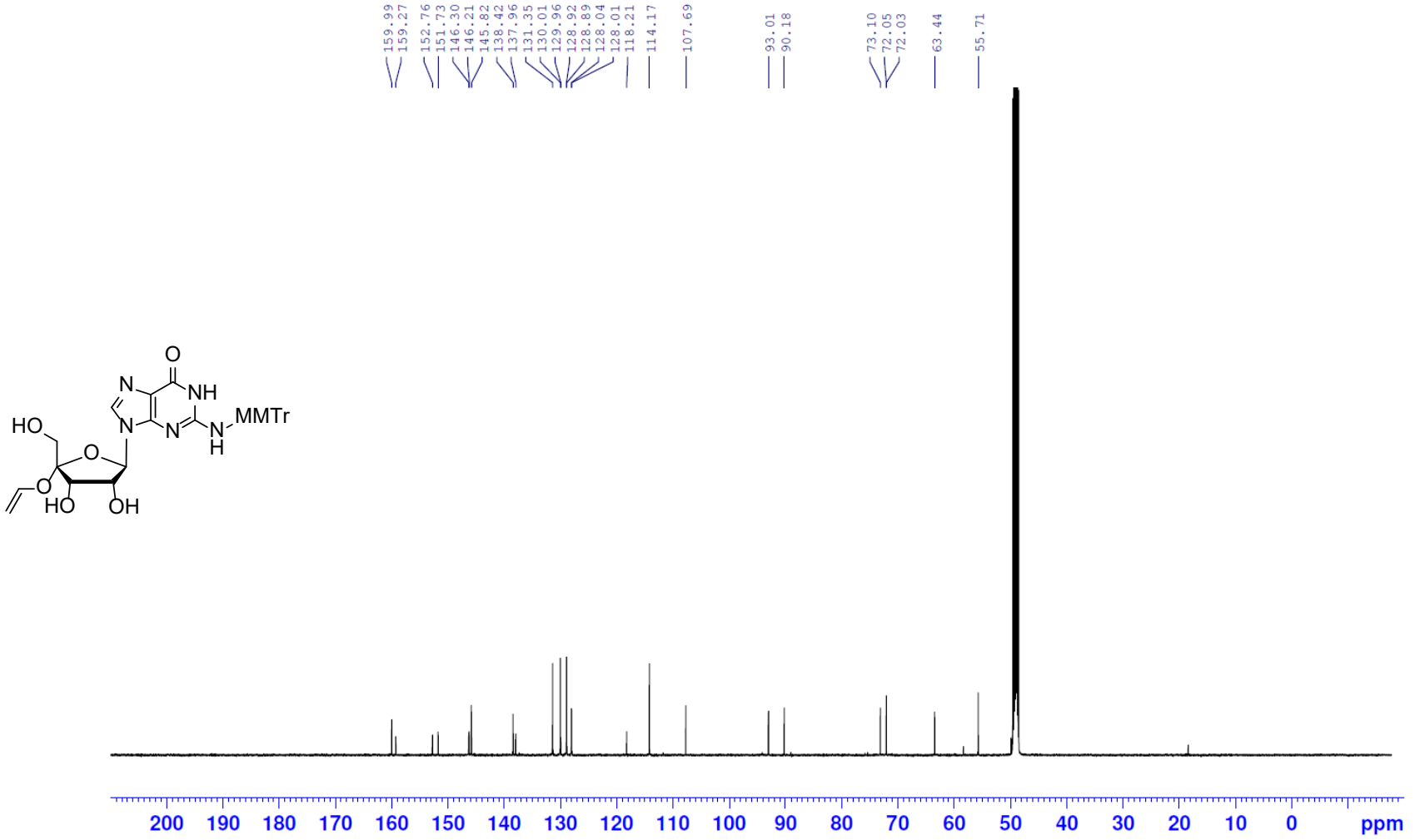
Compound **23** (202 MHz ^{31}P NMR in $CDCl_3$)



