SUPPLEMENTARY INFORMATION

Synthesis of oligonucleotides containing 2-*N*-heteroarylguanine residues and their effect on duplex/triplex stability

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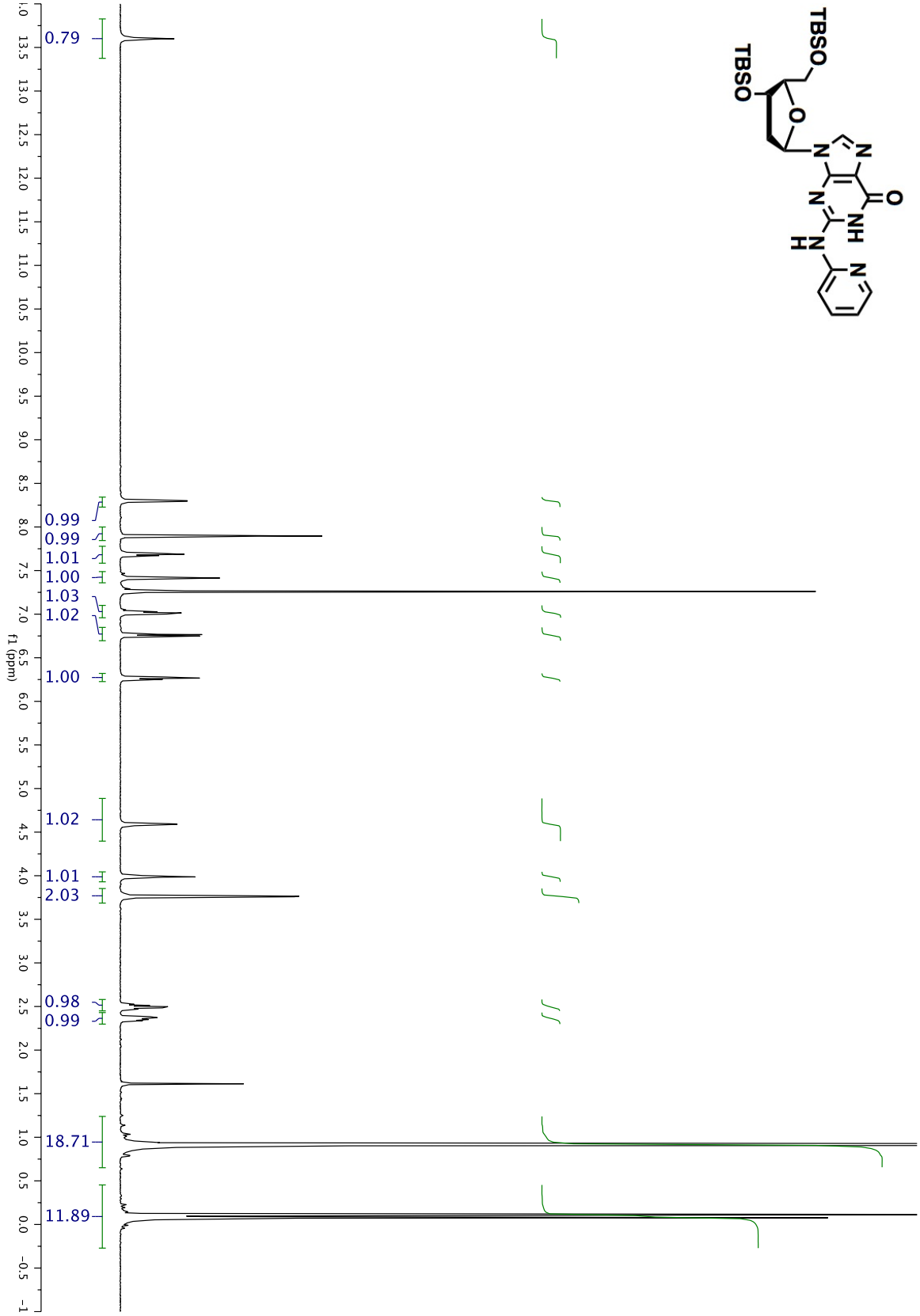
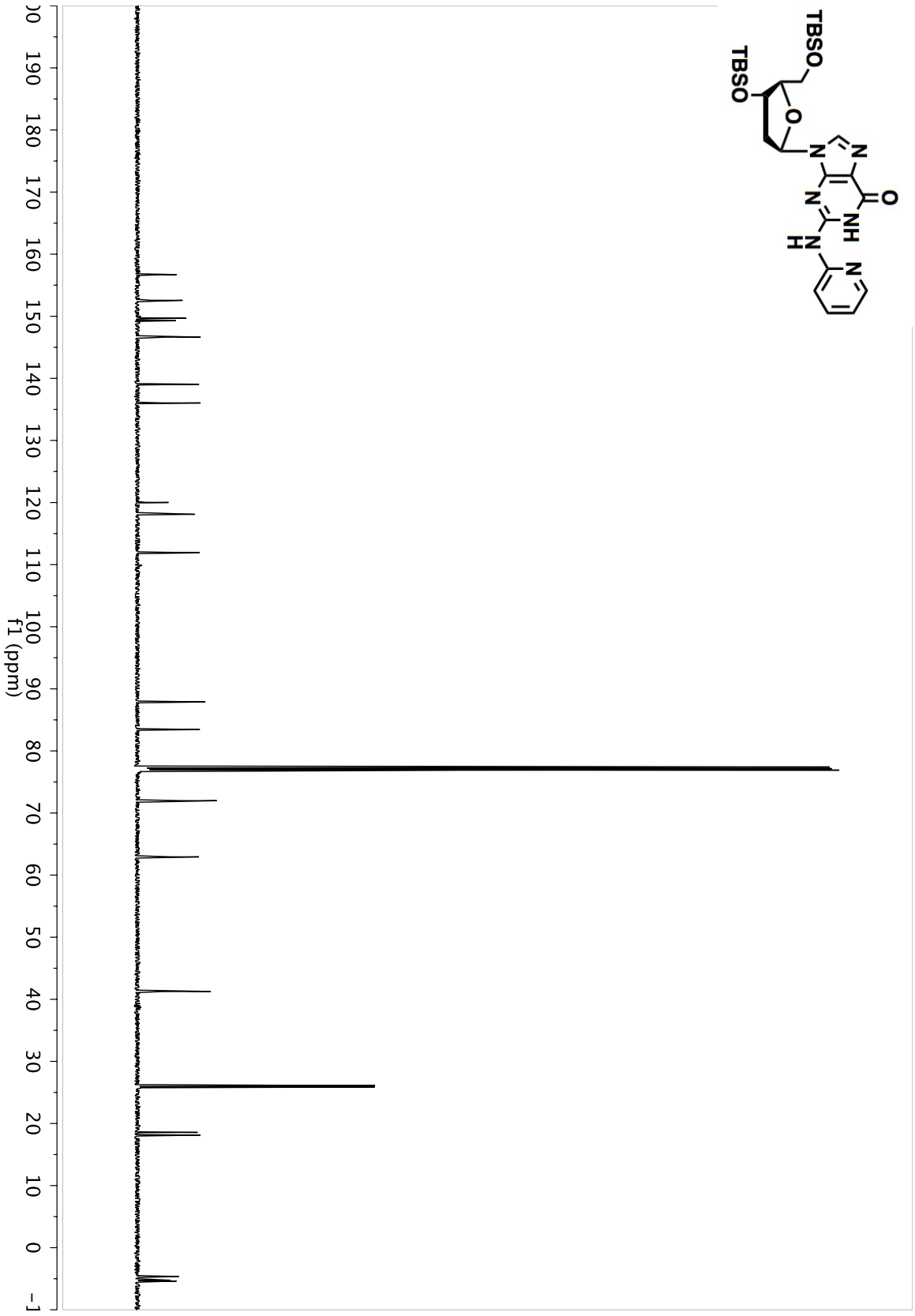
contents

^{1}H NMR of Compound 2a



^{13}C NMR of Compound 2a



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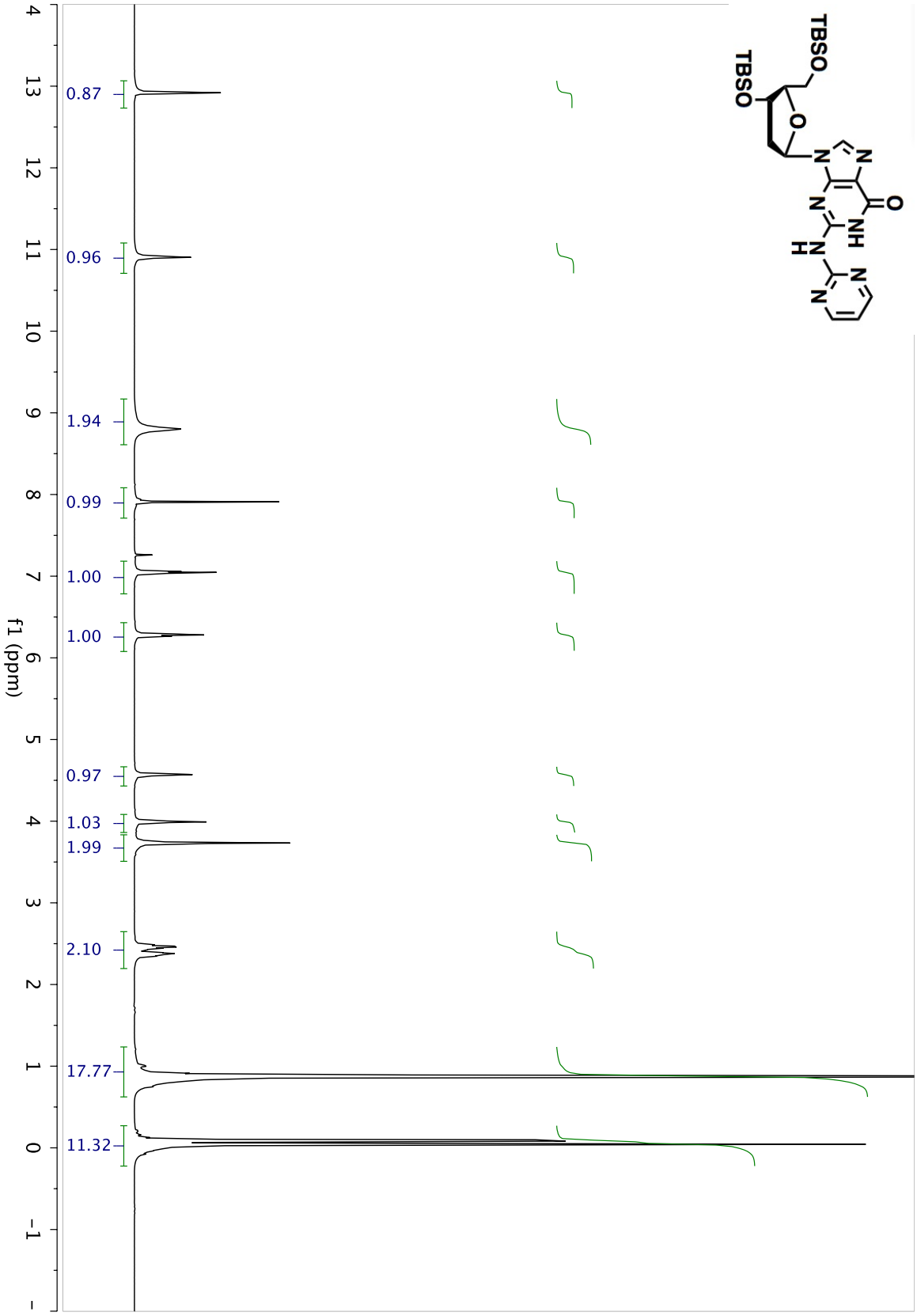

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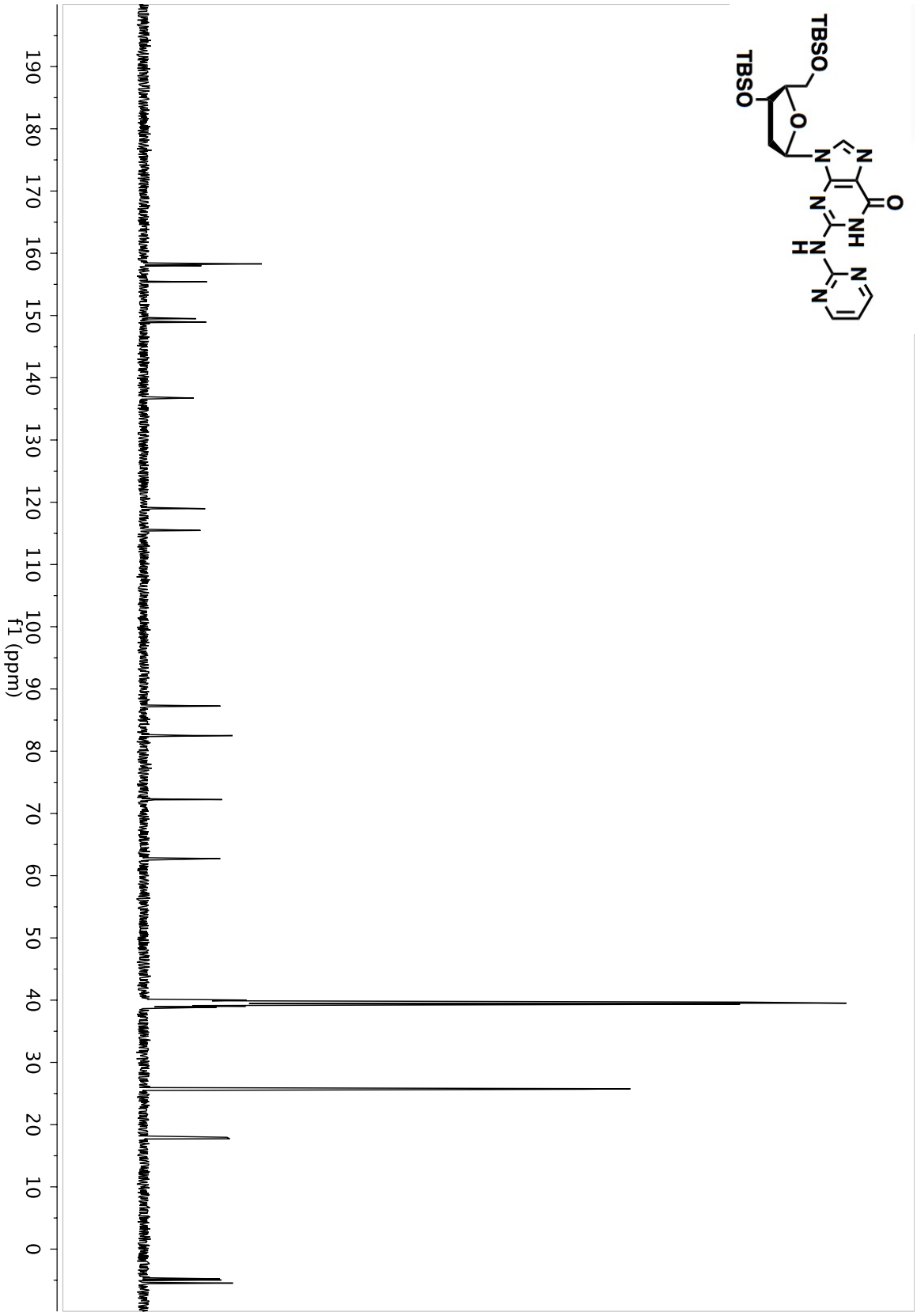

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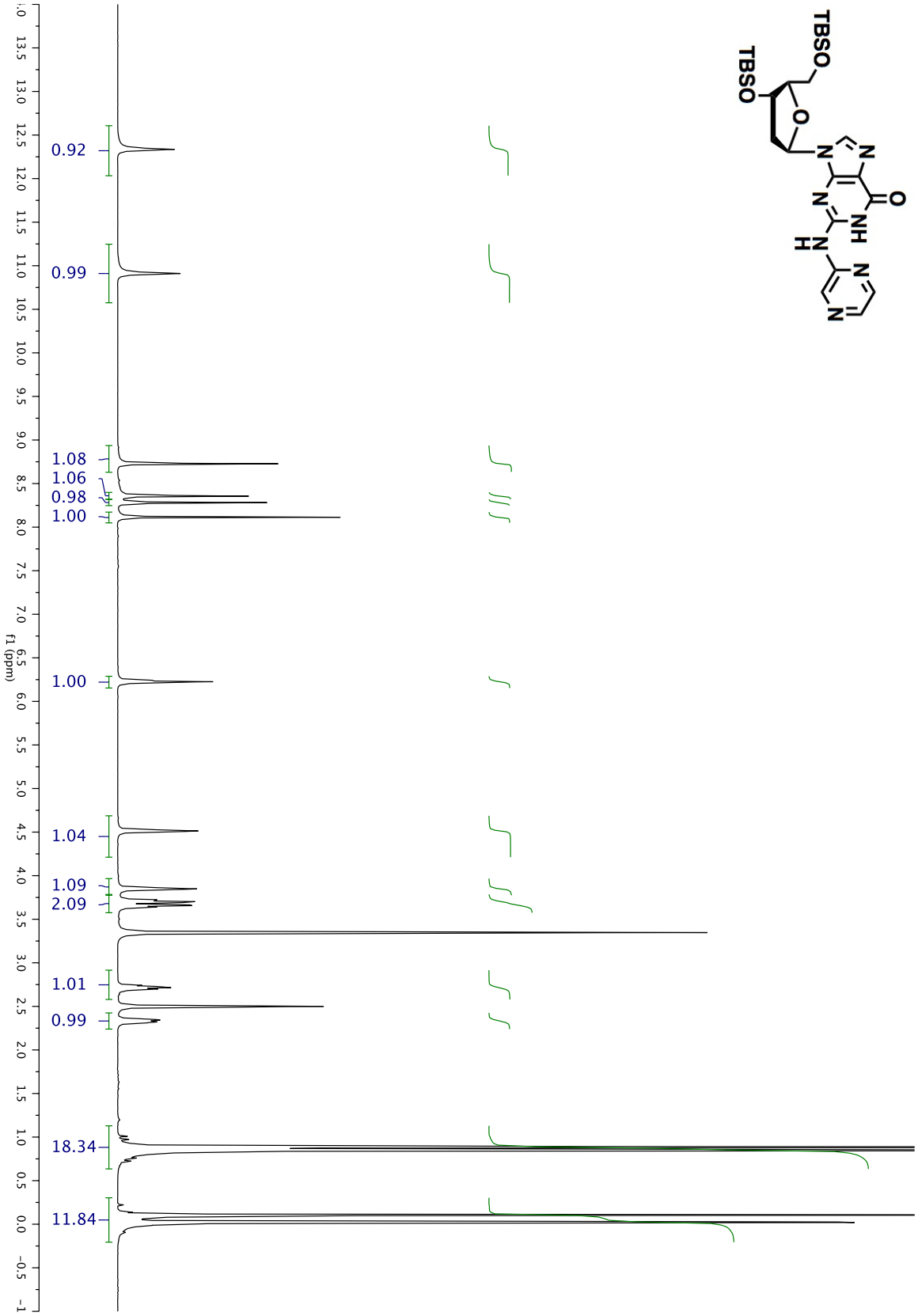

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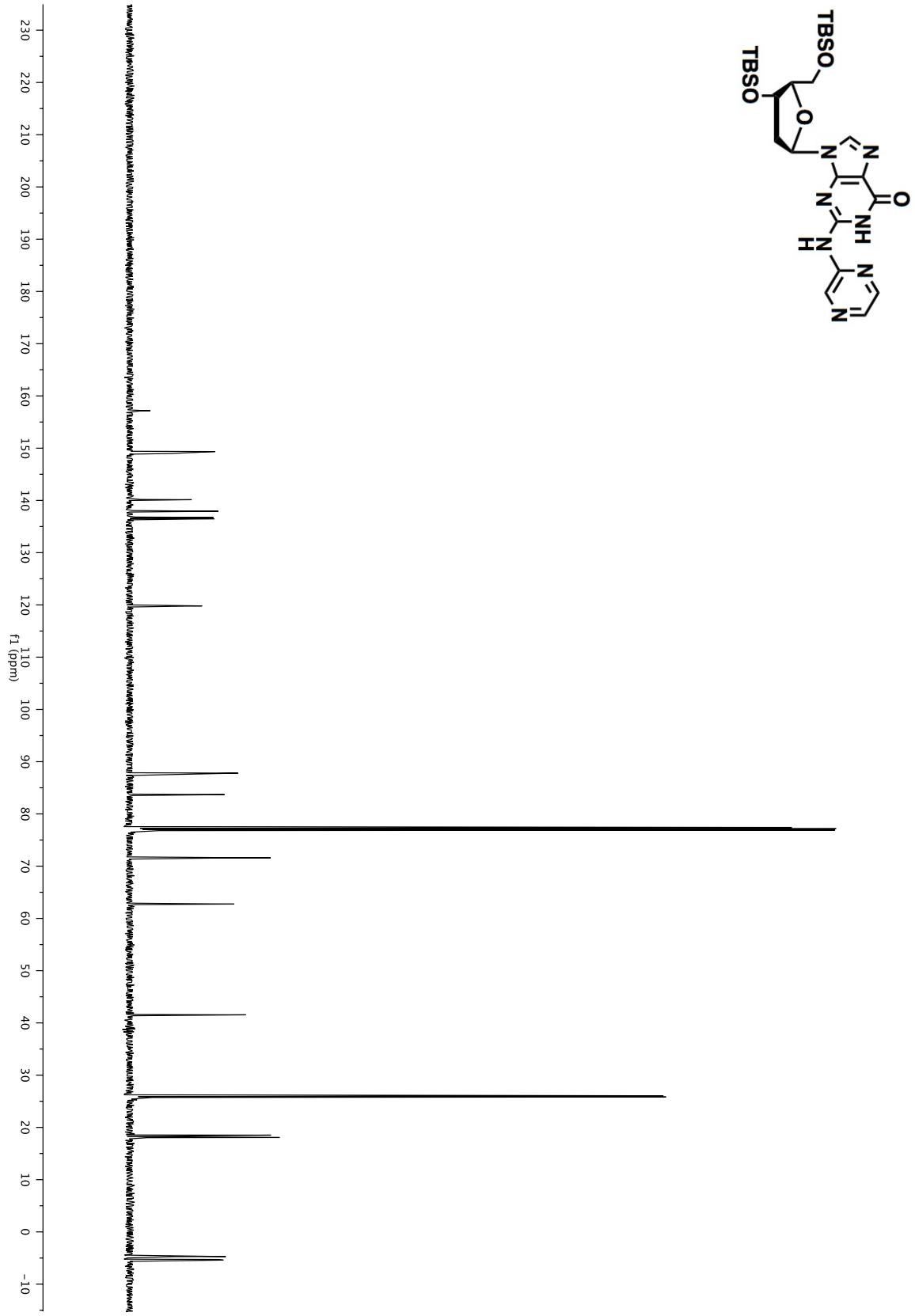