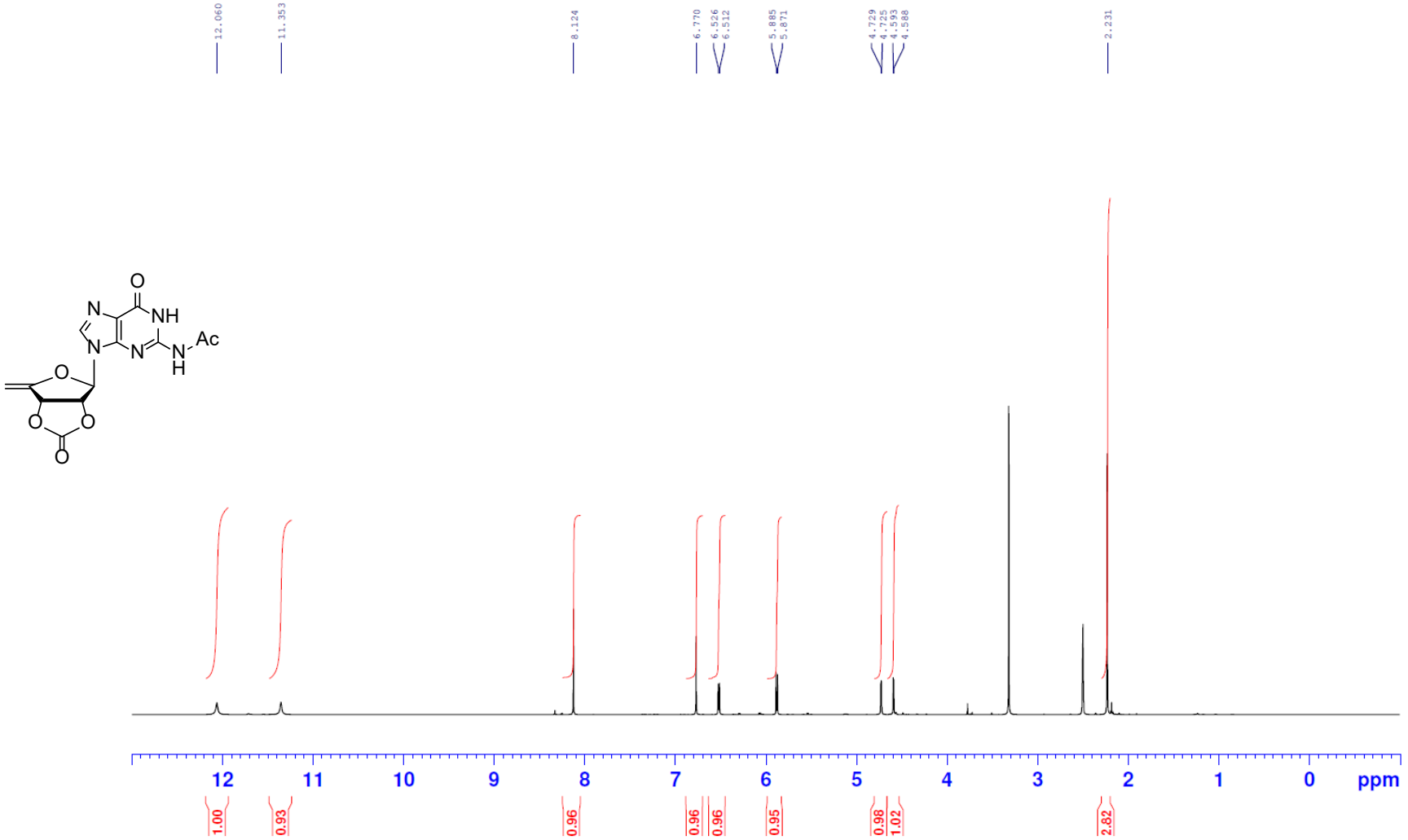
Compound **S1** (500 MHz ^{1}H NMR in CD_3OD)



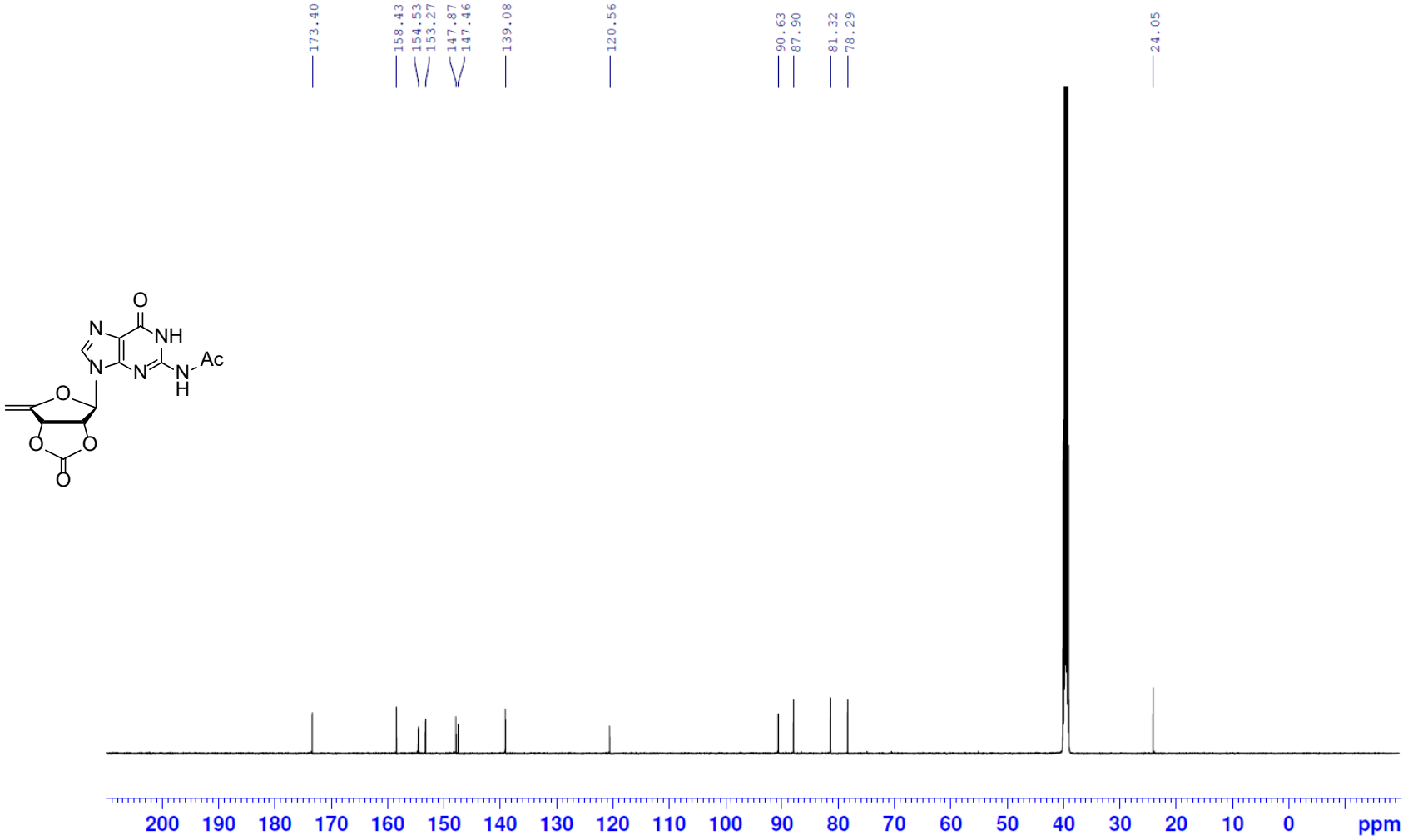
Compound **S1** (125 MHz ^{13}C NMR in CD_3OD)



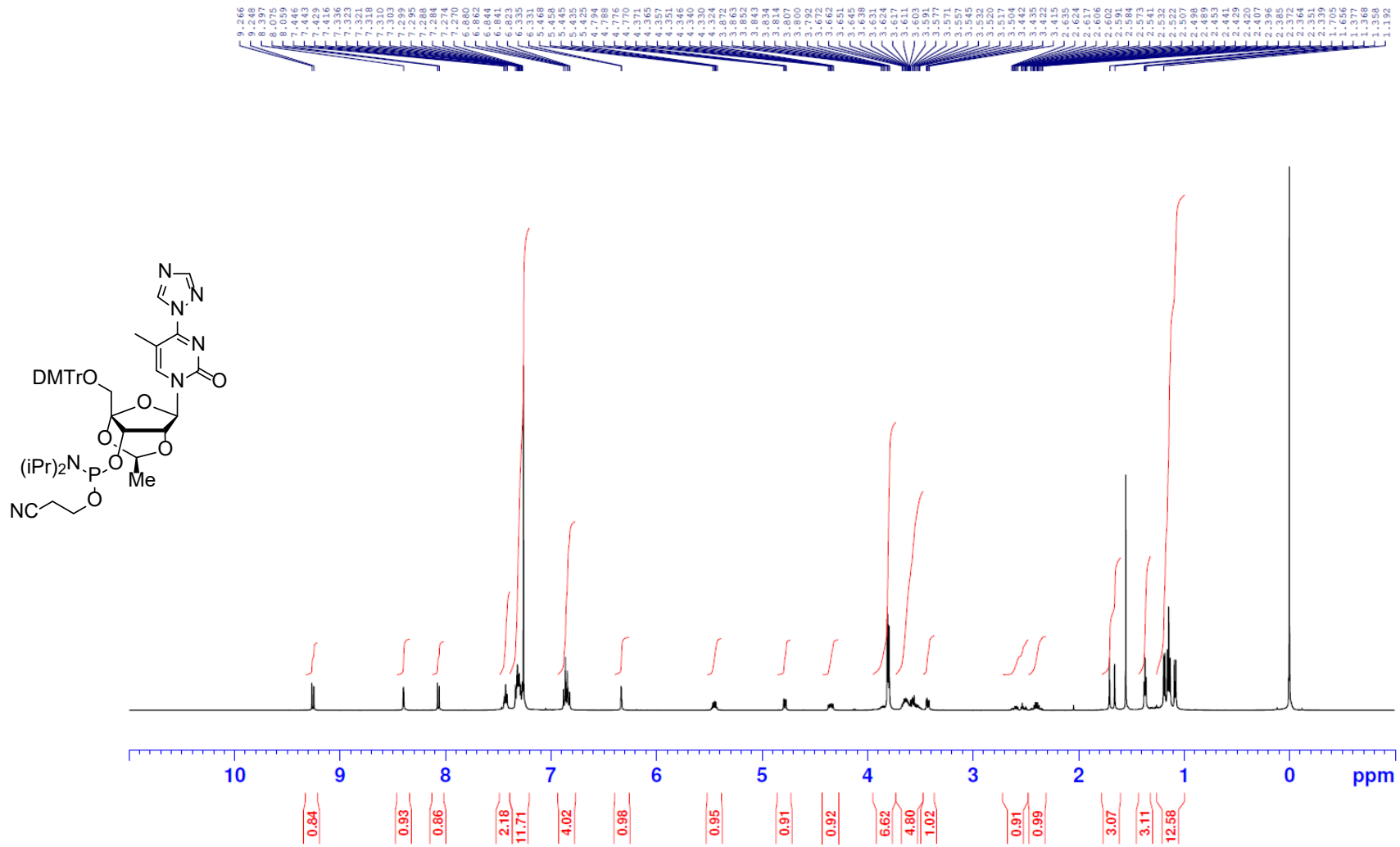
Compound **S2** (500 MHz ^{1}H NMR in DMSO-d_6)