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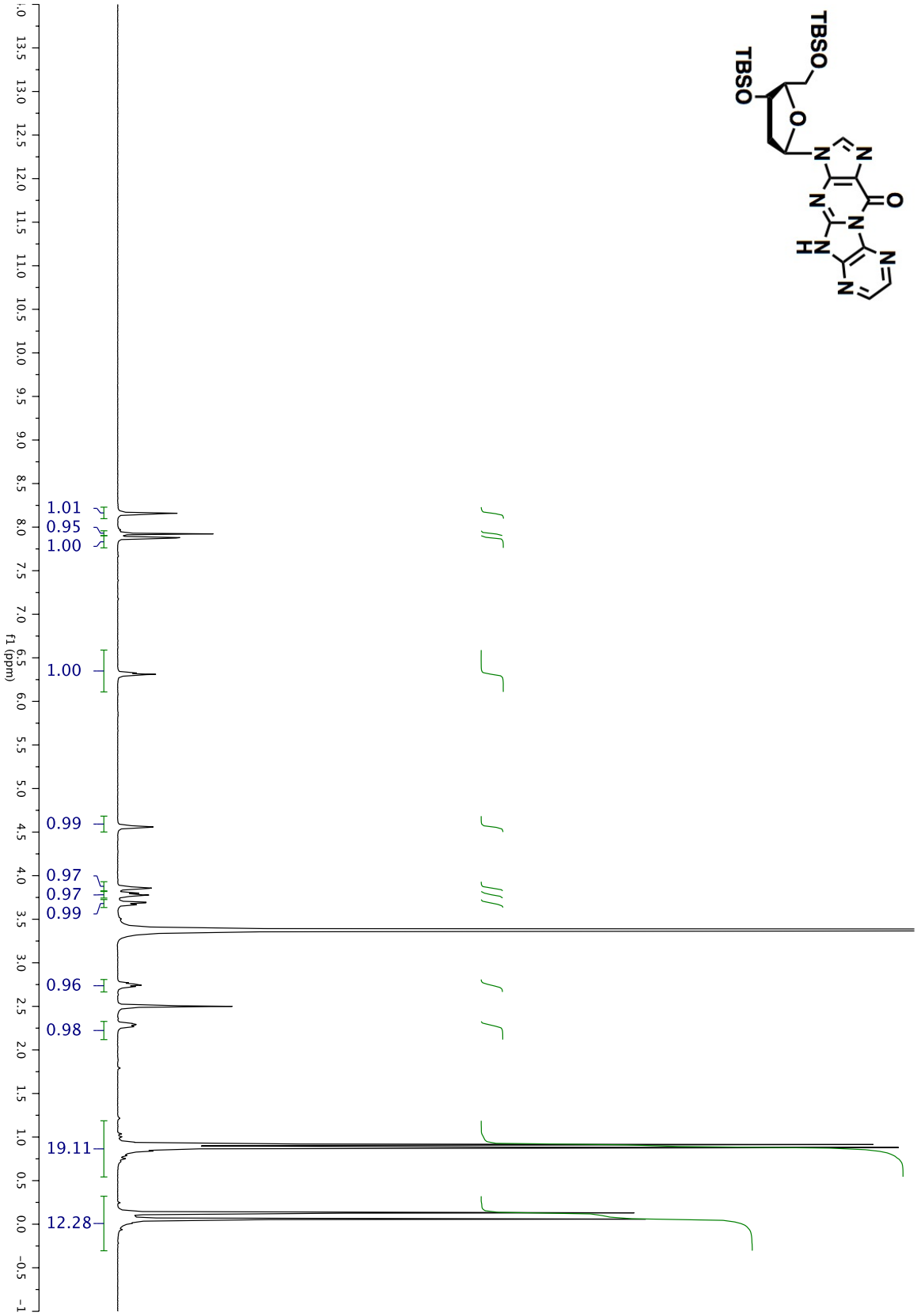

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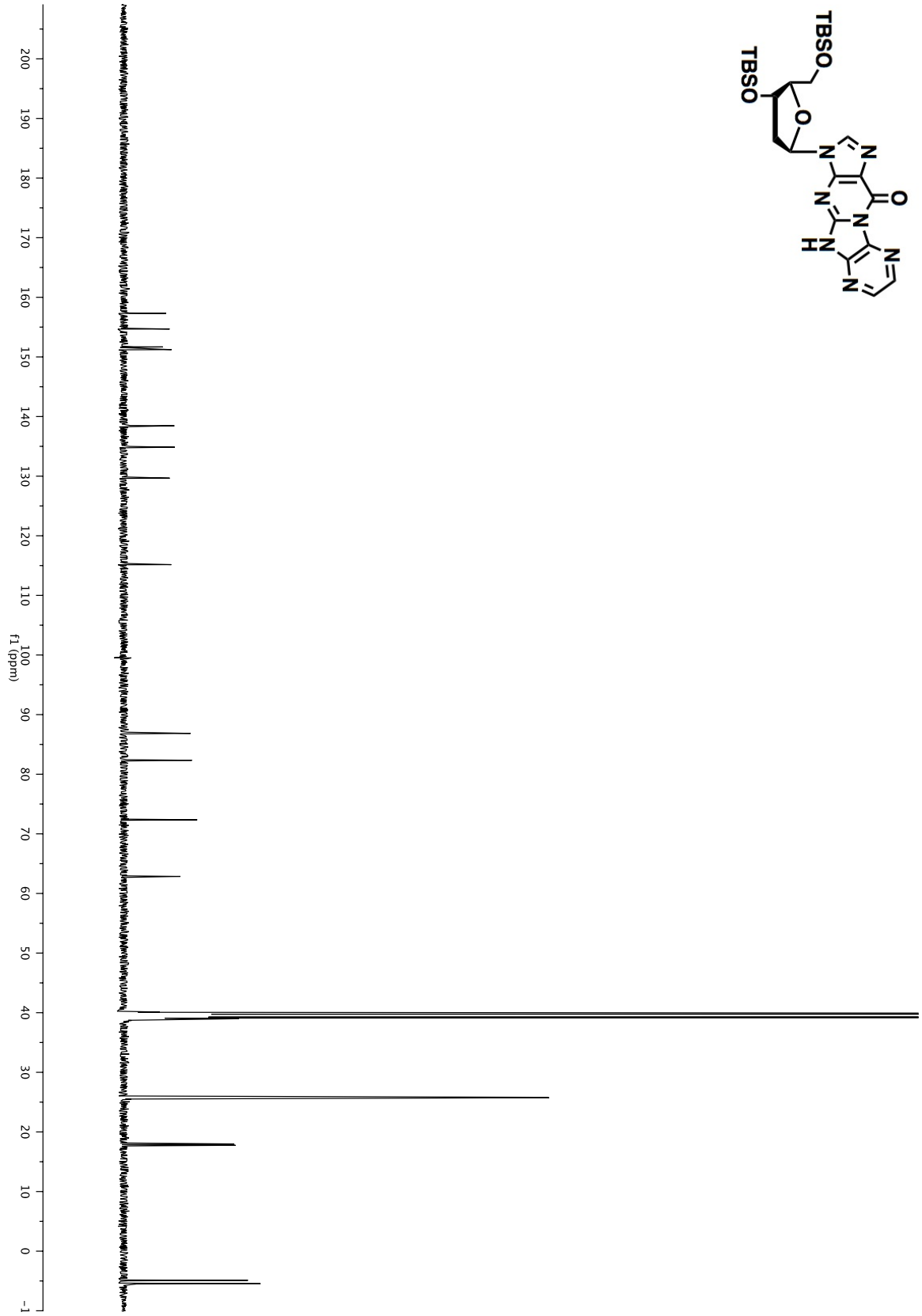

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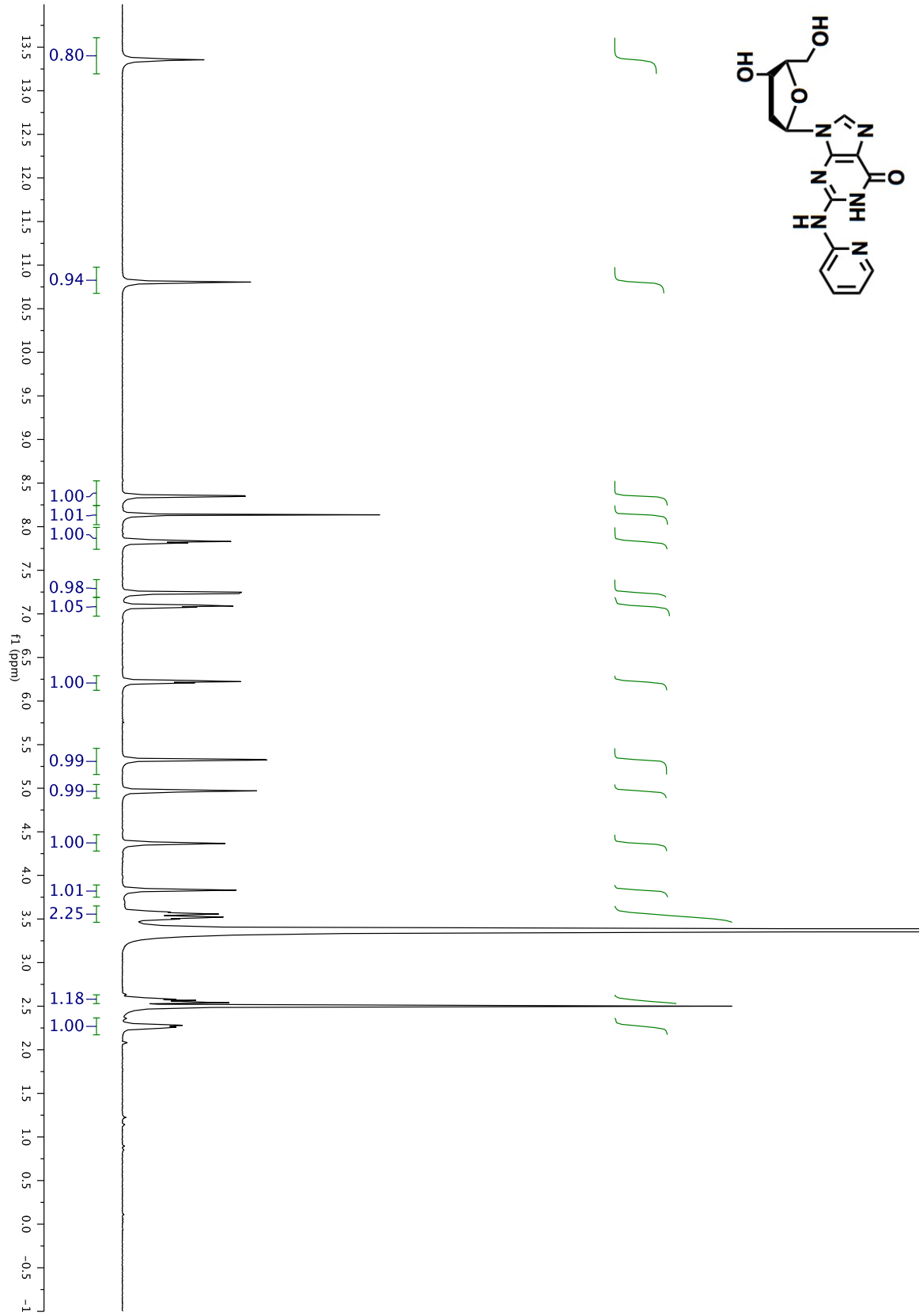

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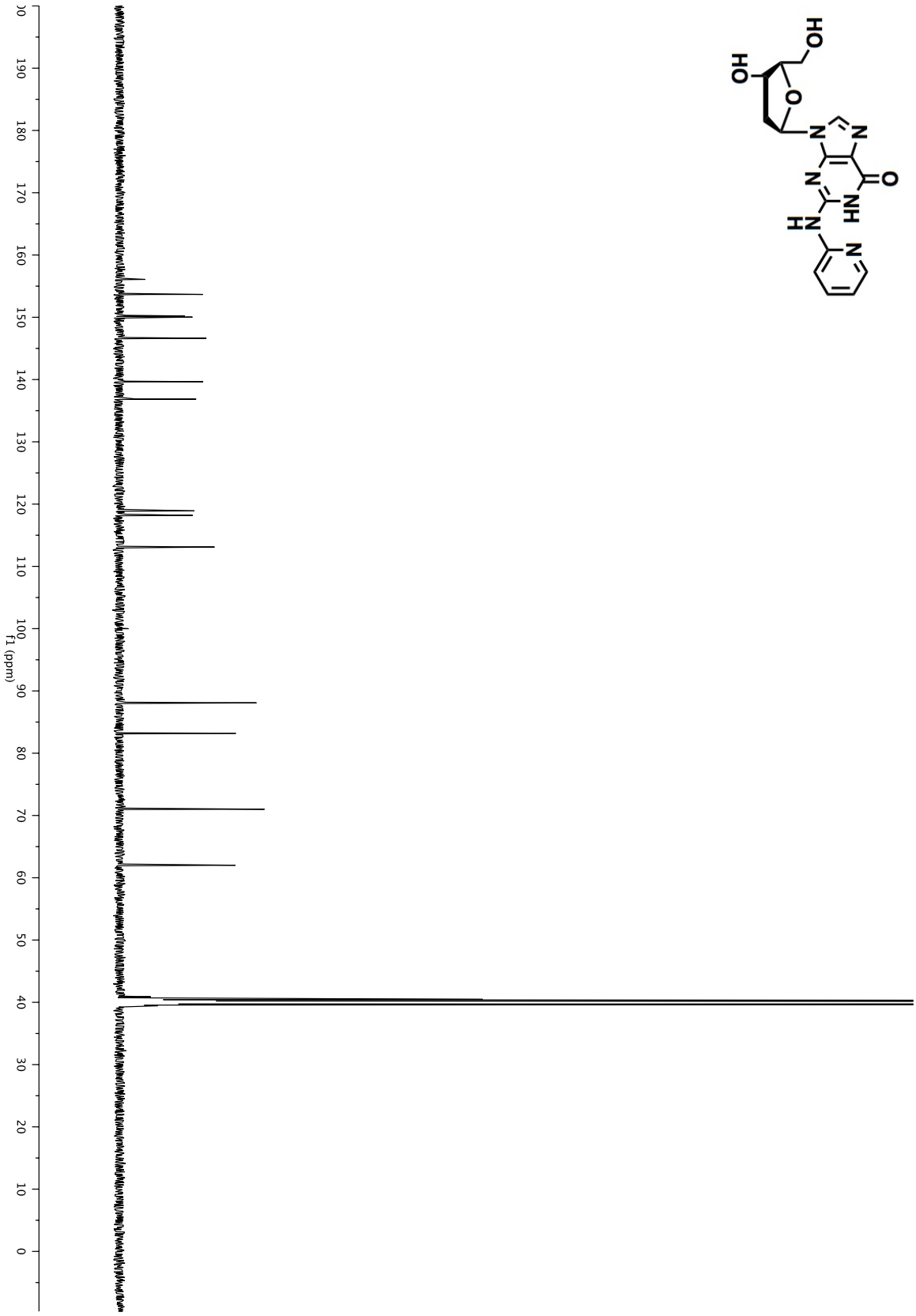

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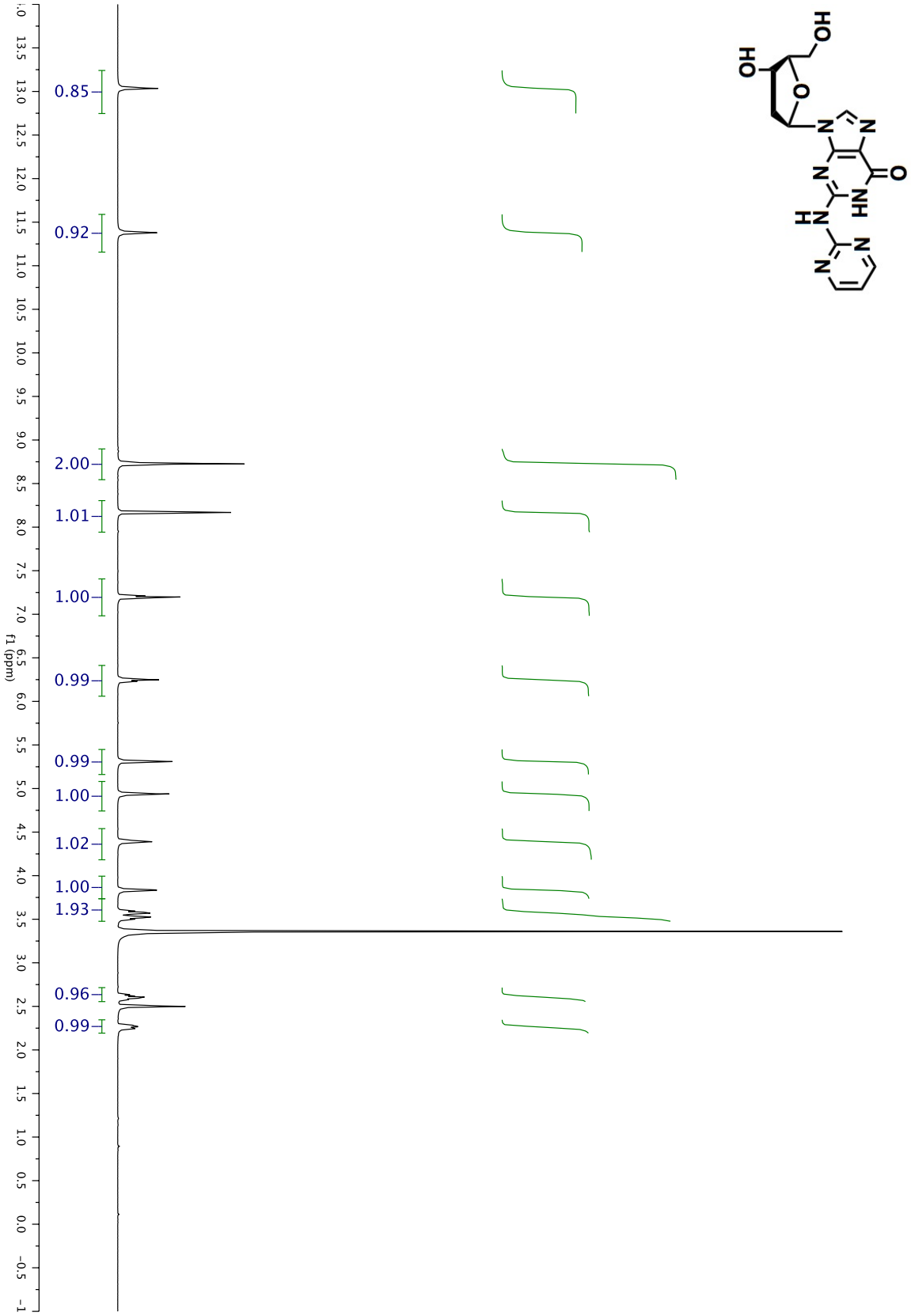

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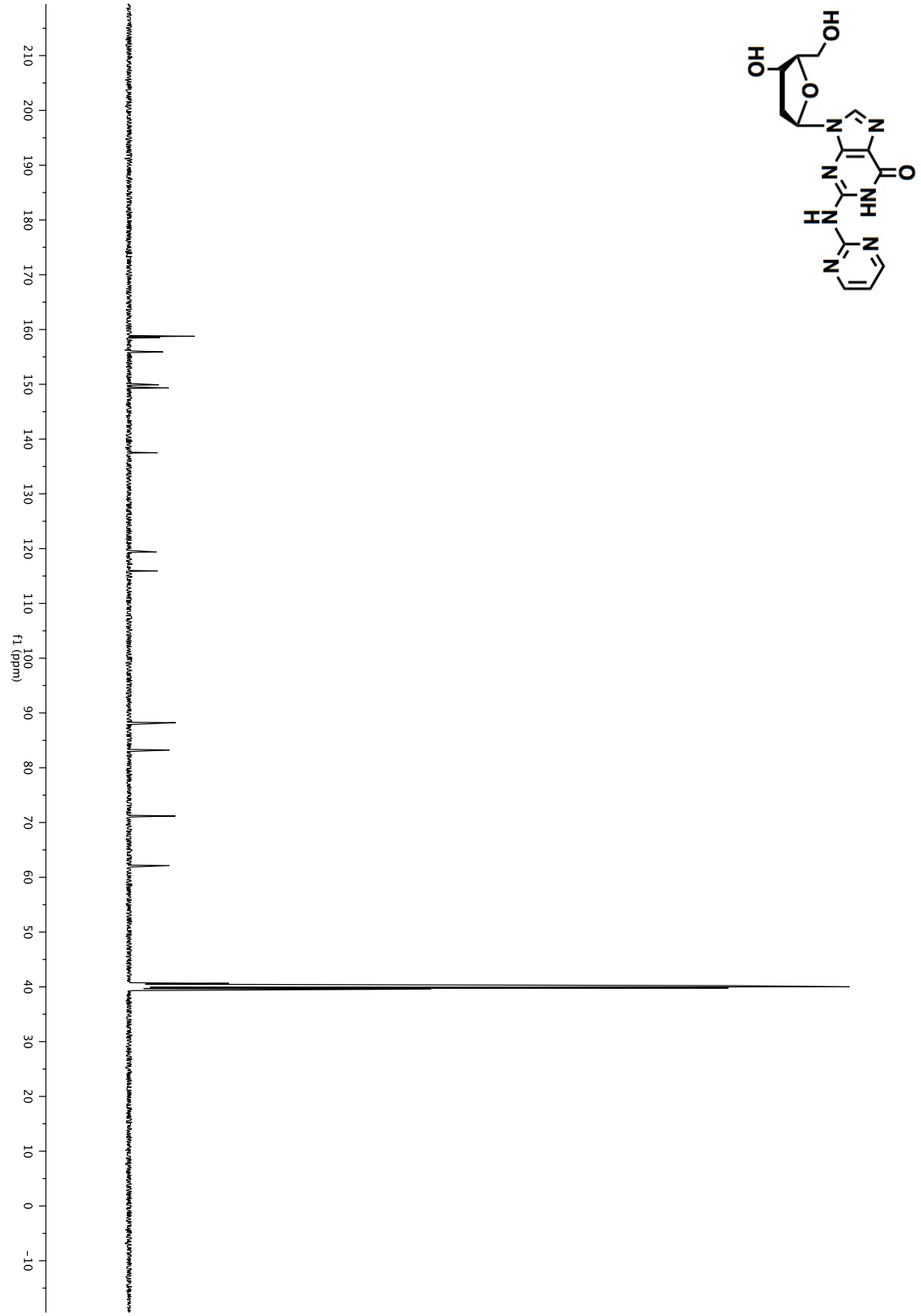