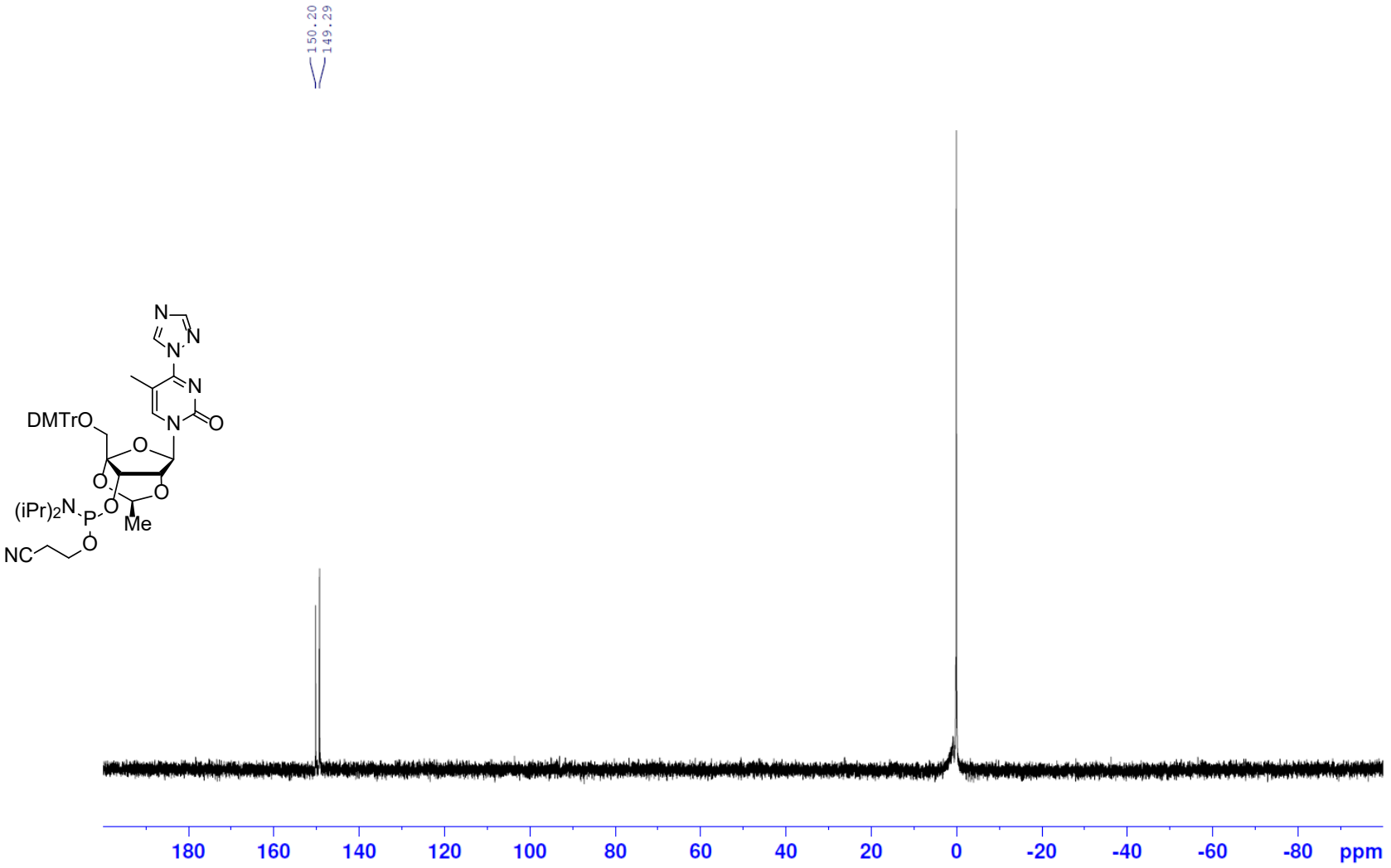
Compound **S2** (125 MHz ^{13}C NMR in DMSO-d_6)



Compound **S3** (500 MHz ^{1}H NMR in $CDCl_3$)

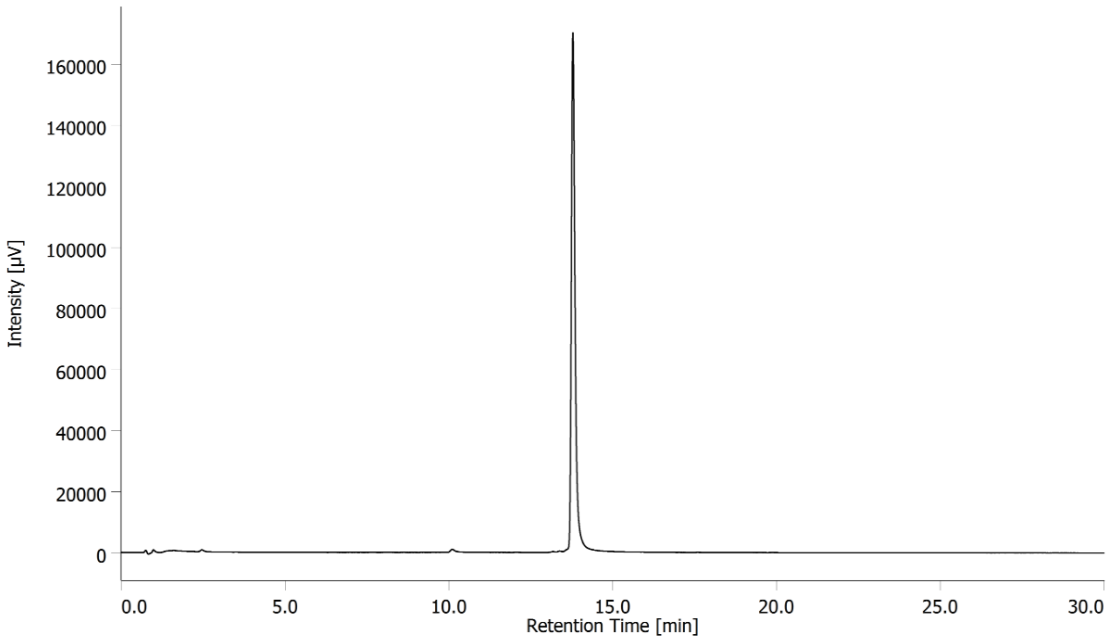


Compound **S3** (202 MHz ^{31}P NMR in $CDCl_3$)

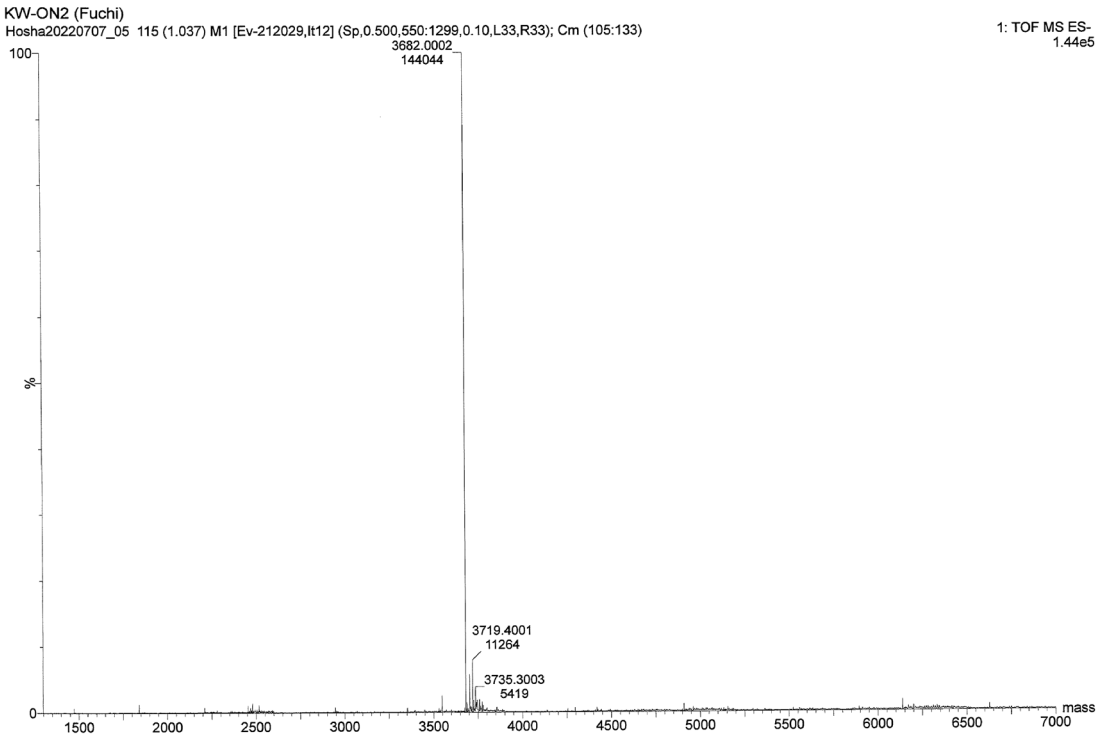


ON1

Column : Waters XBridge® MS C_{18} 5.0 μm, 4.6 × 50 mm.
Gradient : 5-15% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40 ˚C.