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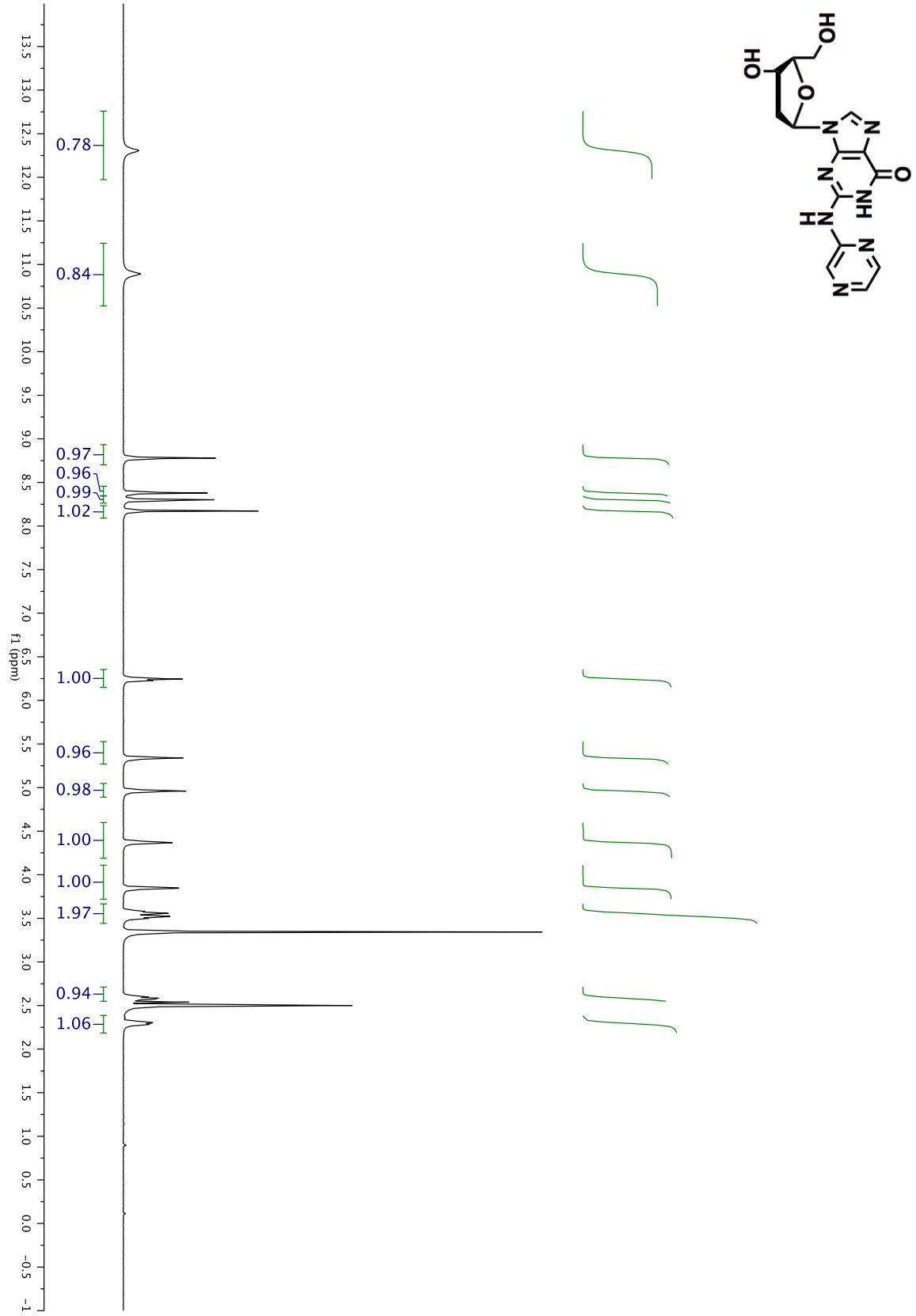

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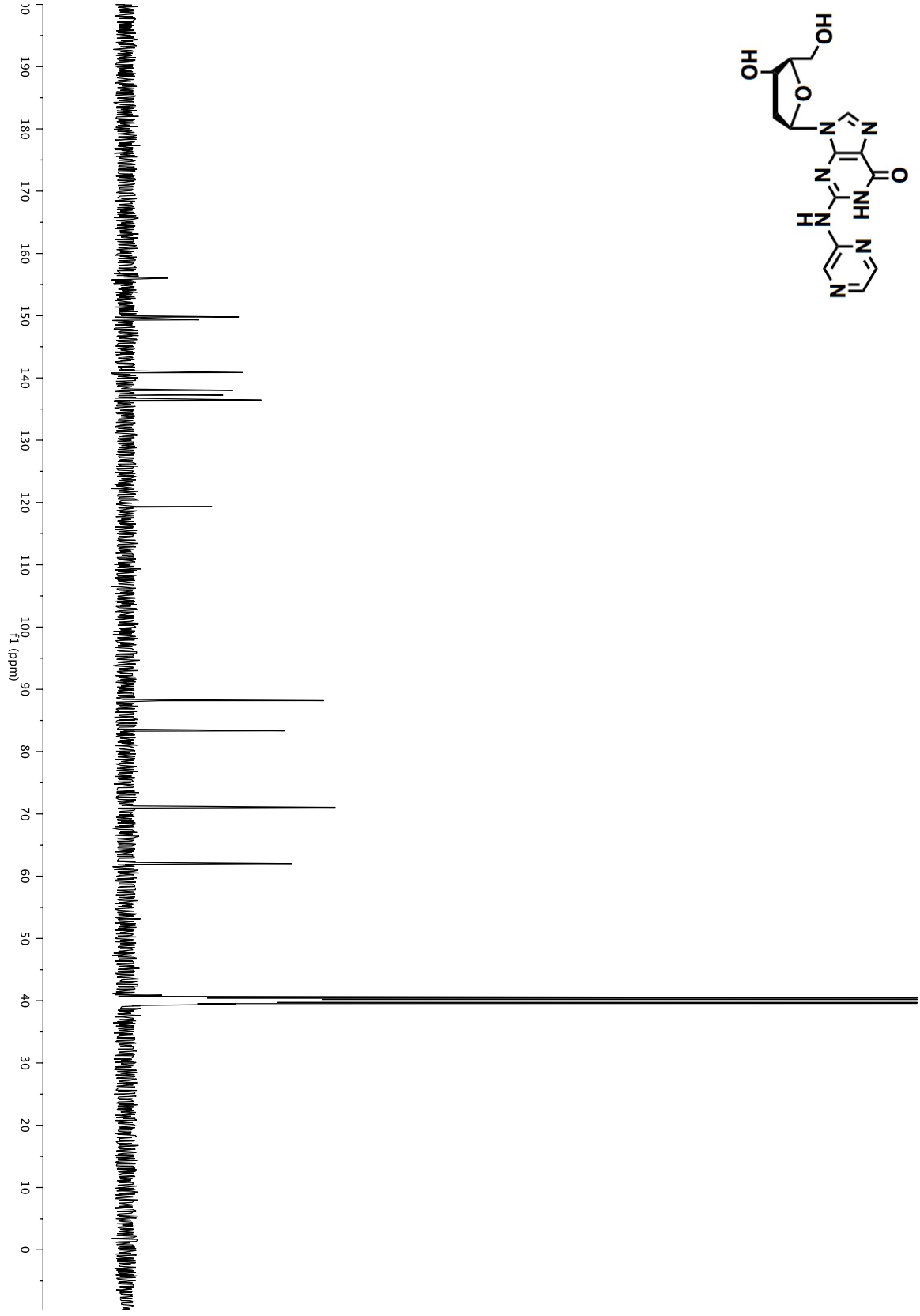

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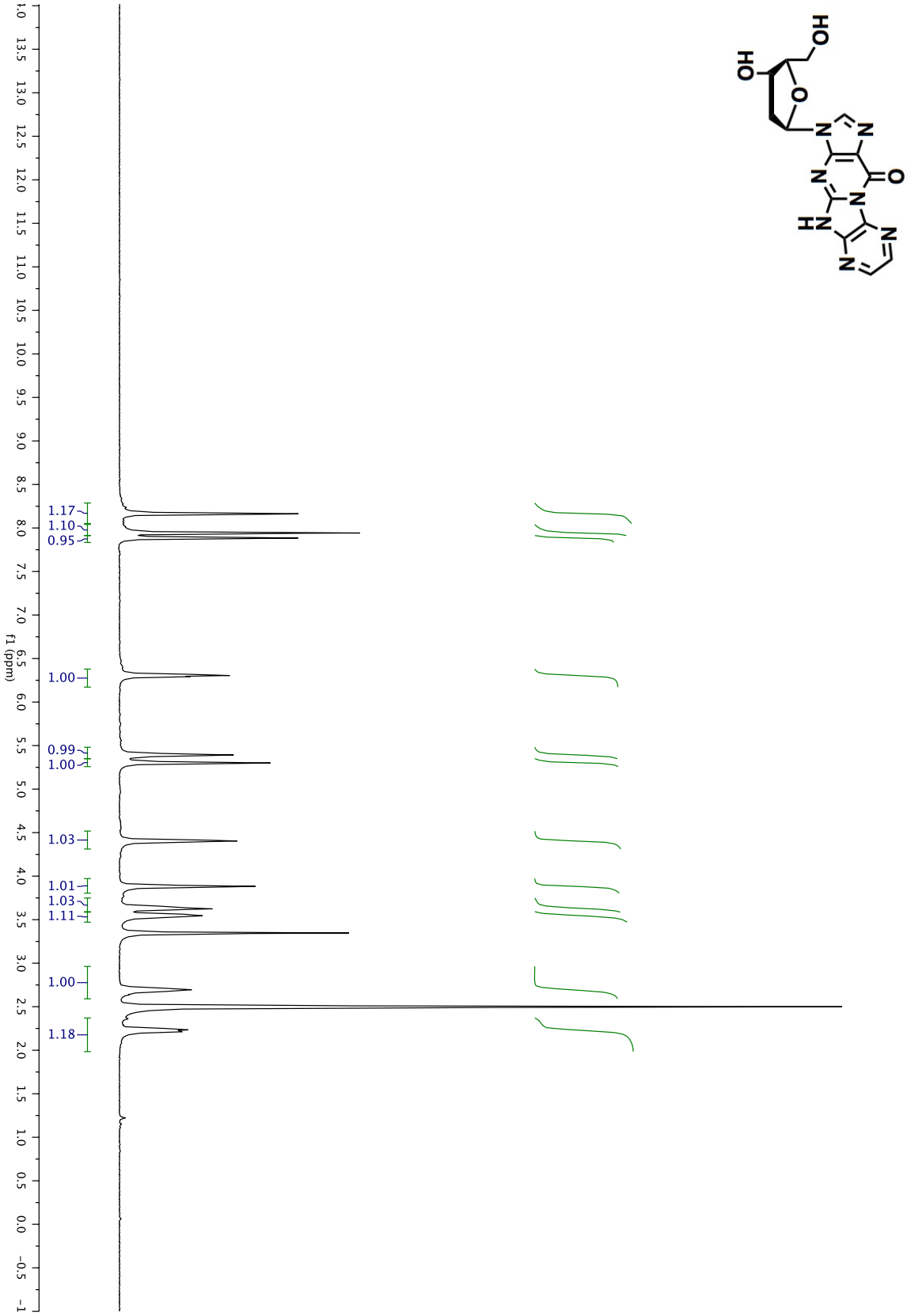

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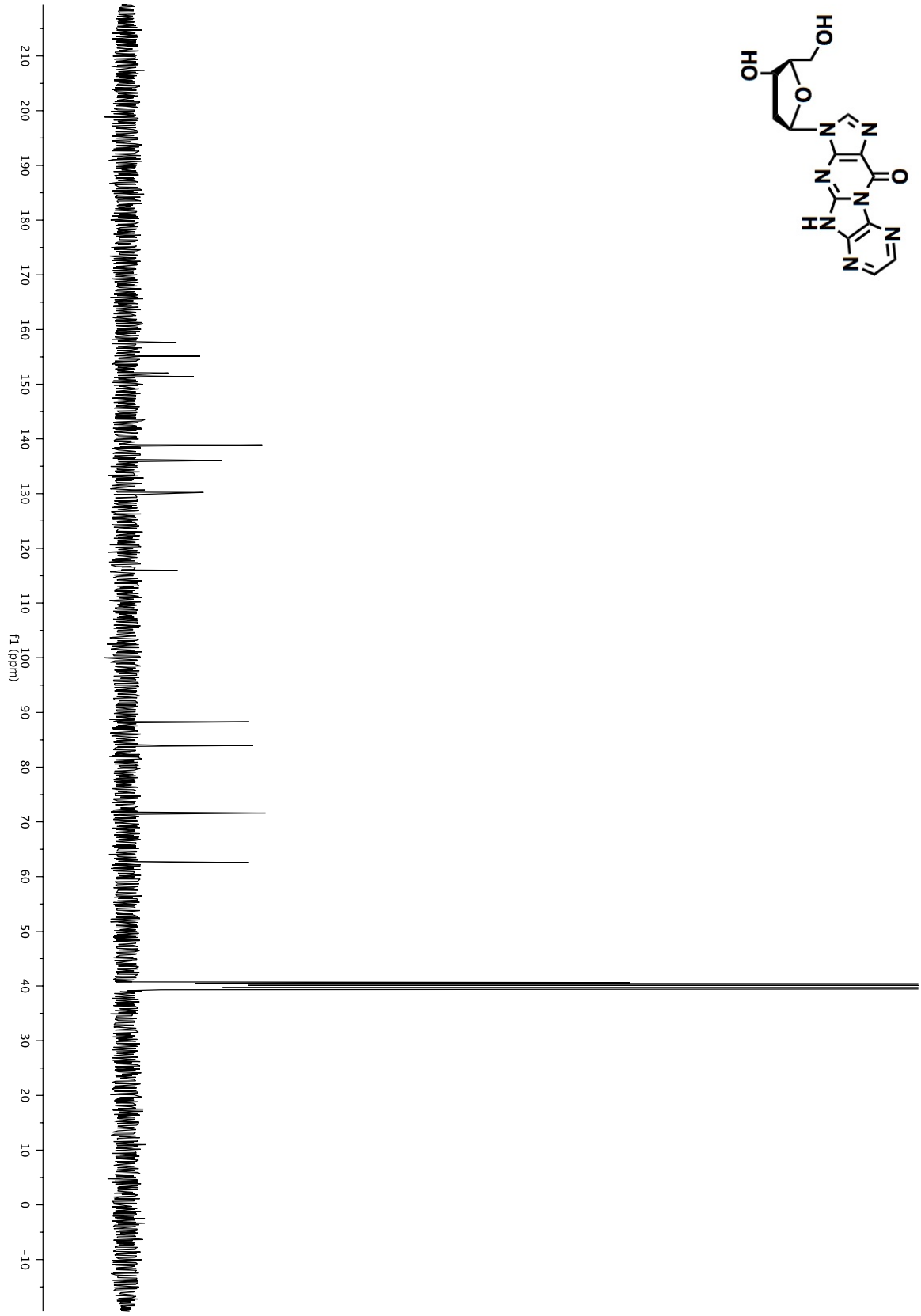

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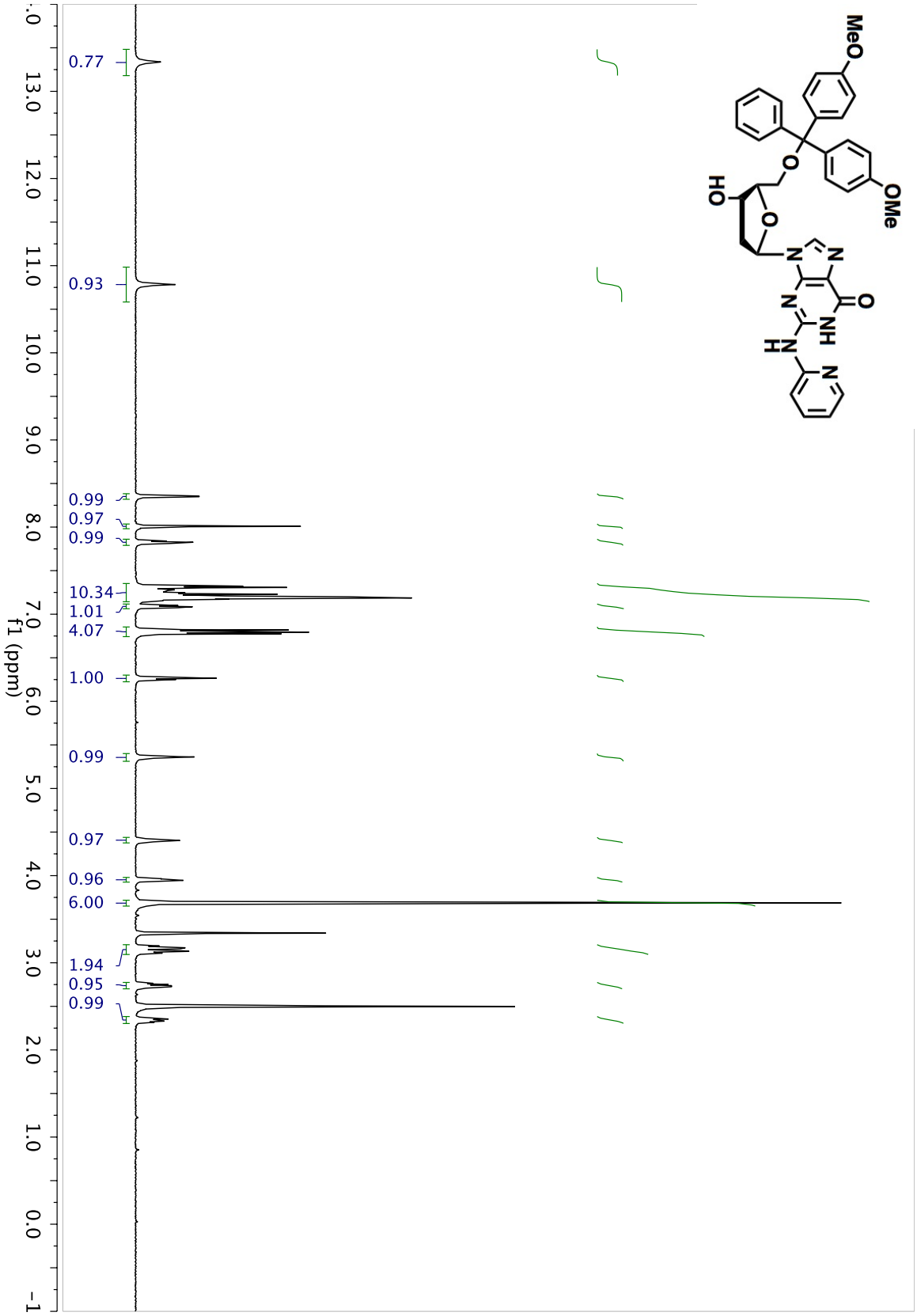

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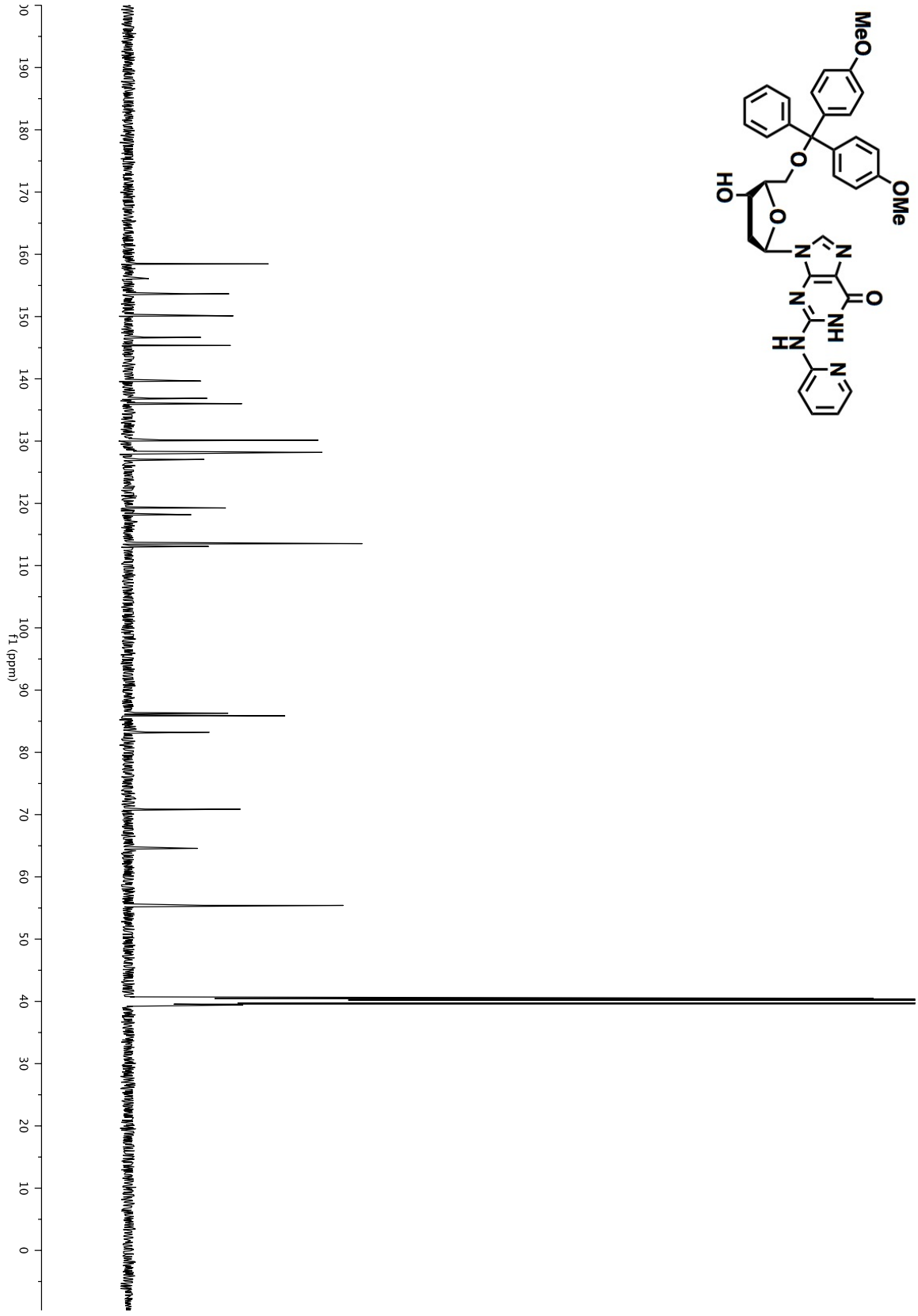