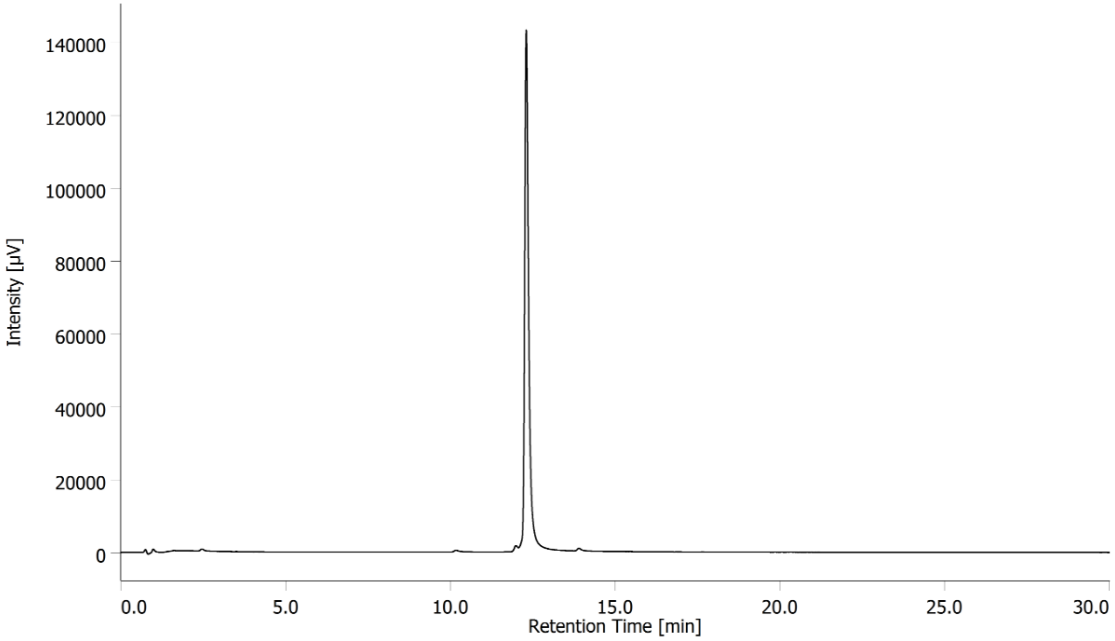


ESI-MS spectrum of **ON1**

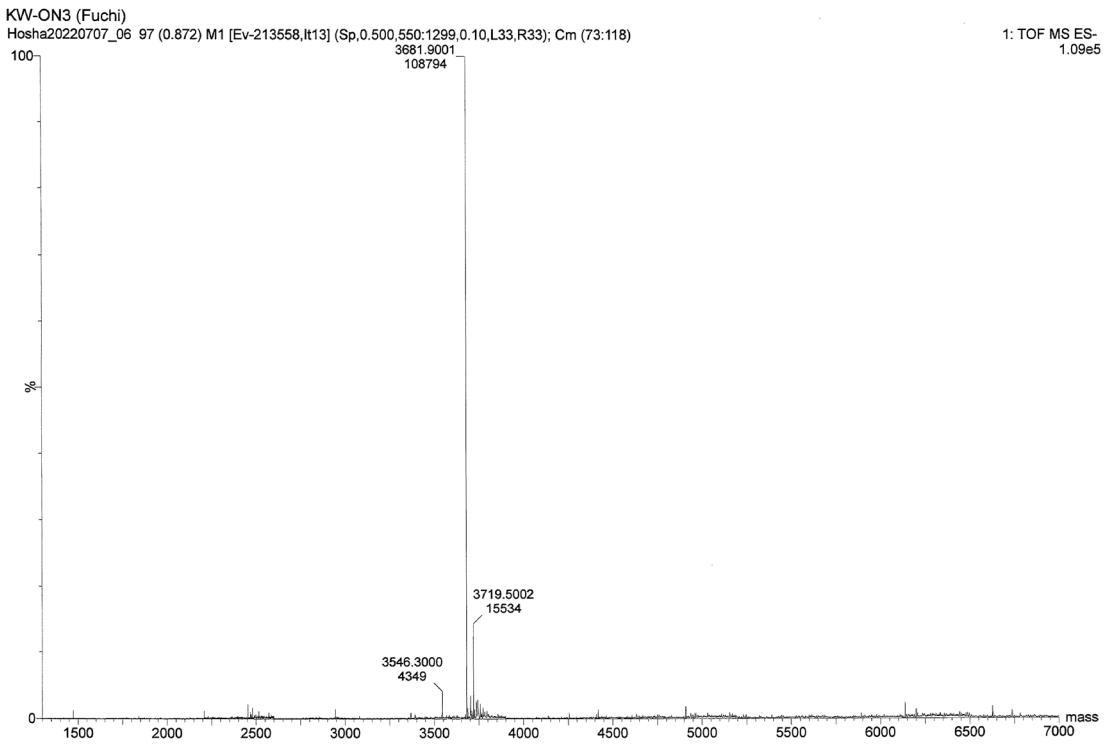


ON2

Column : Waters XBridge® MS C_{18} 5.0 μm, 4.6 × 50 mm.
Gradient : 5-15% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40 ˚C.