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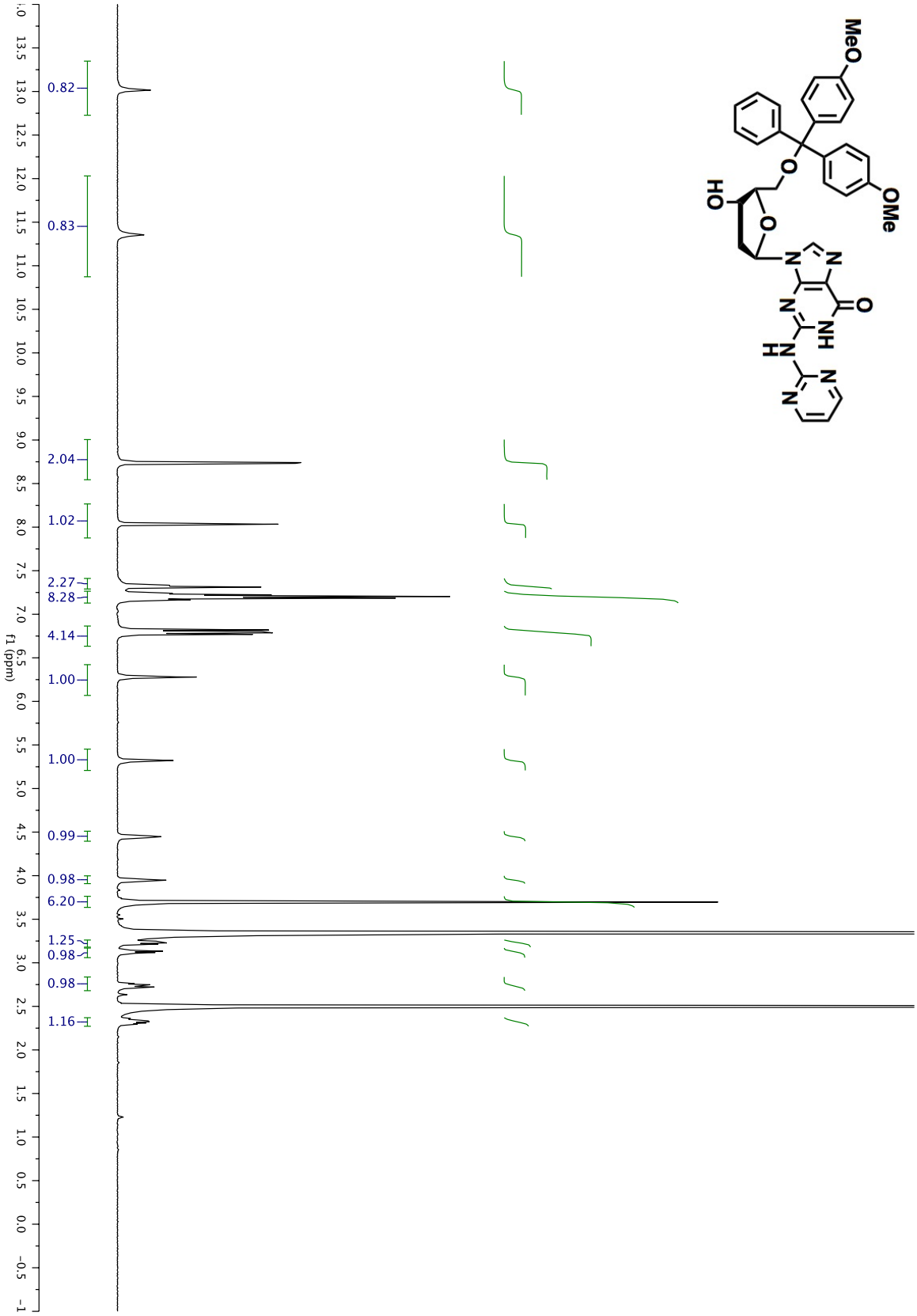

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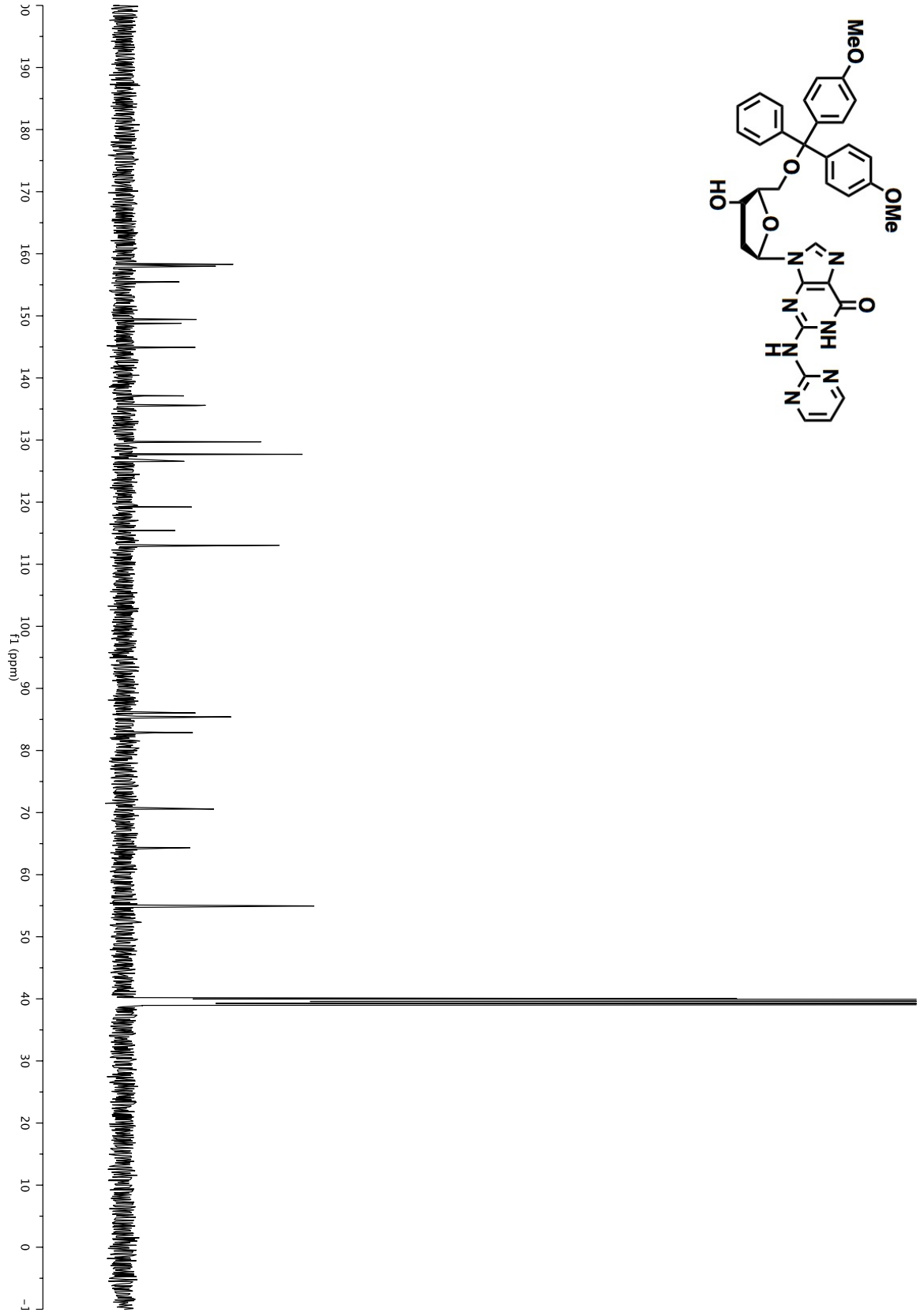

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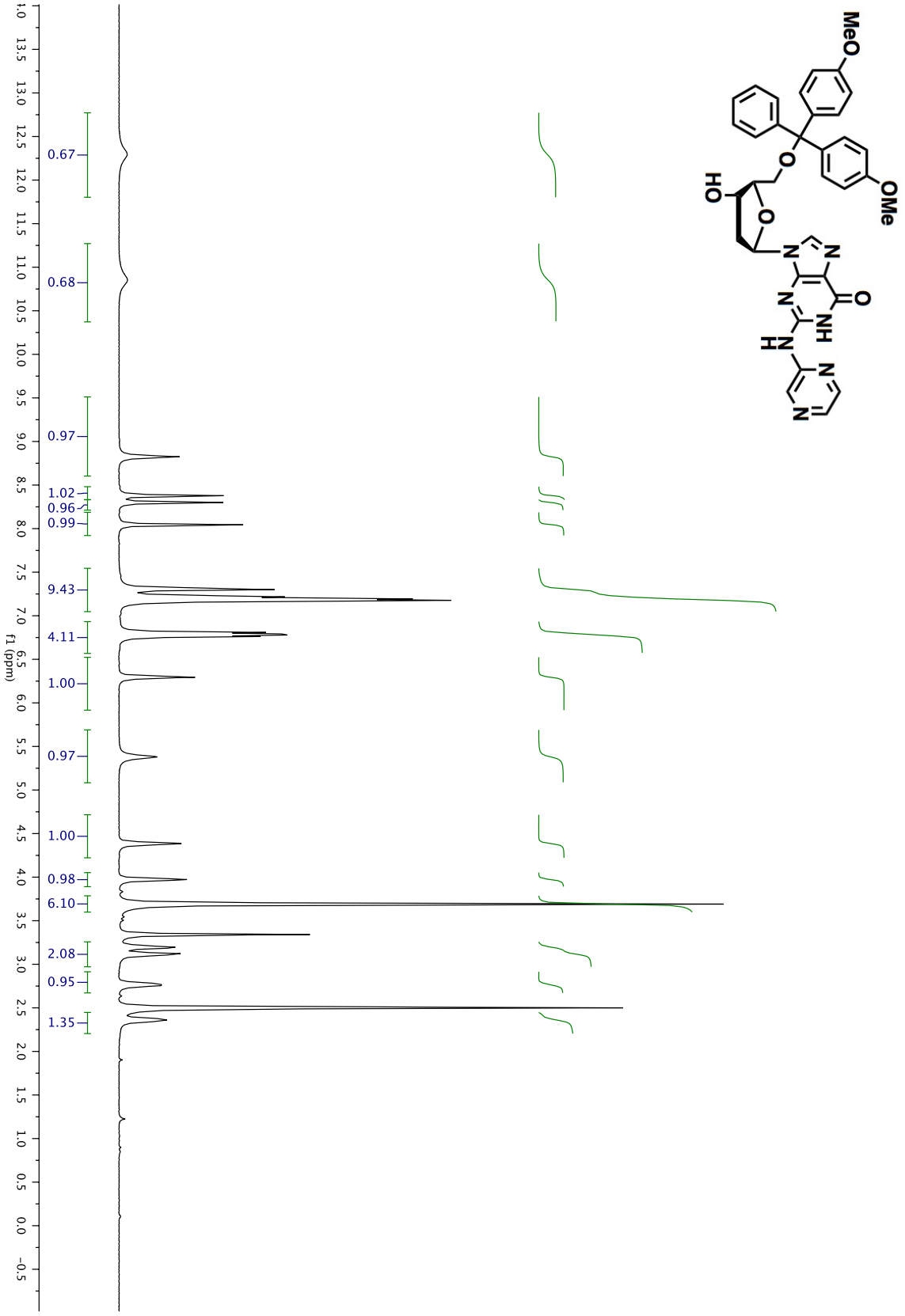

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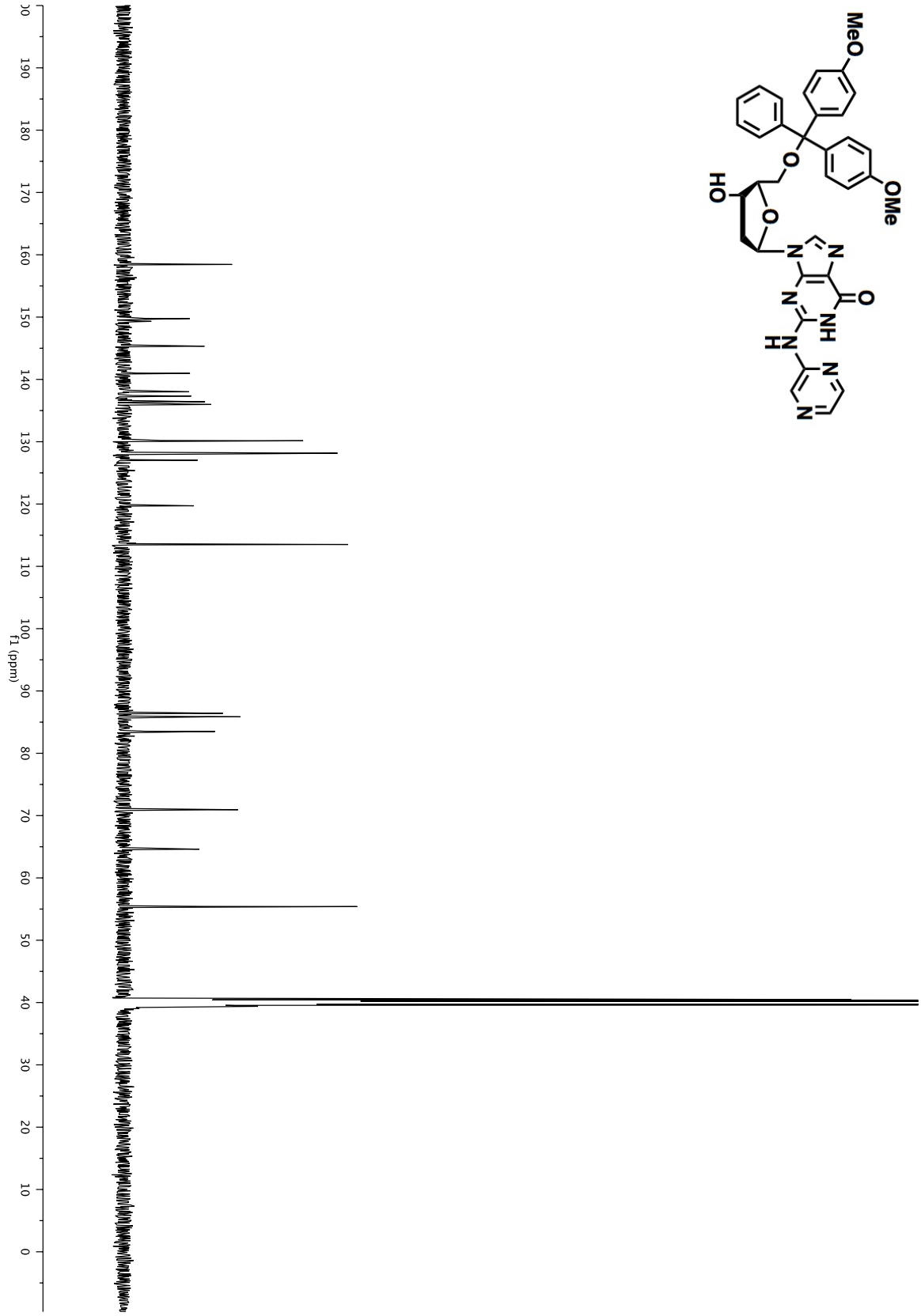

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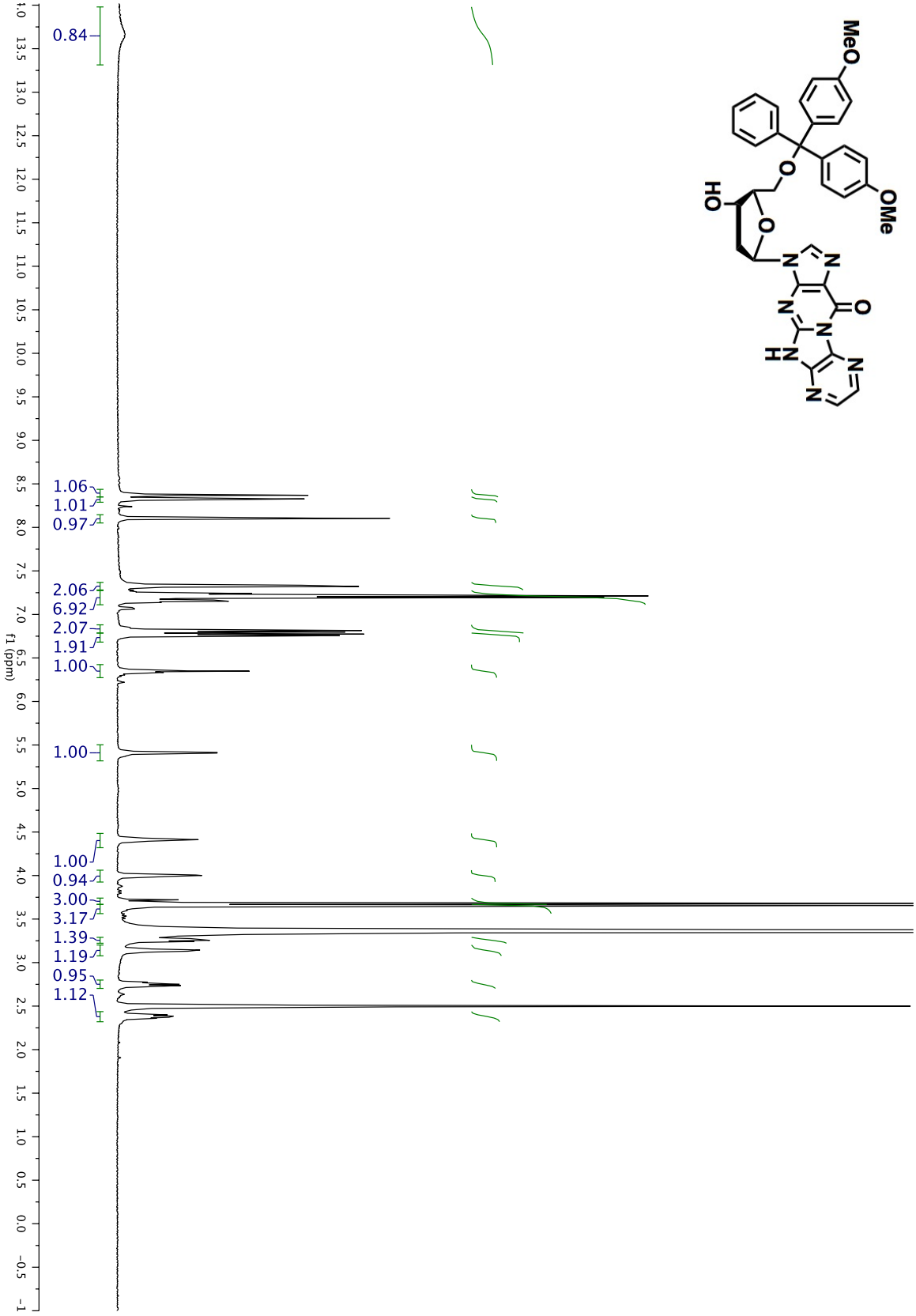

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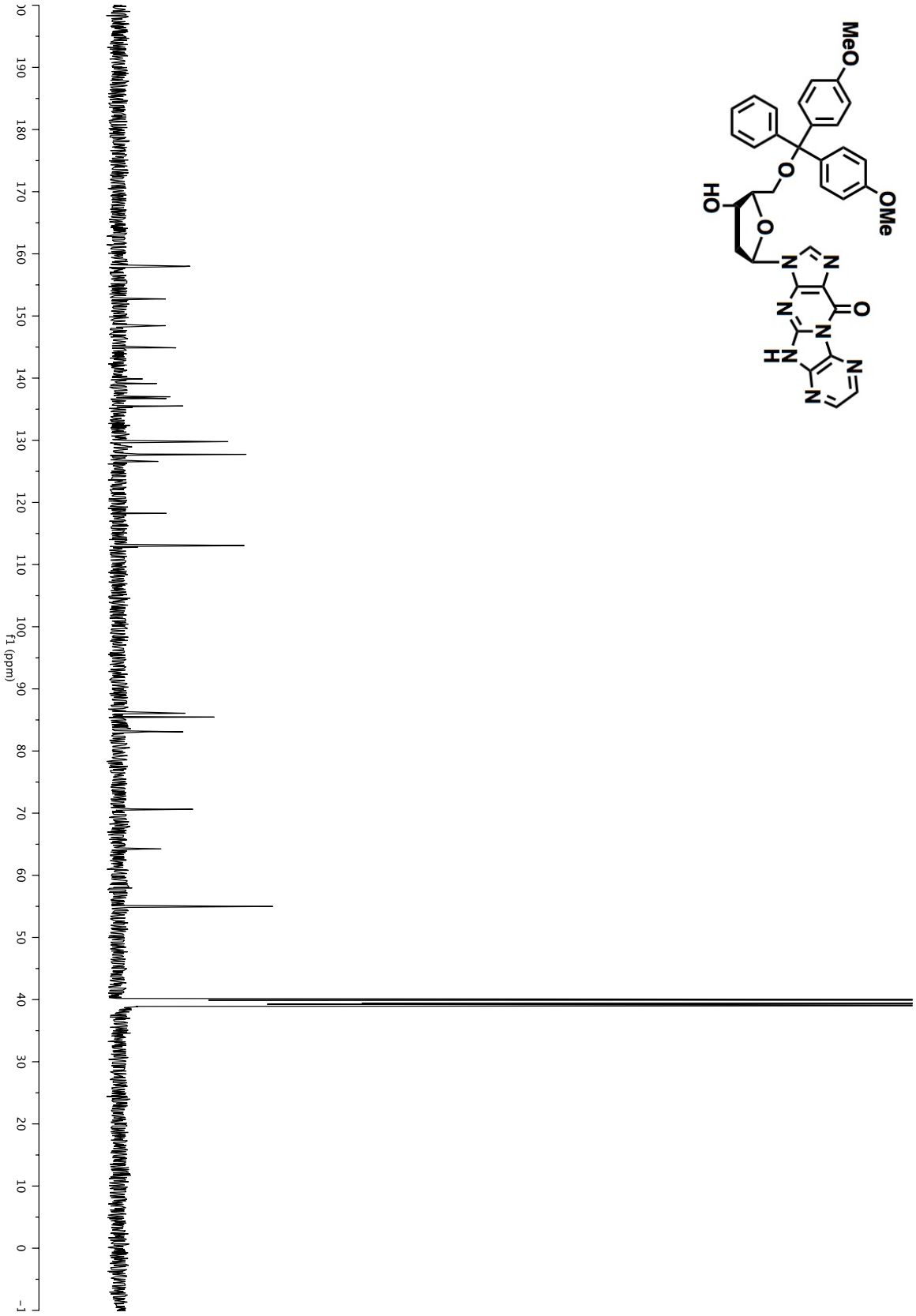