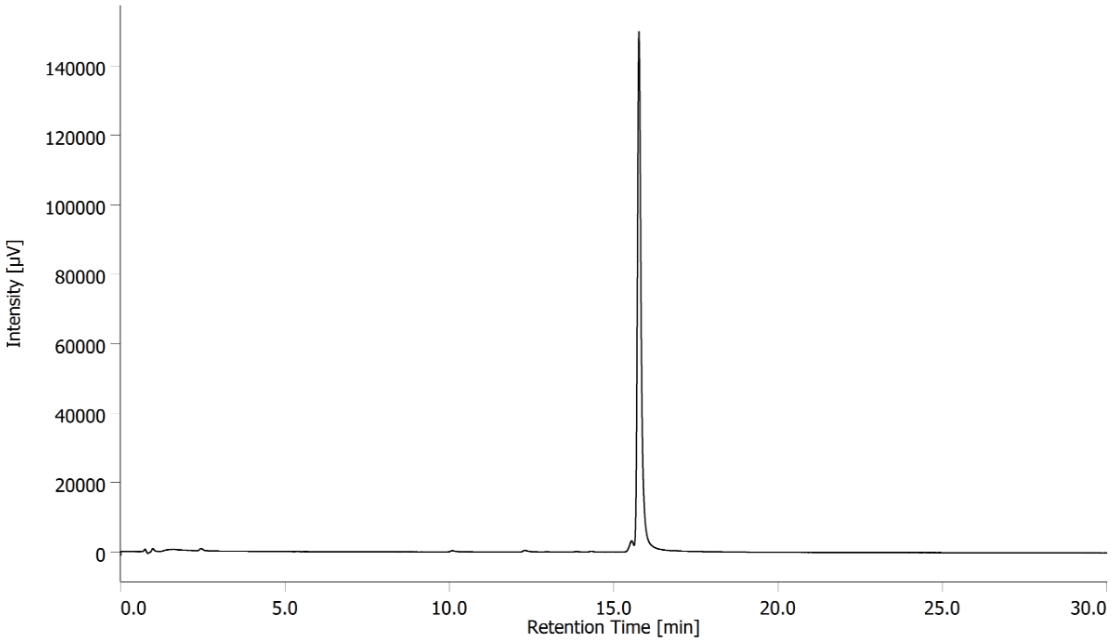


ESI-MS spectrum of **ON2**

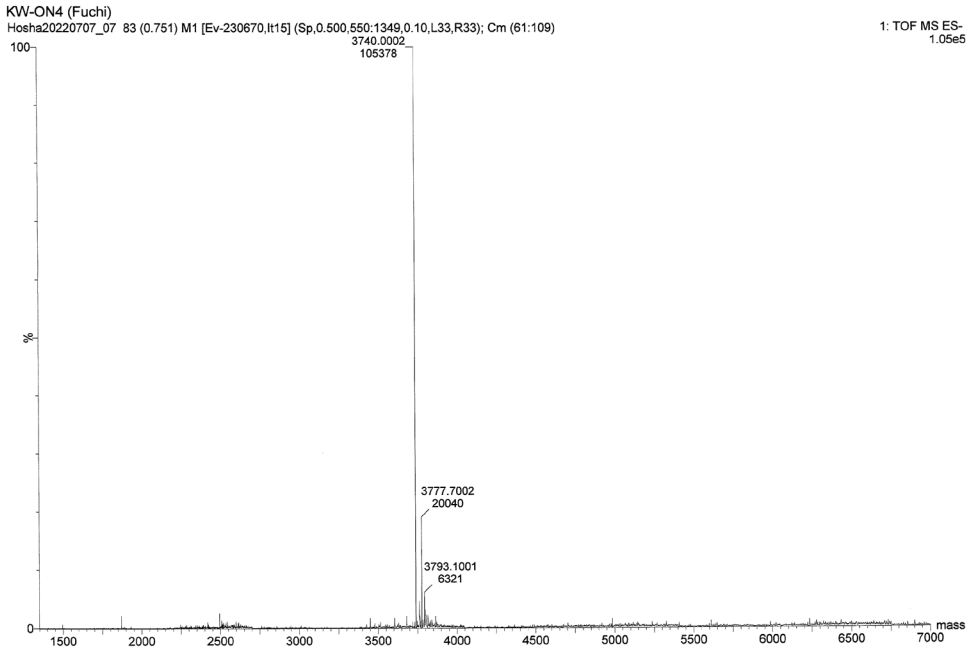


ON3

Column : Waters XBridge® MS C_{18} 5.0 µm, 4.6 × 50 mm.
Gradient : 5-15% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40 ˚C.