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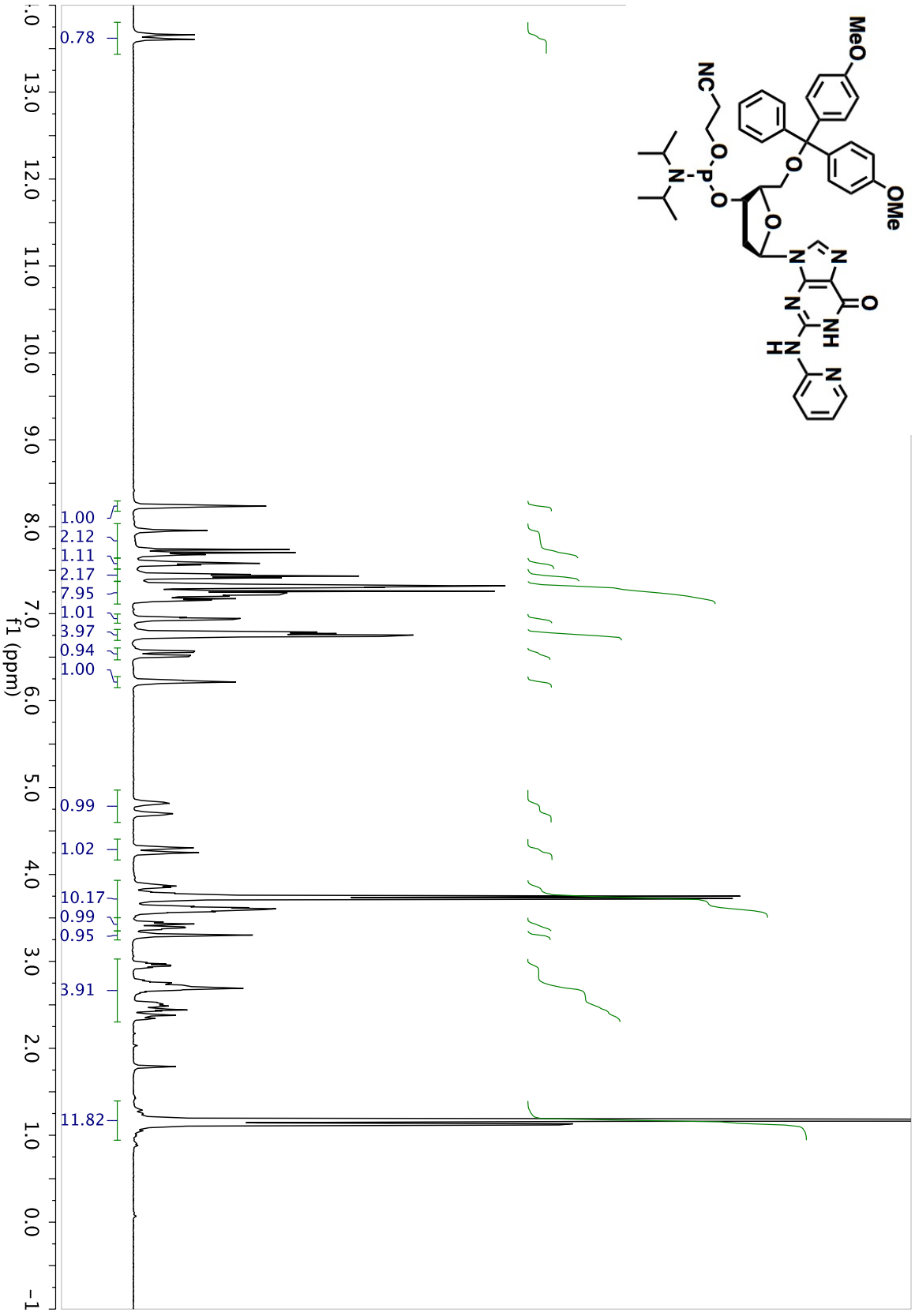

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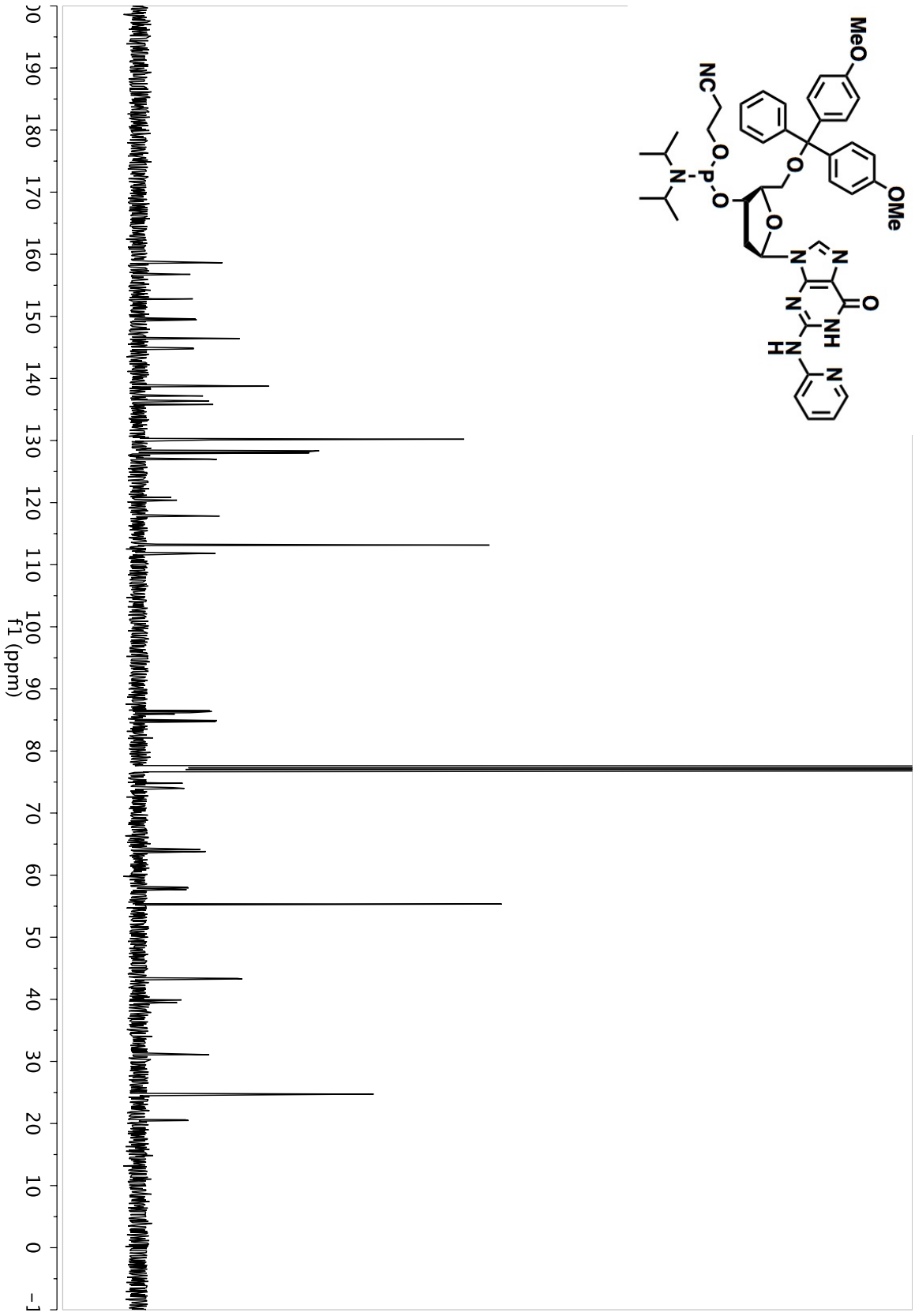

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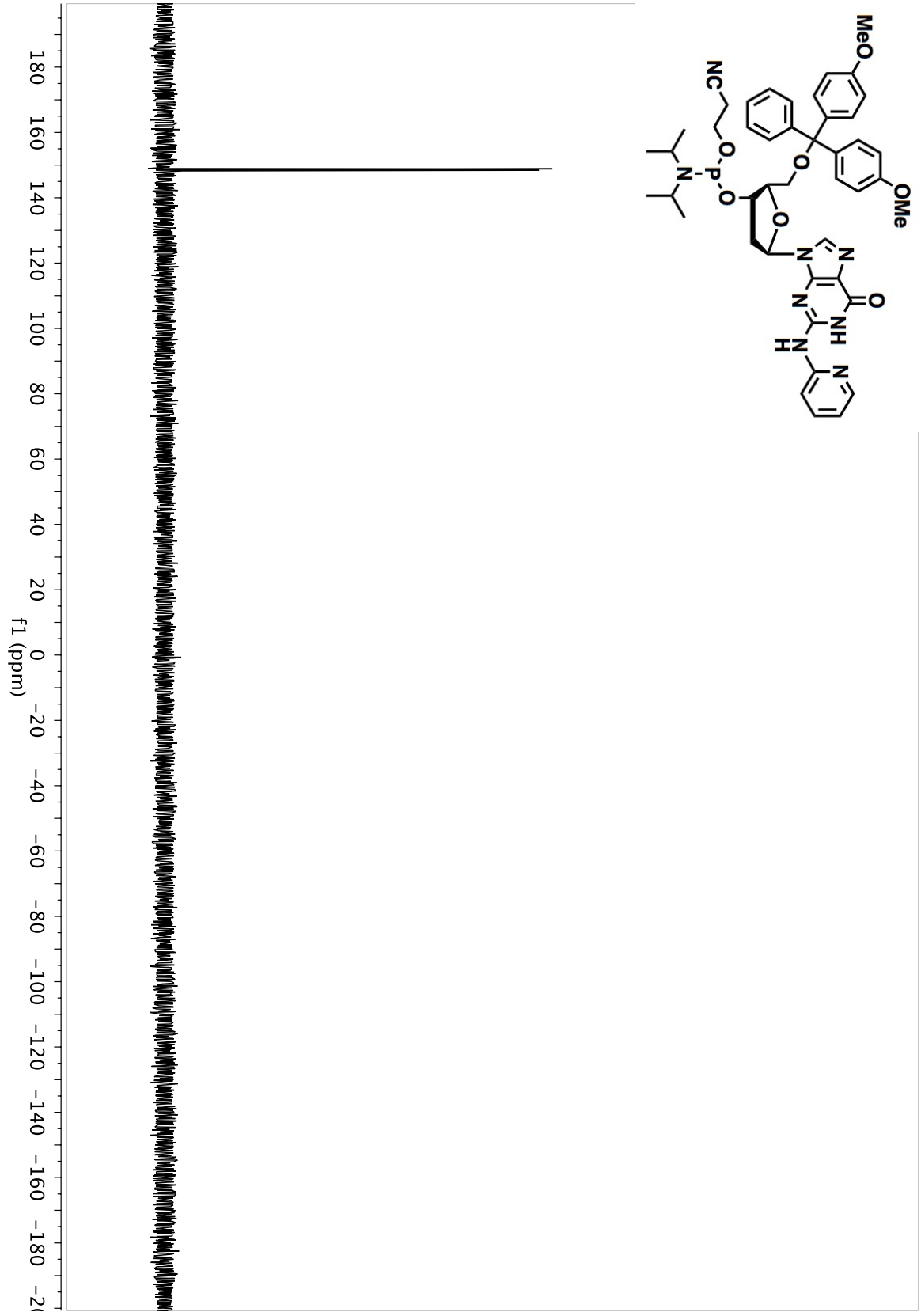

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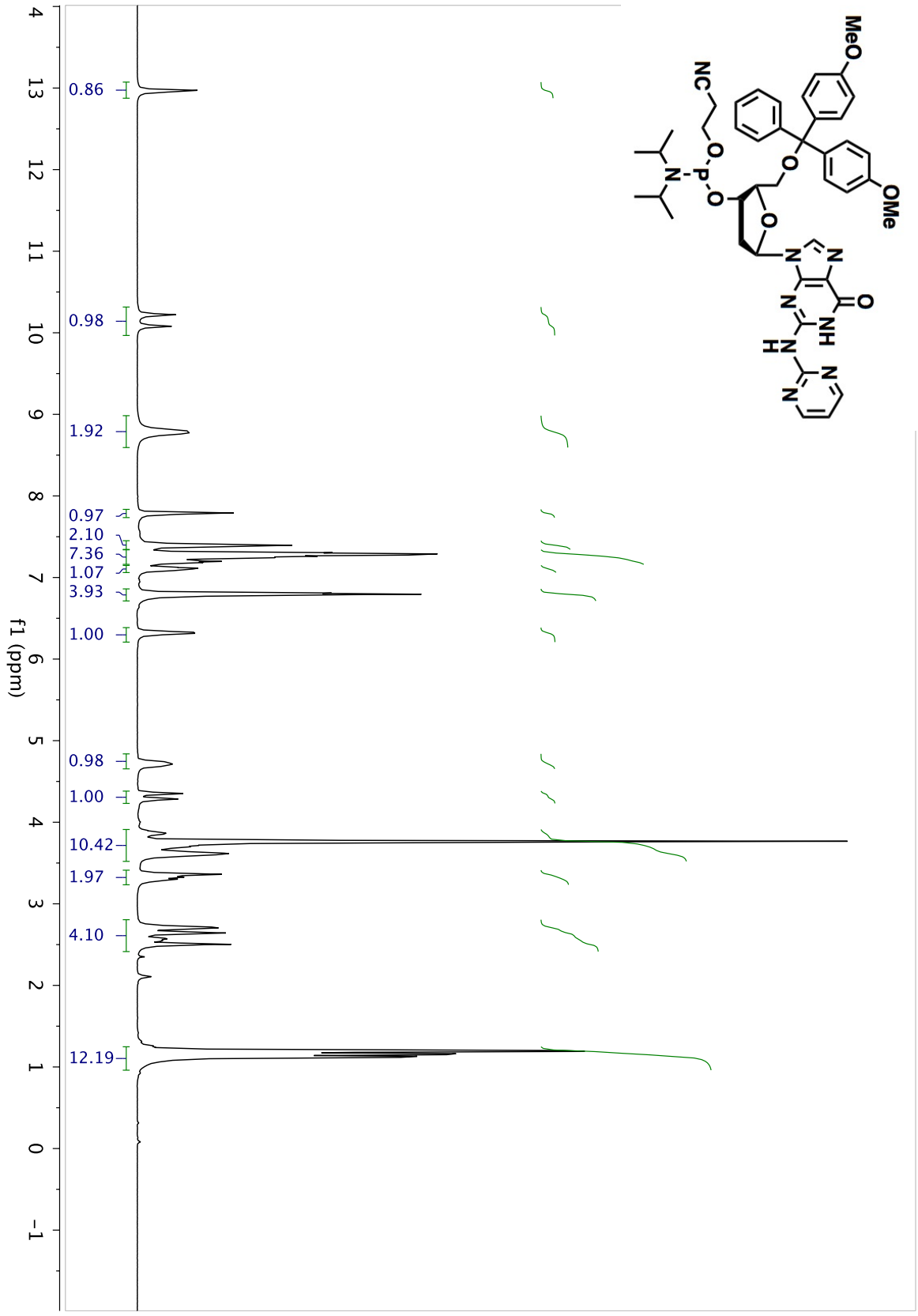

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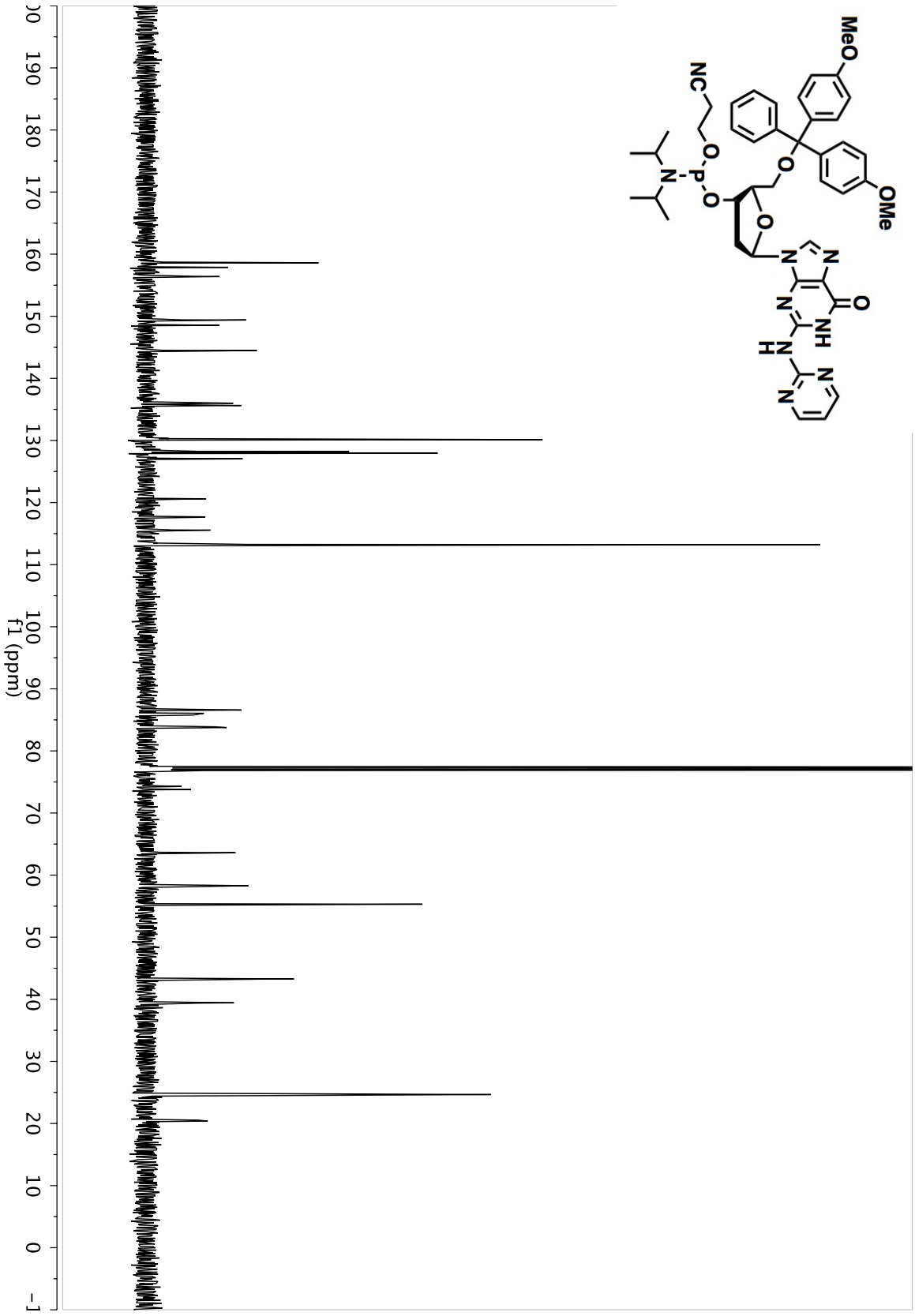

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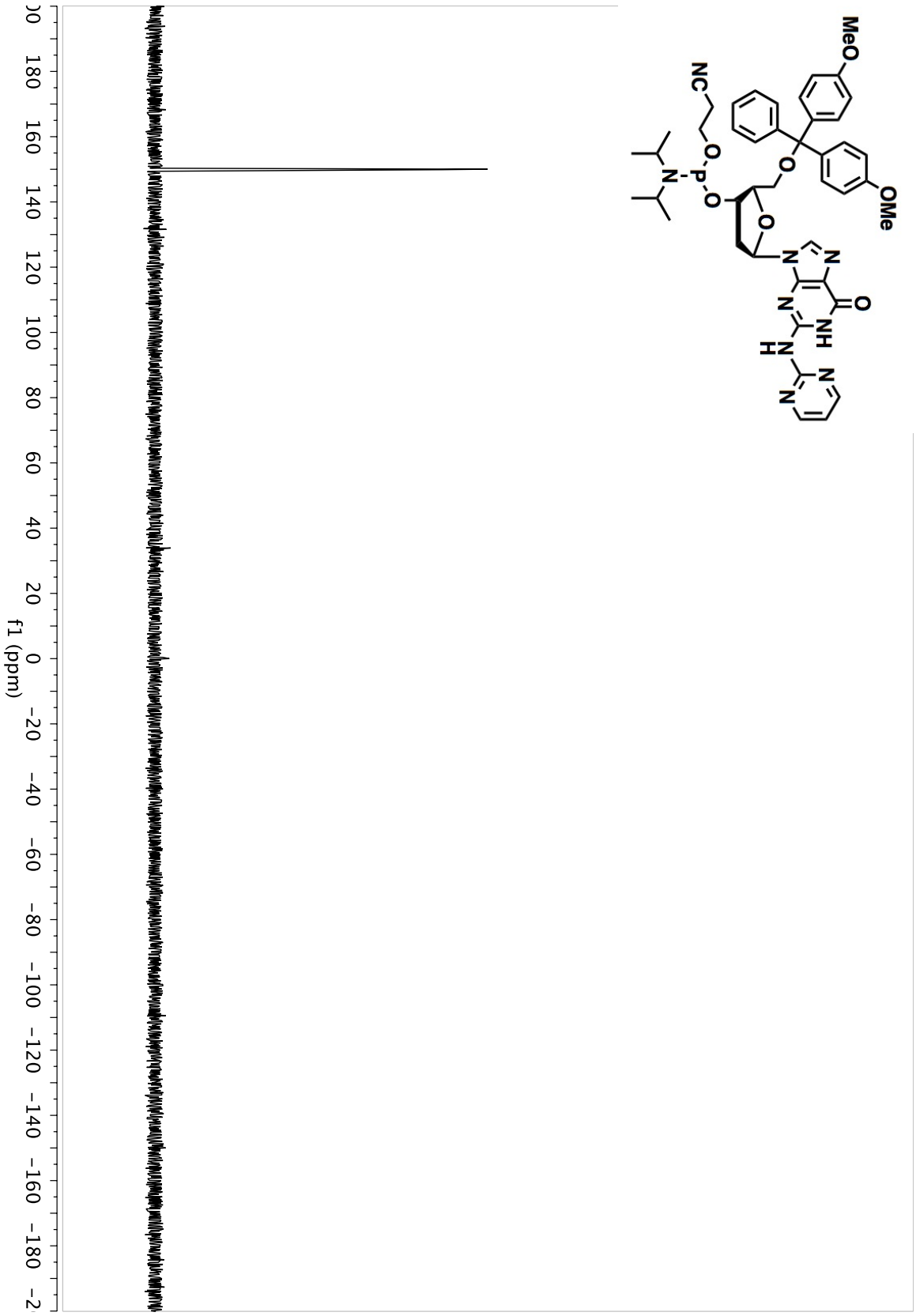

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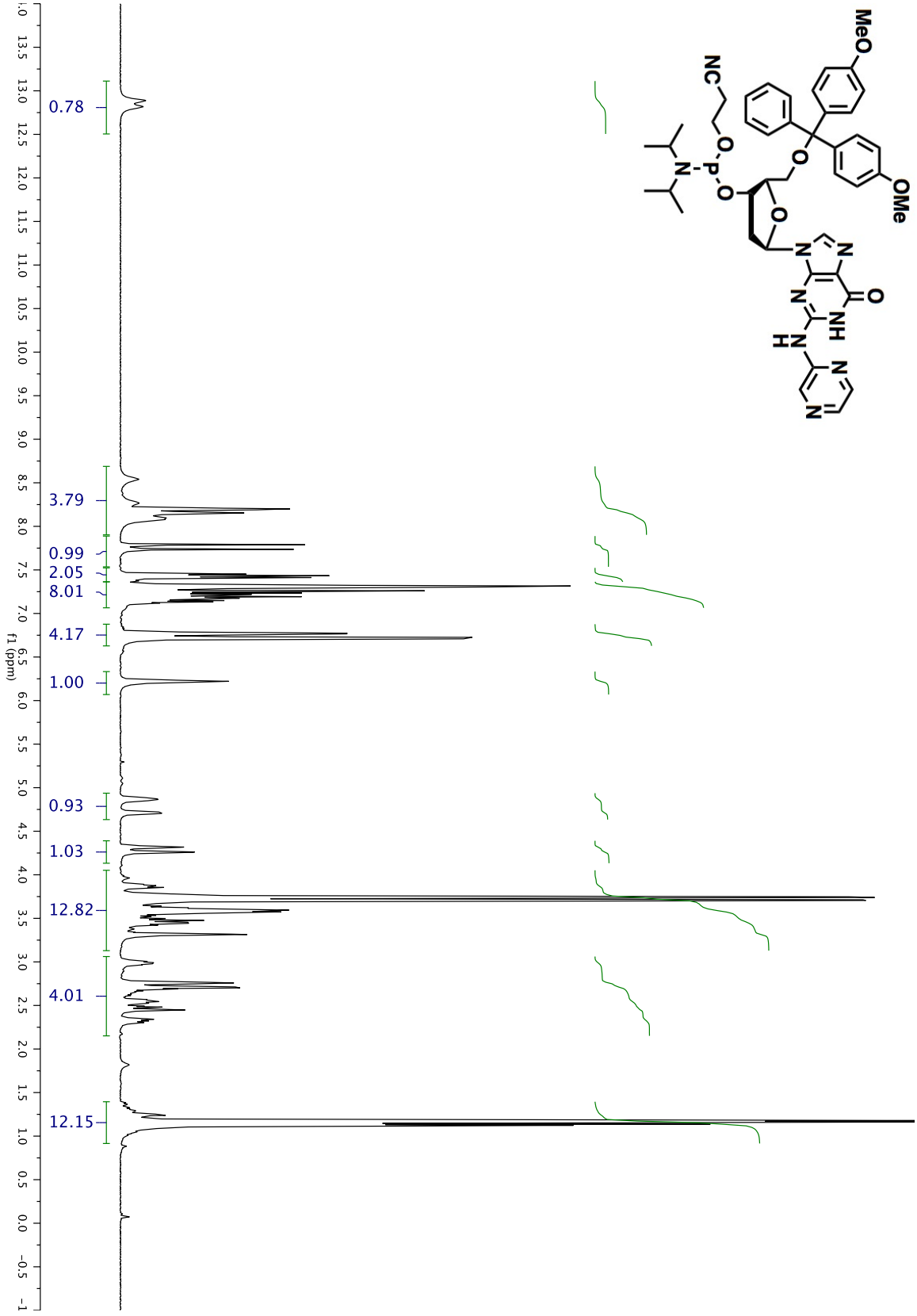

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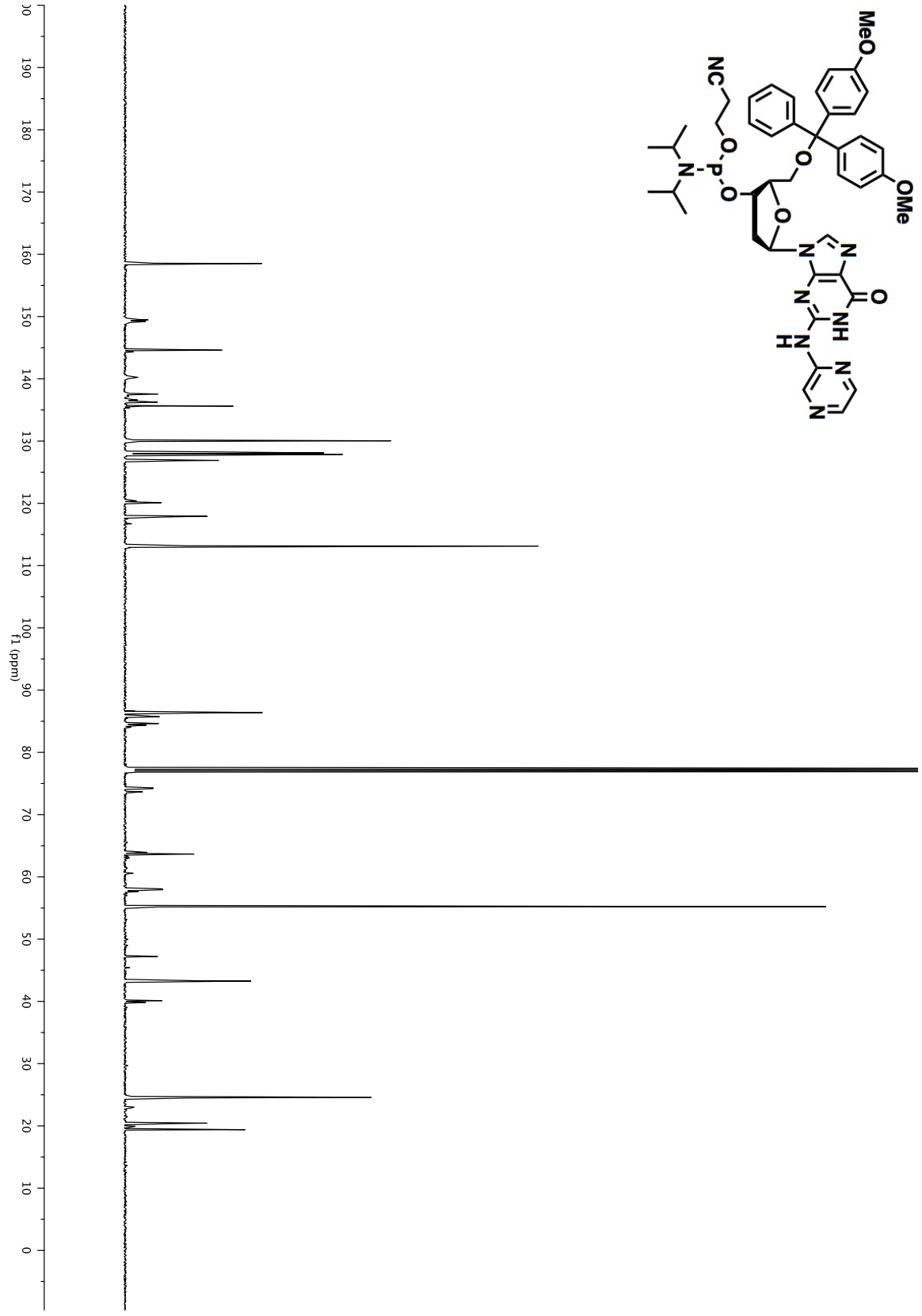

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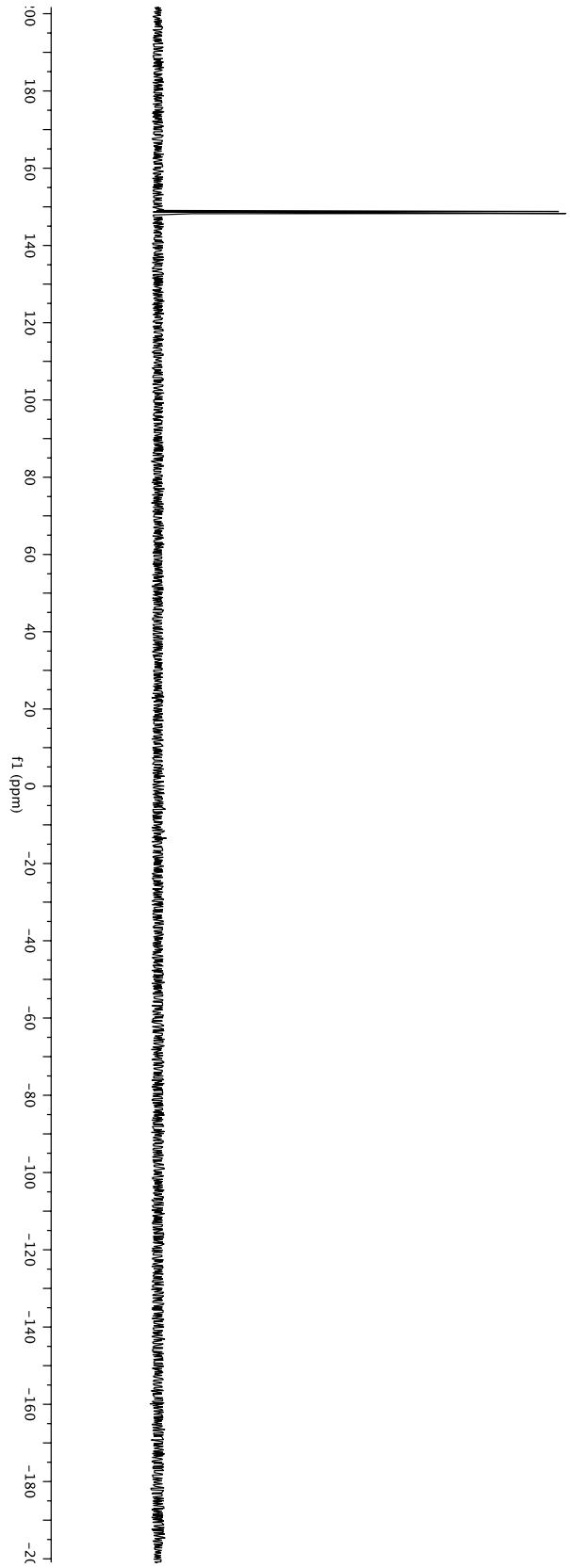

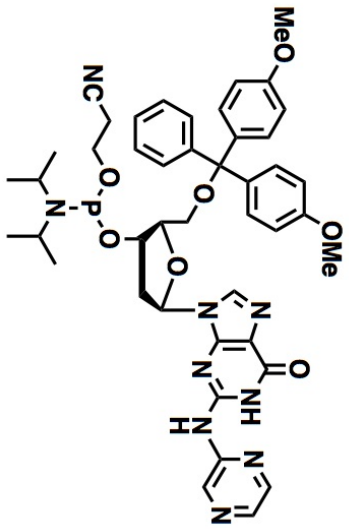

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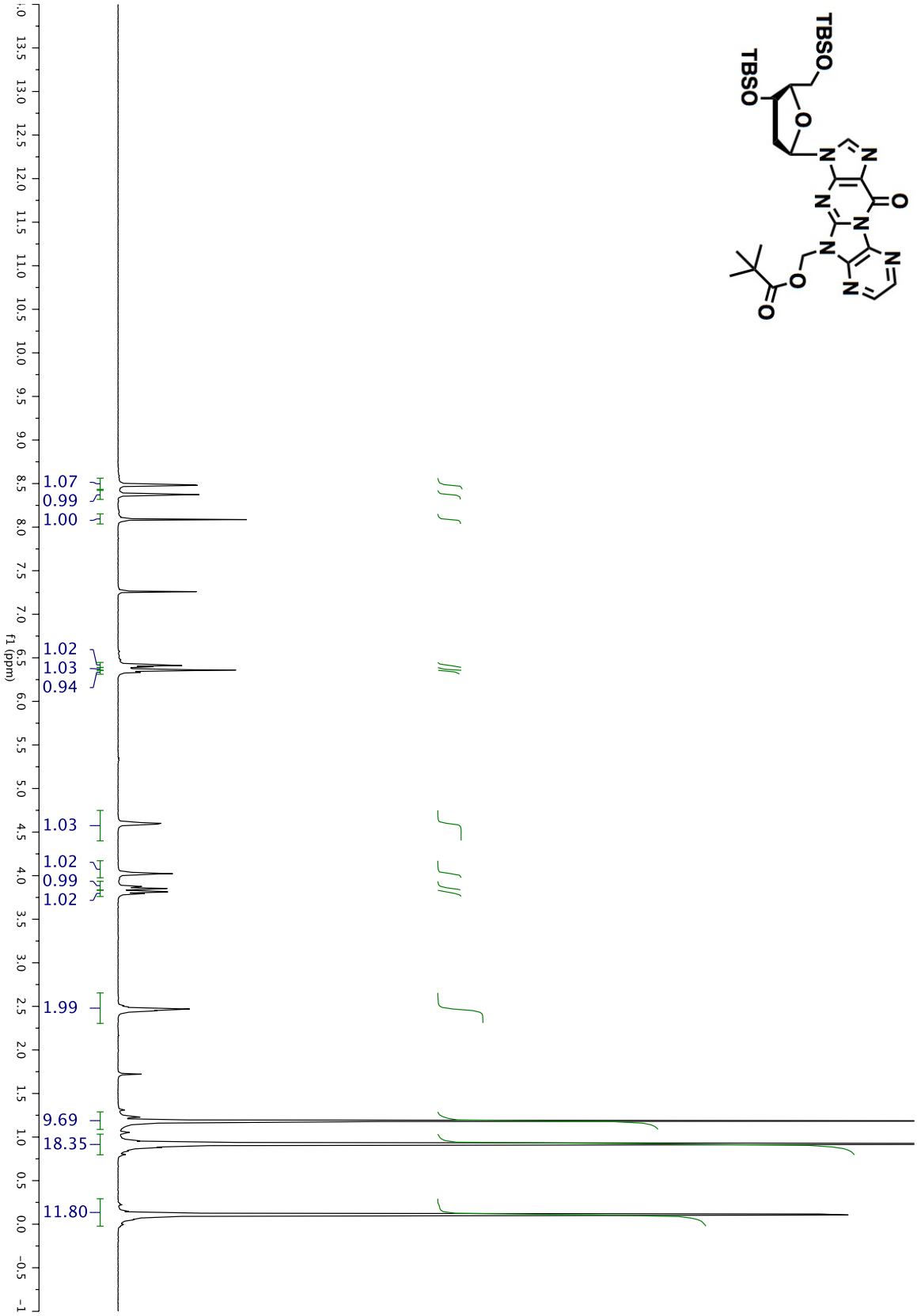

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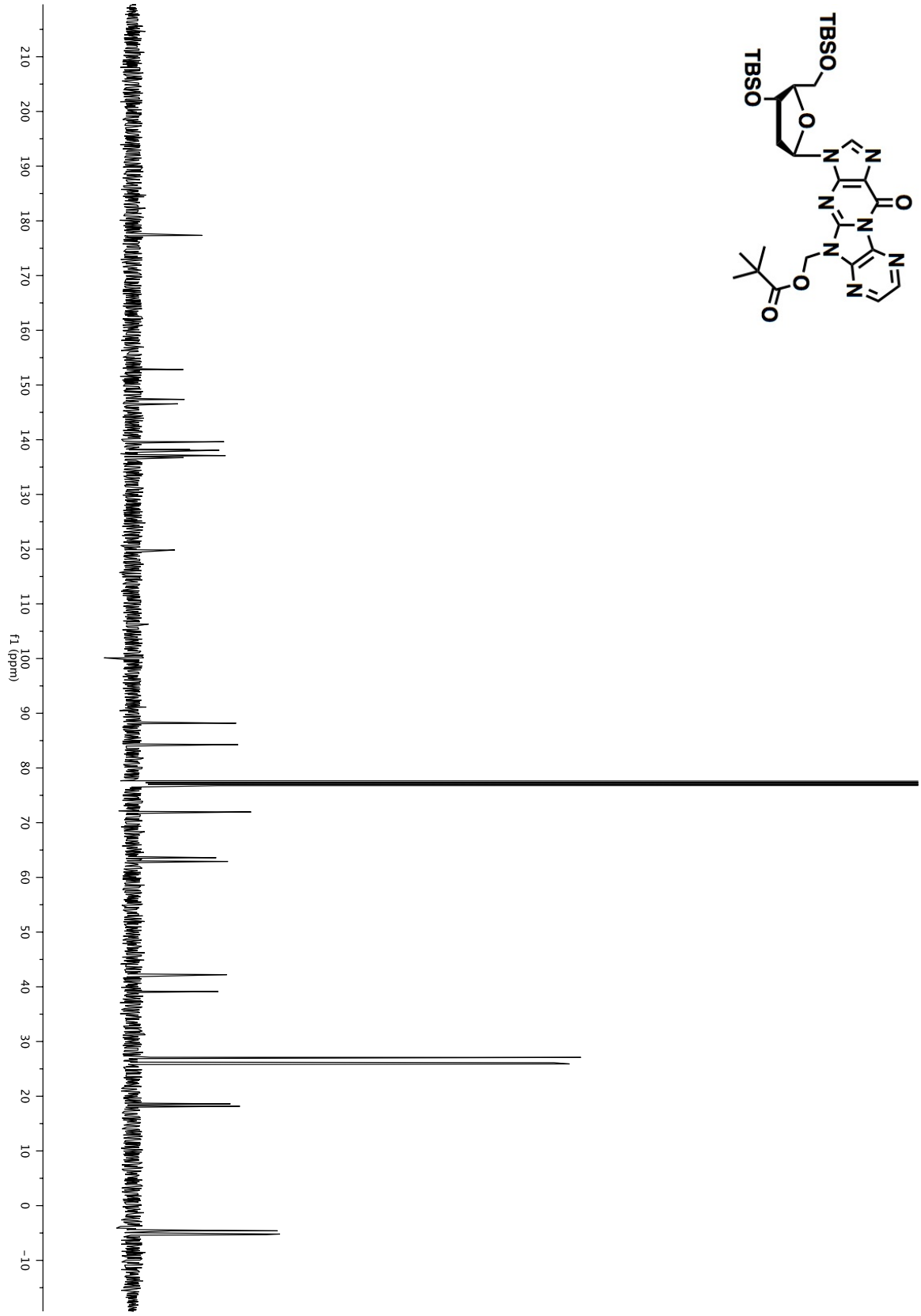

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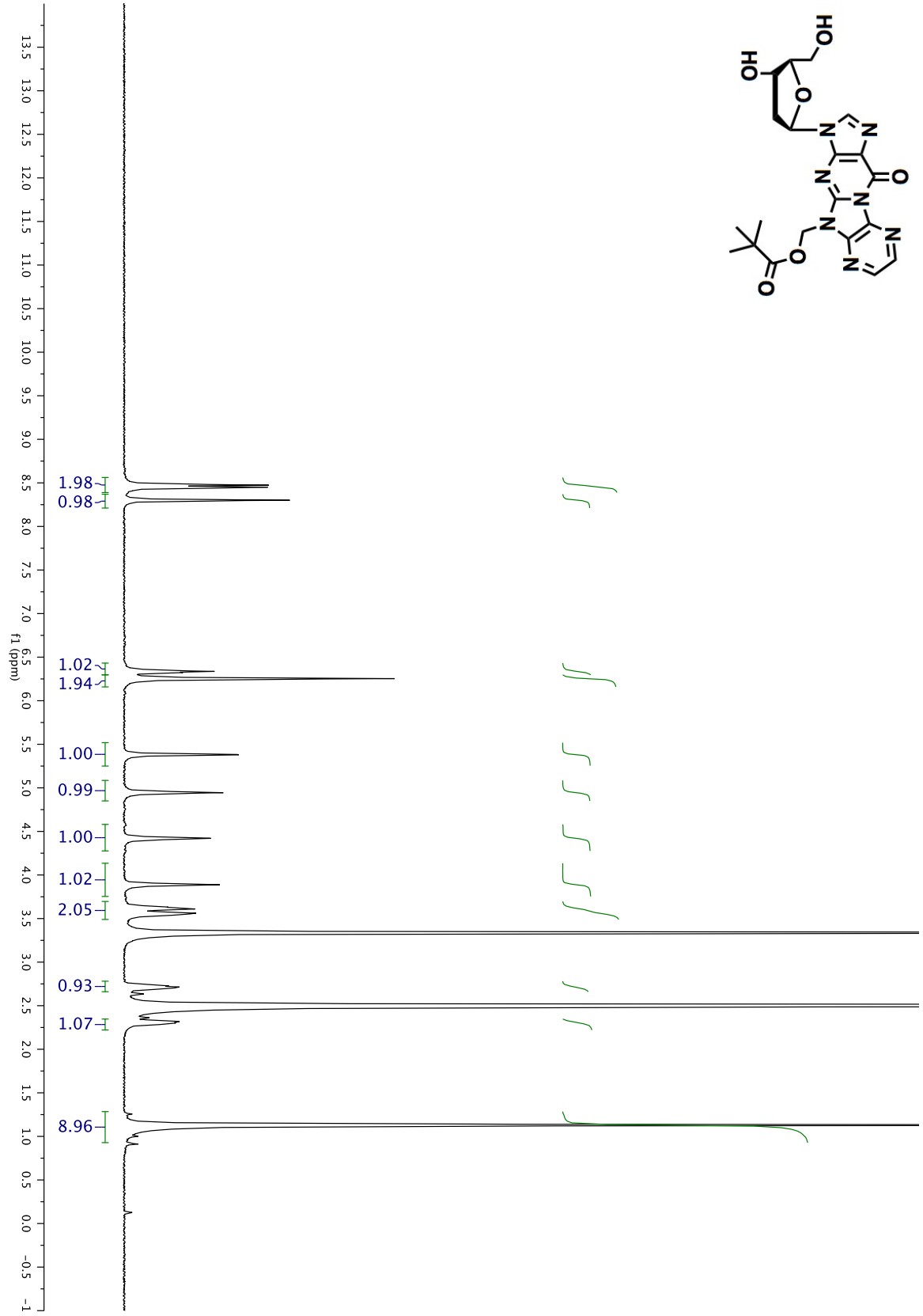

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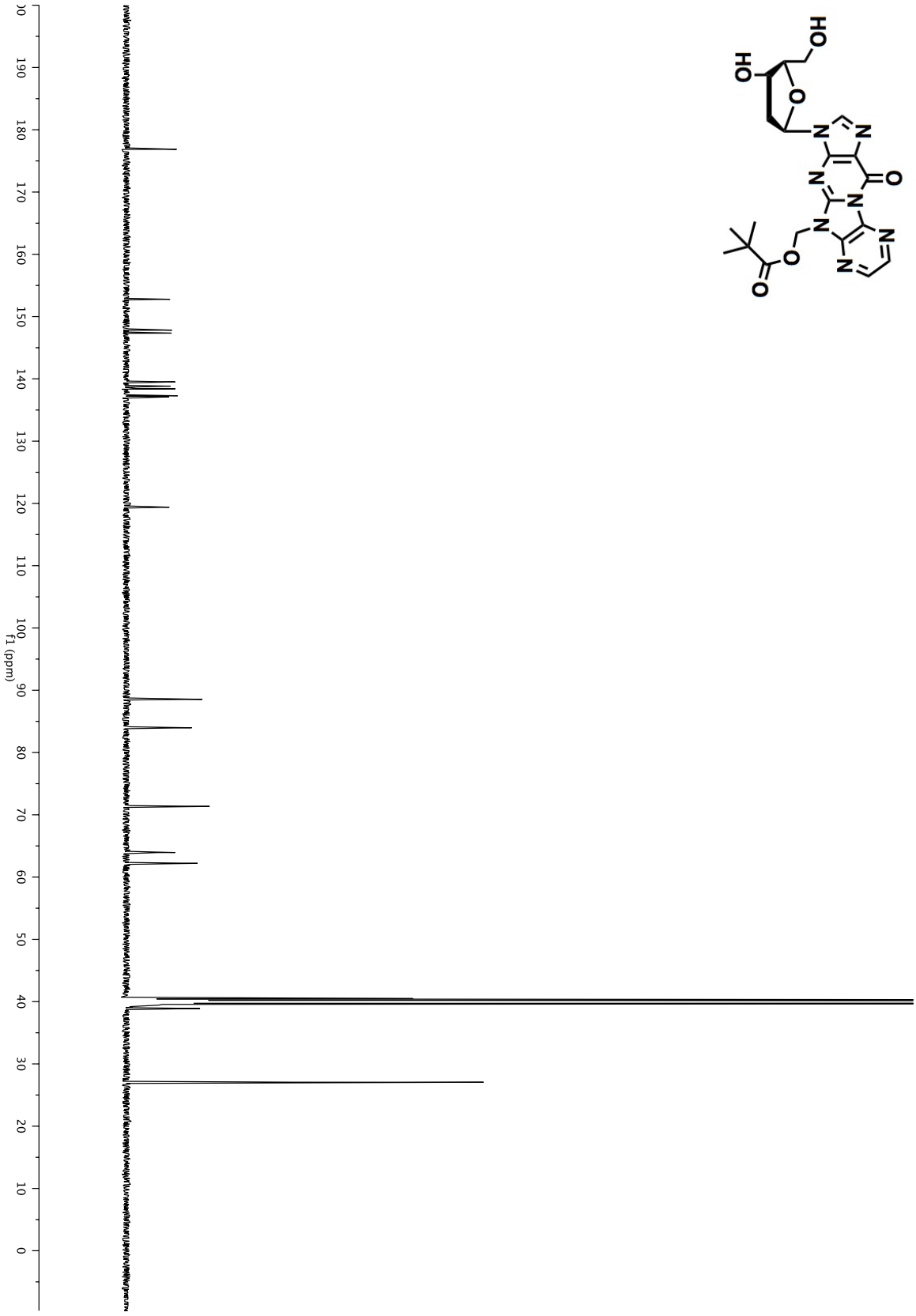