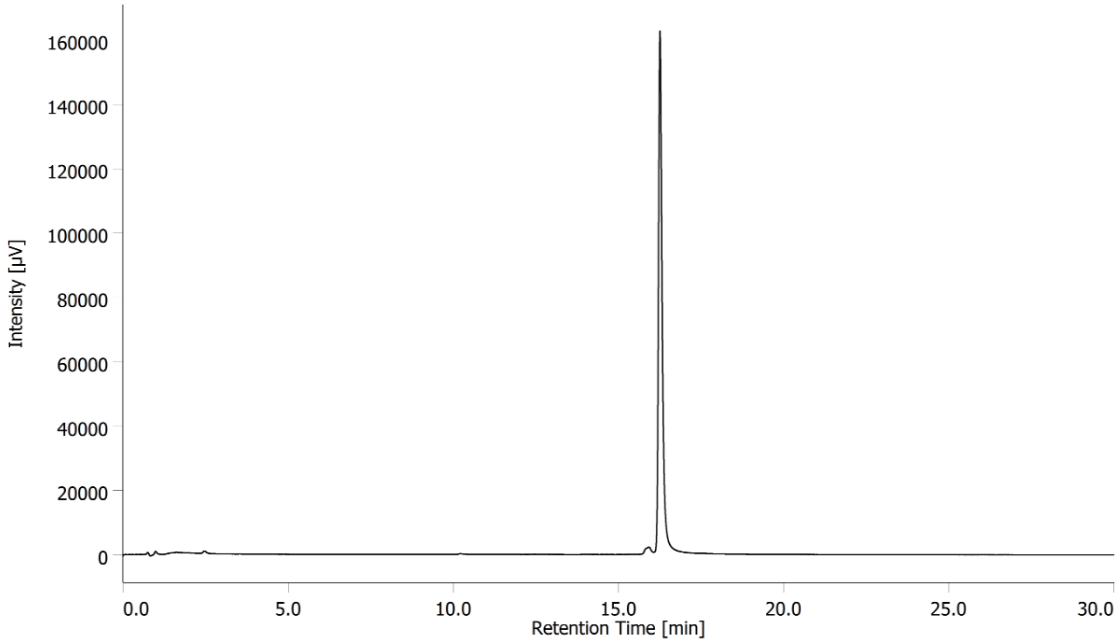


ESI-MS spectrum of **ON3**

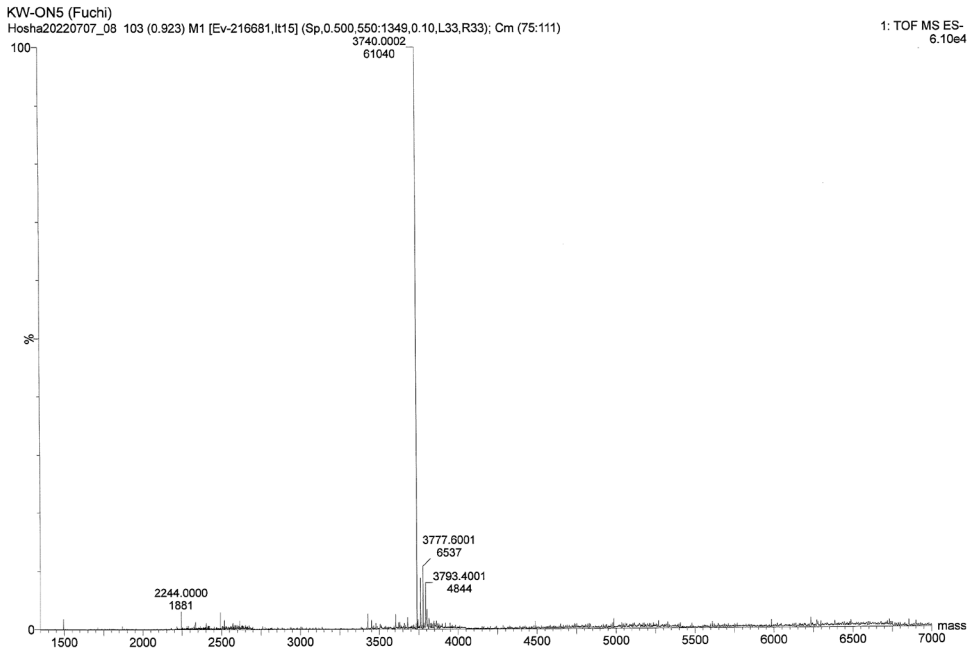


ON4

Column : Waters XBridge® MS C_{18} 5.0 µm, 4.6 × 50 mm.
Gradient : 5-15% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40 ˚C.

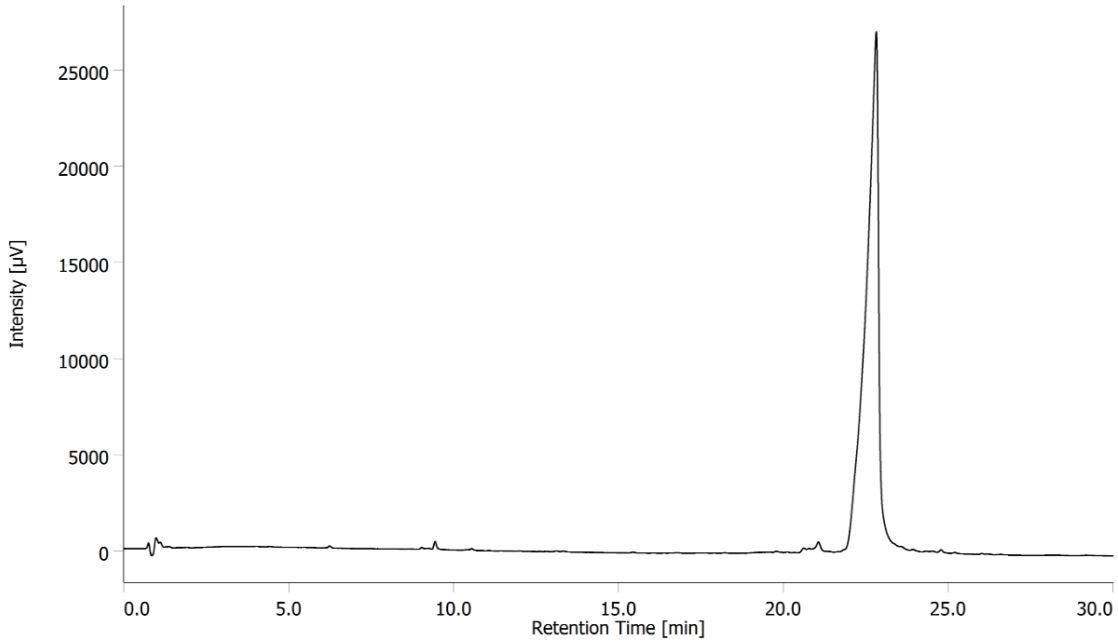


ESI-MS spectrum of **ON4**



ON5

Column : Waters XBridge® MS C_{18} 5.0 μm, 4.6 × 50 mm.
Gradient : 5-25% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 60 ˚C.



ESI-MS spectrum of **ON5**