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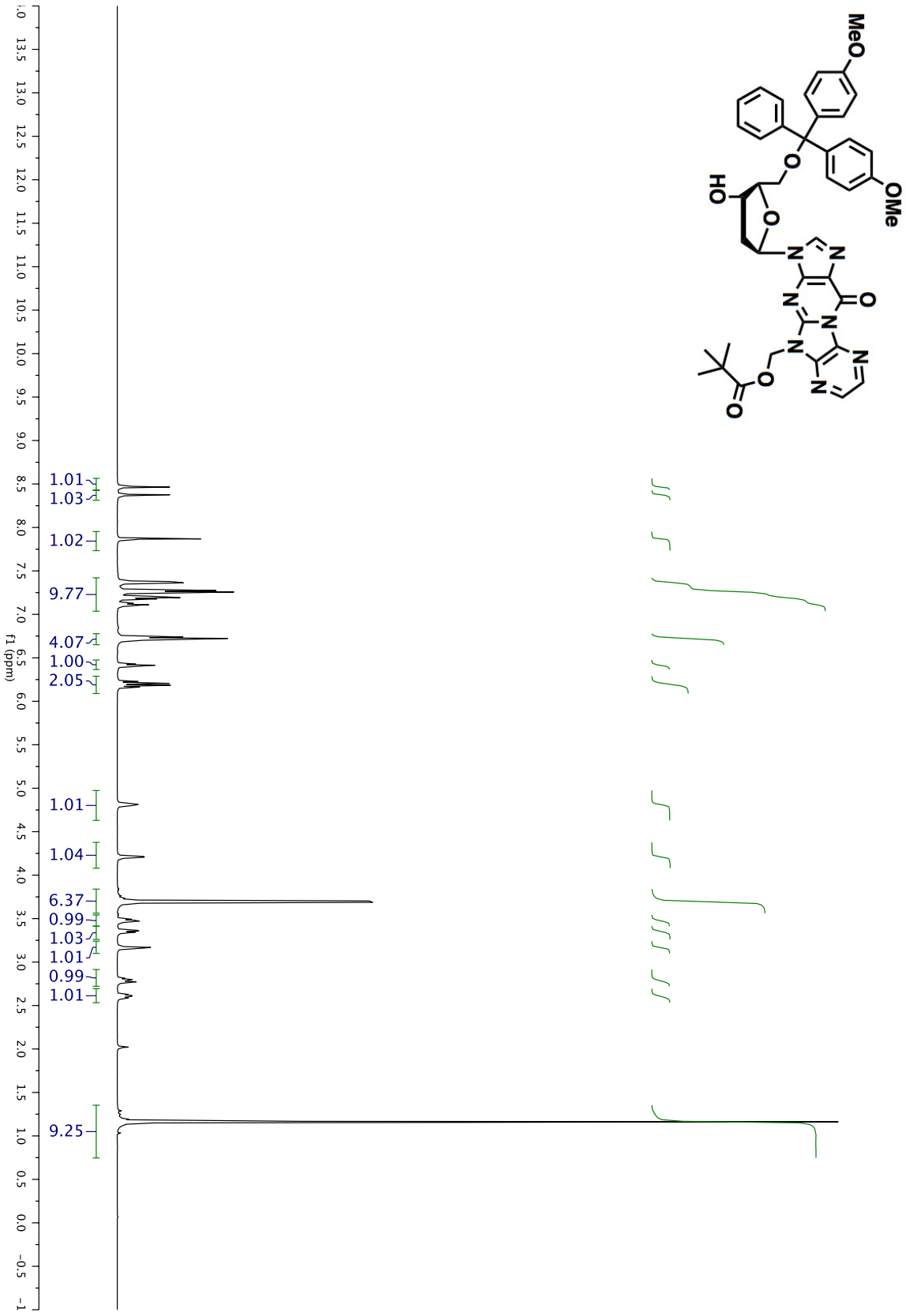

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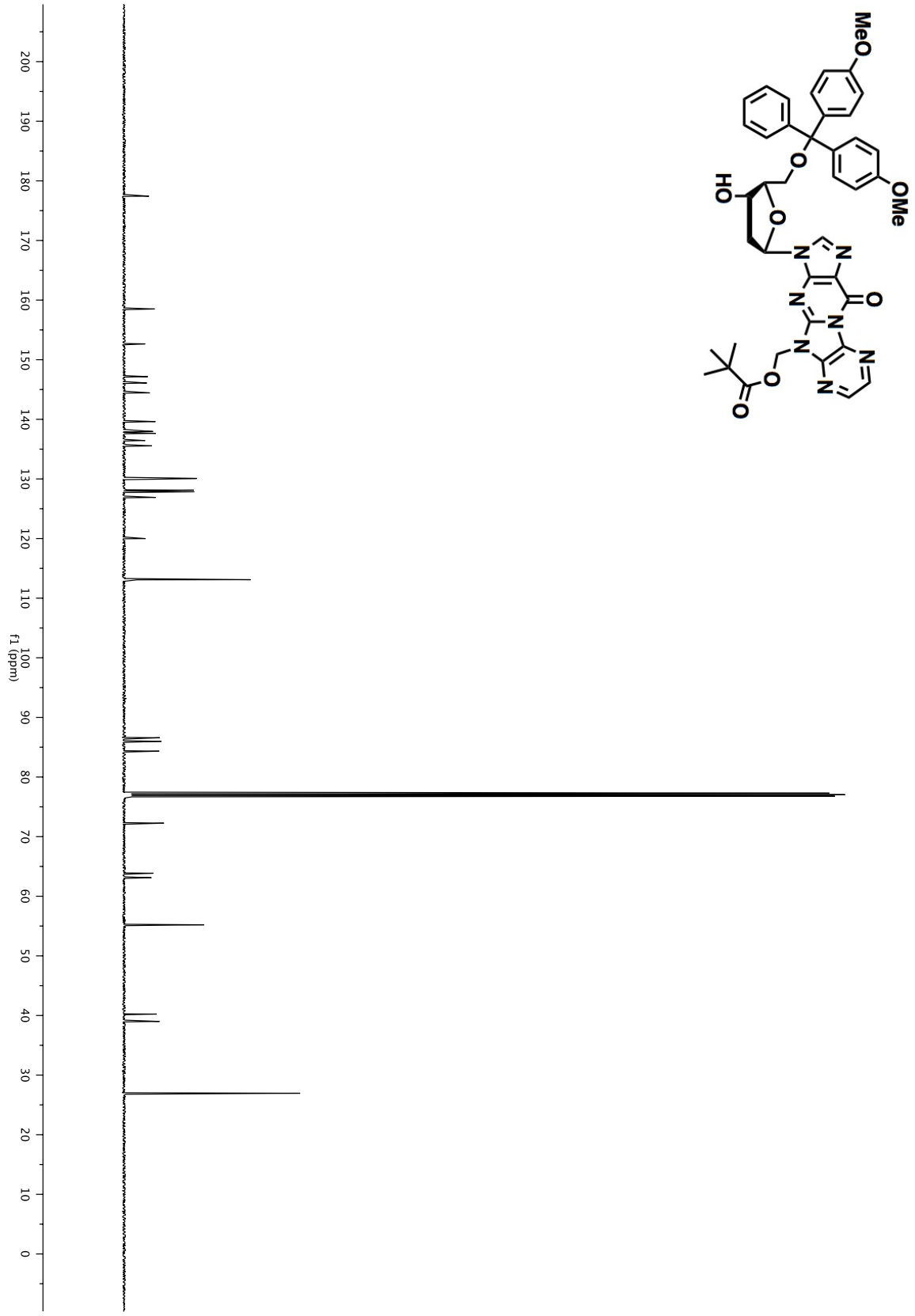

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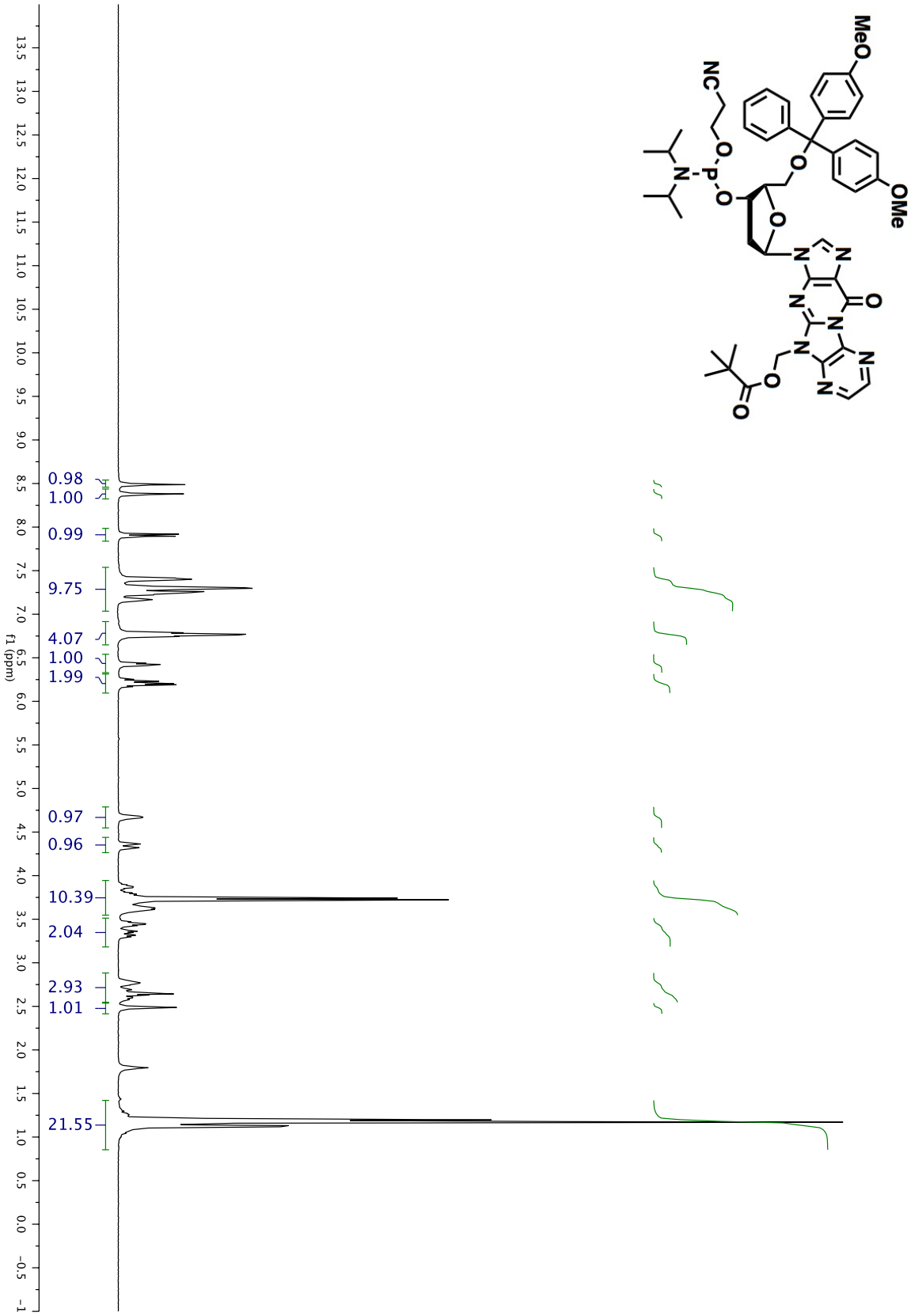

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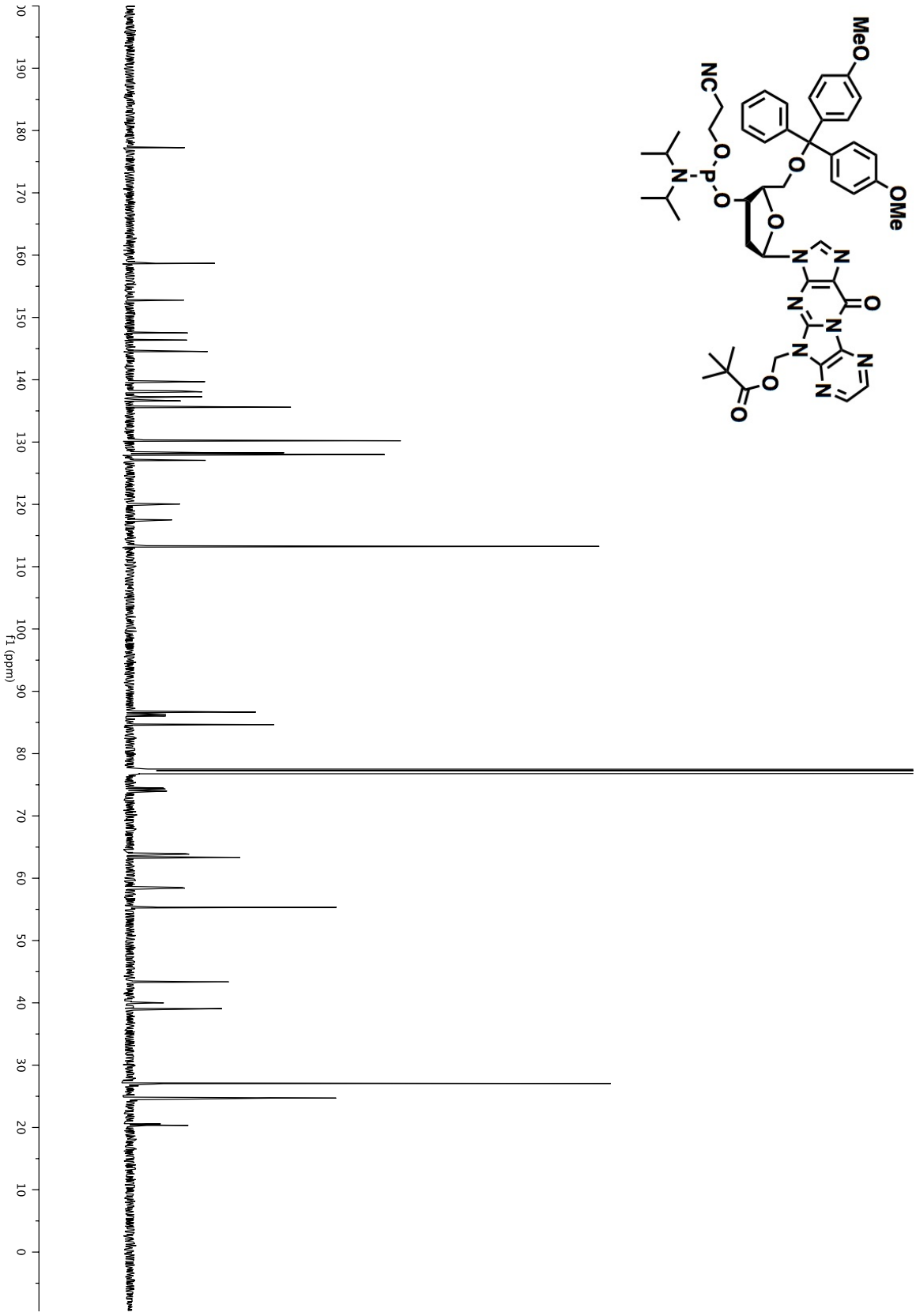

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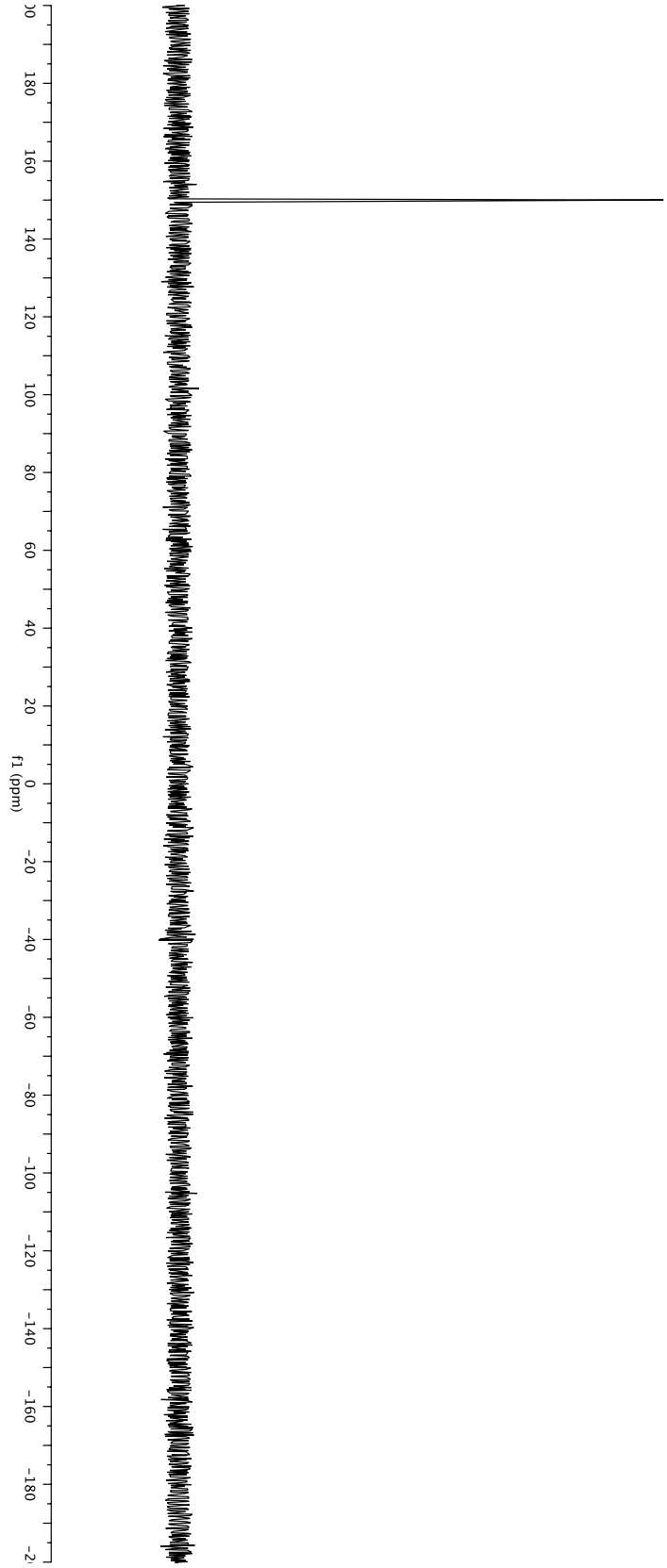
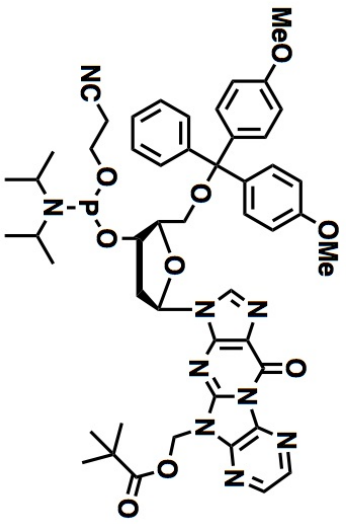
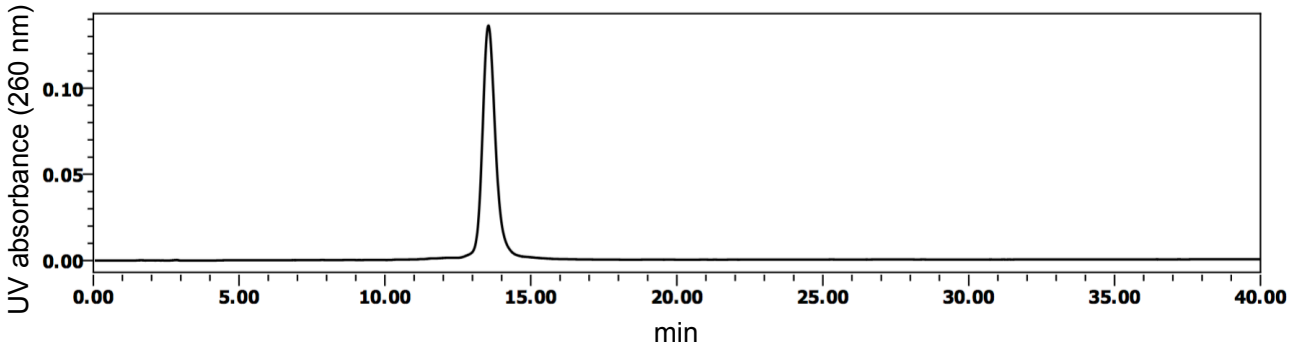




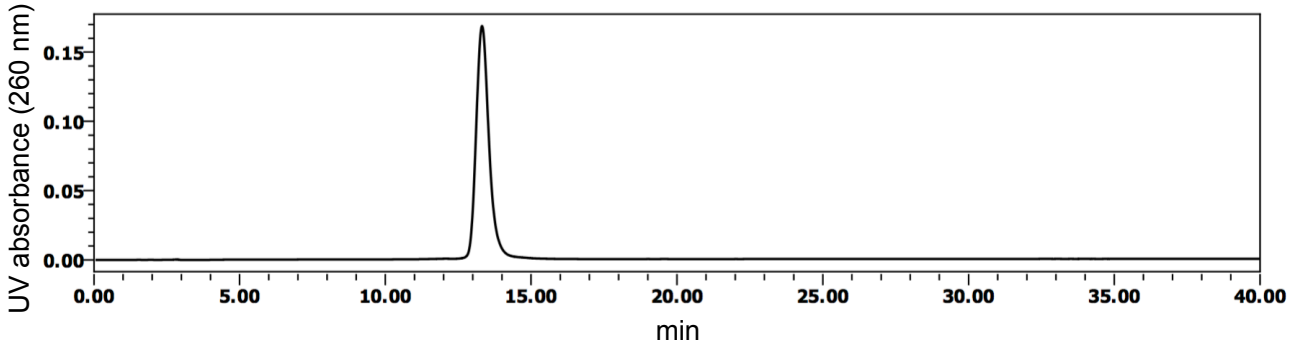
HPLC analysis of ODN1-4

Reversed phase HPLC was performed on a Waters Alliance system with a Waters 3D UV detector and a Waters XBridge™ C_{18} column (4.6 × 150 mm). A linear gradient (0-20%, 0.5 %/min) of solvent I (MeCN) in solvent II (0.03 M ammonium acetate buffer) was used at 30 °C at a flow rate of 1.0 mL/min for 40 min.

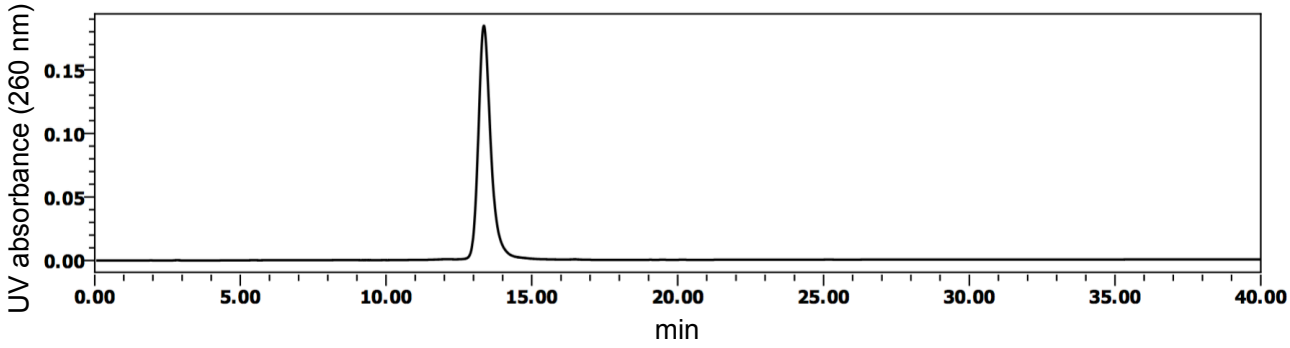
ODN1: 5´-TTTTCTTCTCTT **G^{Py}** TTCTT-3´



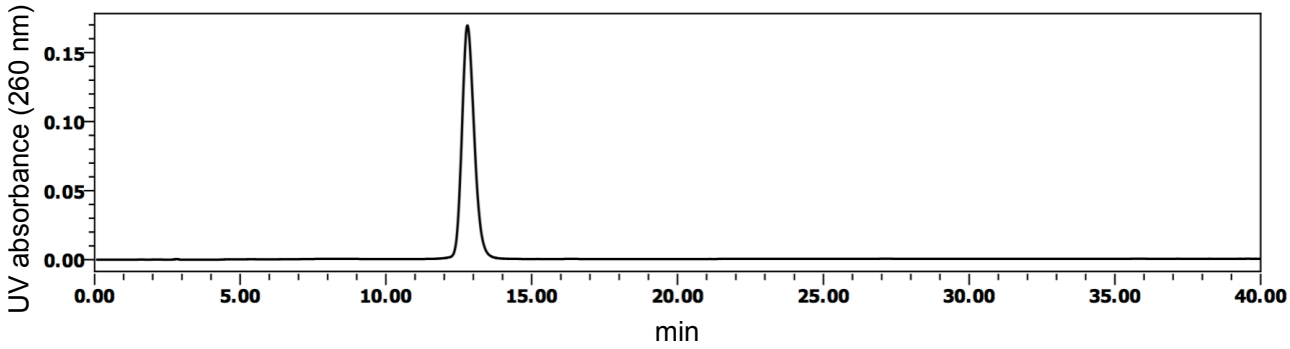
ODN2: 5´-TTTTCTTCTCTT **G^{Pym}** TTCTT-3´



ODN3: 5´-TTTTCTTCTCTT **G^{Pyra}** TTCTT-3´

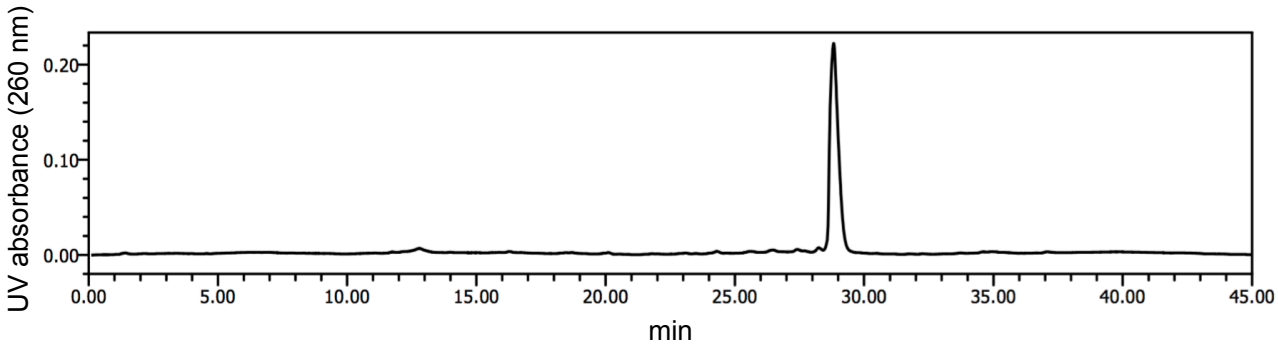


ODN4: 5´-TTTTCTTCTCTT **PIP** TTCTT-3´

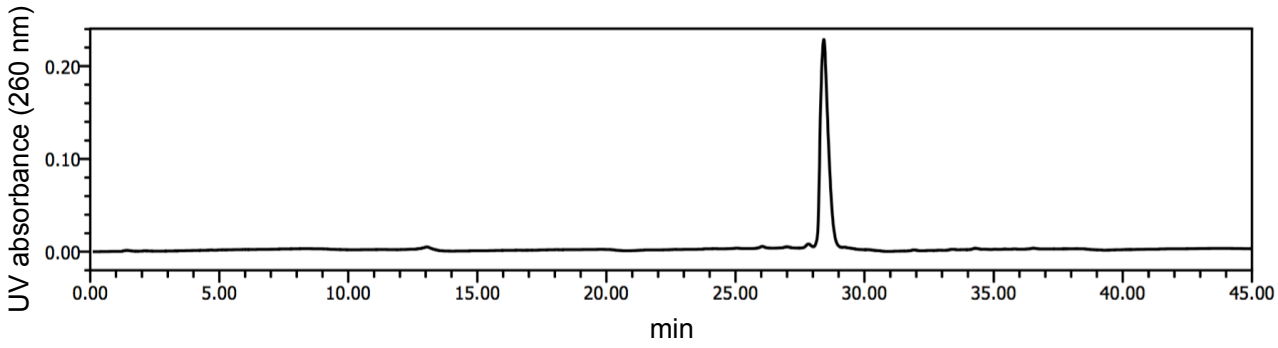


Anion exchange HPLC was performed on a Waters Alliance system with a Waters 3D UV detector and a DNA-Pac PA100 column (4 × 250 mm). A linear gradient (0-60%, 1.3 %/min) of solvent I (25 mM sodium phosphate buffer, pH 6.0, 1 M NaCl, 10% MeCN (v/v)) in solvent II (25 mM sodium phosphate buffer, pH 6.0, 10% MeCN (v/v)) was used at 50 °C at a flow rate of 1.0 mL/min for 45 min.

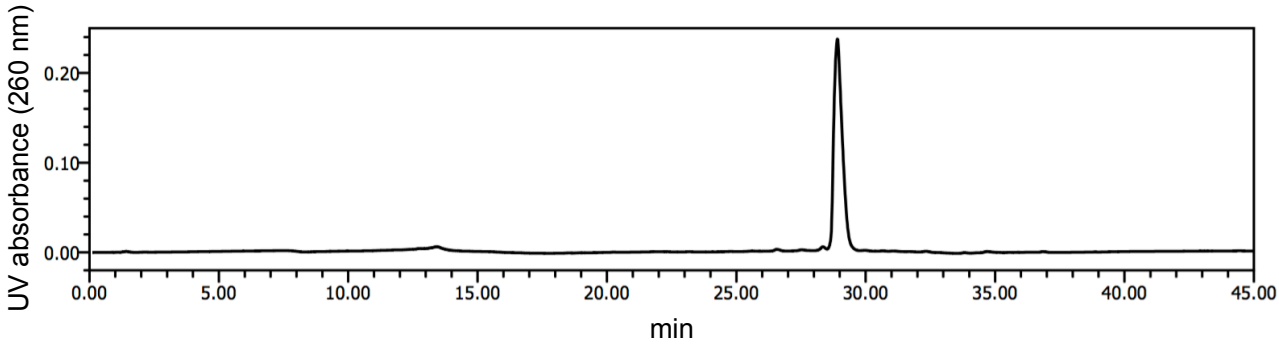
ODN1: 5´-TTTTCTTCTCTT $\mathbf{G^{Py}}$ TTCTT-3´



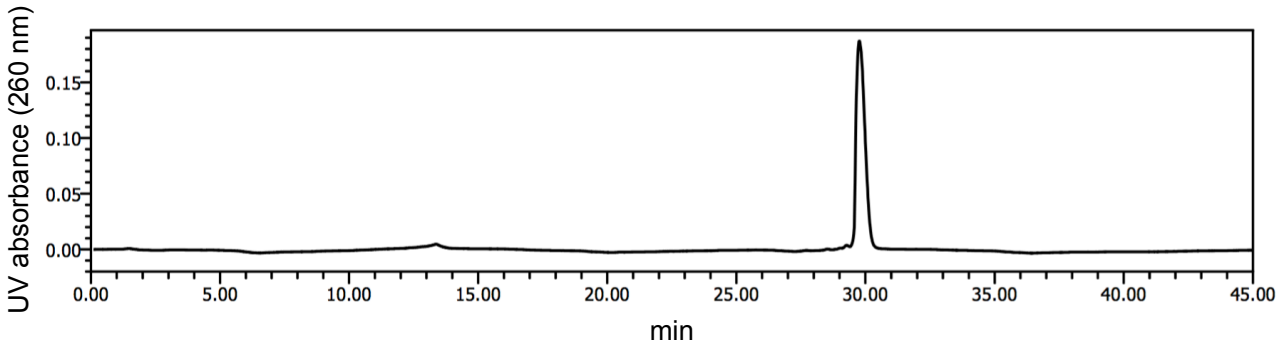
ODN2: 5´-TTTTCTTCTCTT $\mathbf{G^{Pym}}$ TTCTT-3´



ODN3: 5´-TTTTCTTCTCTT **G**$^{\mathbf{Pyra}}$ TTCTT-3´



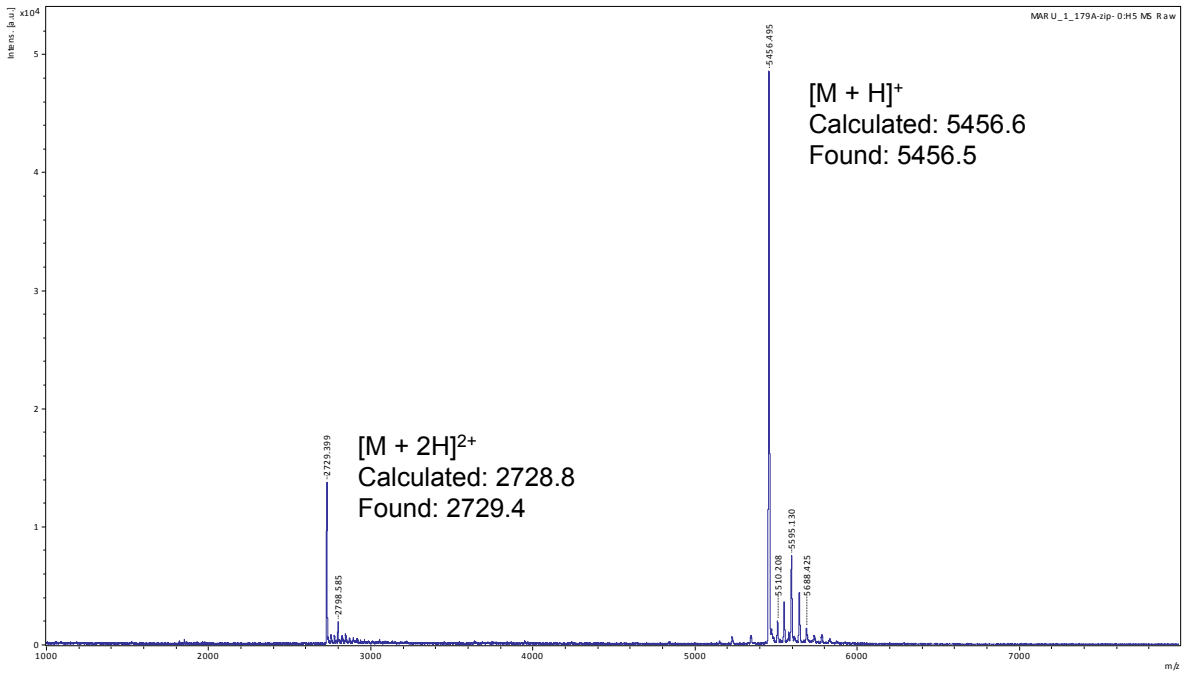
ODN4: 5´-TTTTCTTCTCTT **PIP** TTCTT-3´



MALDI-TOF Mass spectrometry of ODN1-4

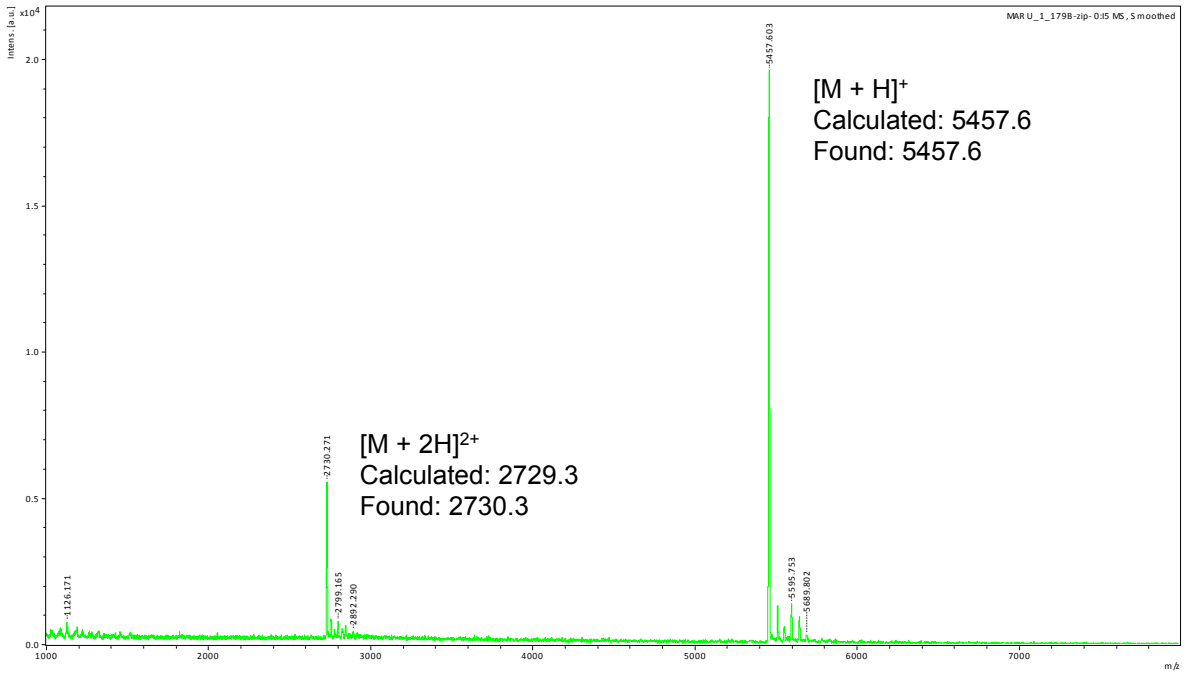
ODN1: 5´-TTTTCTTCTCTT **G^{Py}** TTCTT-3´

calculated for $[M + H]^+$: 5456.6, found 5456.5.



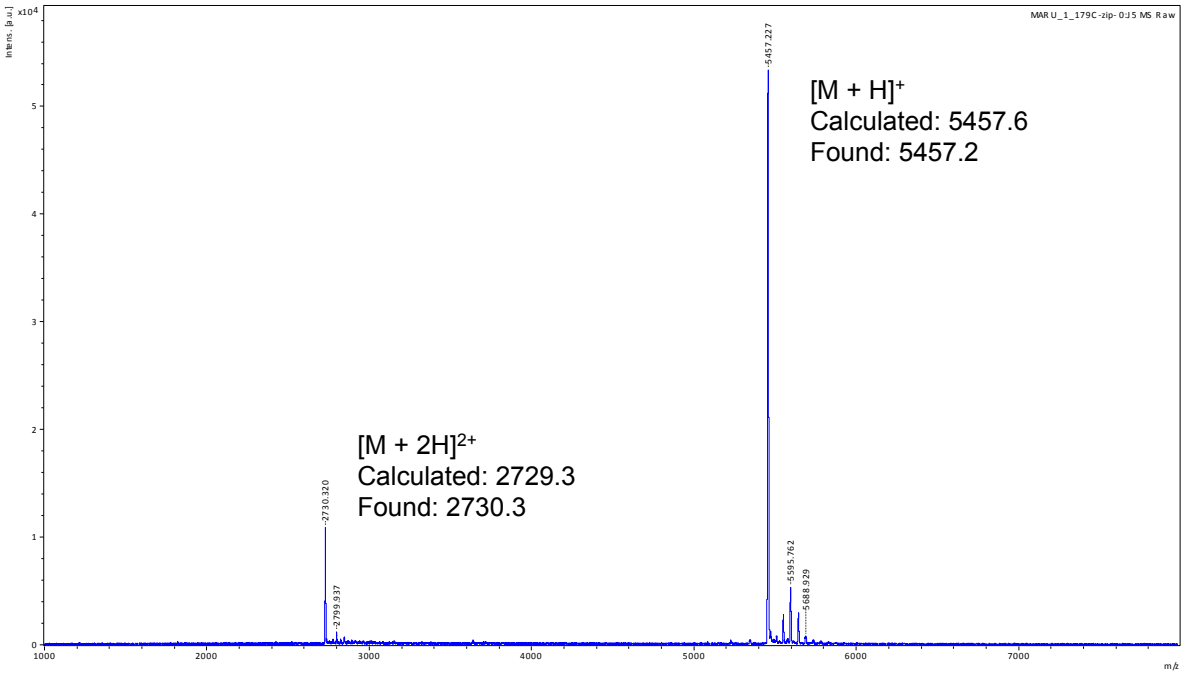
ODN2: 5´-TTTTCTTCTCTT **G^{Pym}** TTCTT-3´

calculated for $[M + H]^+$: 5457.6 found 5457.6.



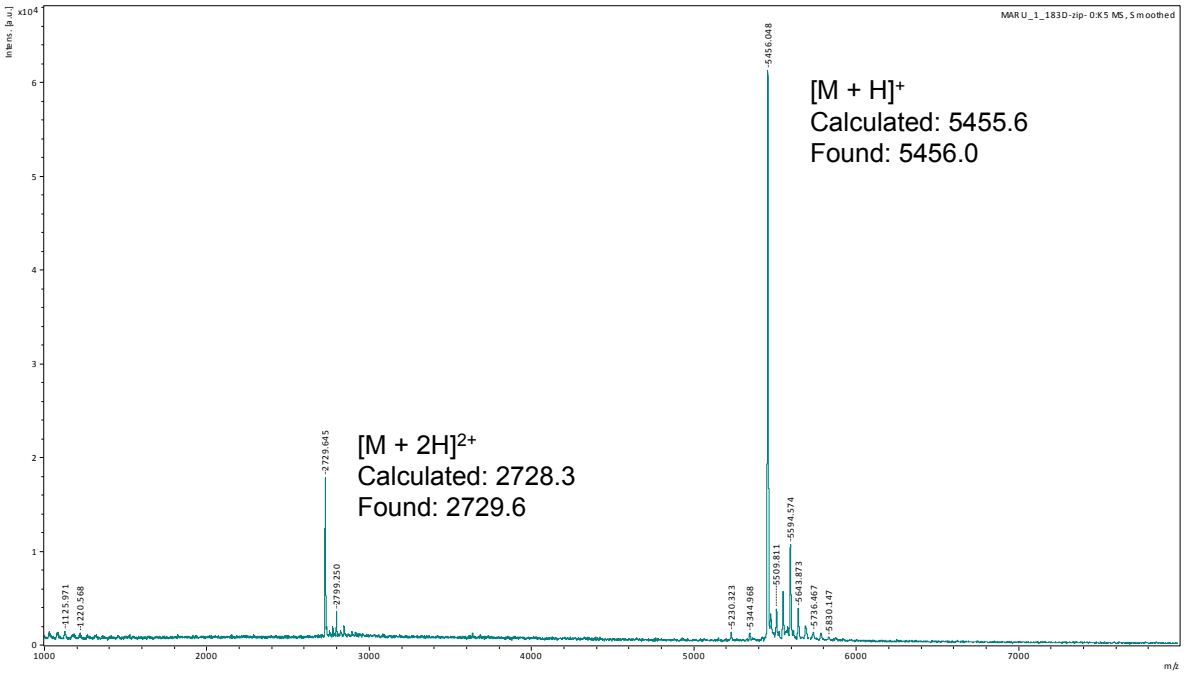
ODN3: 5´-TTTTCTTCTCTT **G^{Pyra}** TTCTT-3´

calculated for $[M + H]^+$: 5457.6, found 5457.2.



ODN4: 5´-TTTTCTTCTCTT **PIP** TTCTT-3´

calculated for $[M + H]^+$: 5455.6, found 5456.0.



p*K*a Curve fitting of PIP nucleoside (3d)

We assumed that **3d** and the deprotonated form of **3d** were in the equilibrium,

$$[PIP-H] \leftrightarrows [PIP^{-}] + [H^{+}] \quad (1)$$

where [PIP-H] and [PIP^{-}] represent the concentration of **3d** and deprotonated form of **3d**, respectively. Here, acid dissociation constant K_a and the total concentration of **3d** can be written as Equation (2) and (3).

$$K_a = \frac{[PIP^{-}][H^{+}]}{[PIP-H]} \quad (2)$$

$$[PIP_{total}] = [PIP-H] + [PIP^{-}] \quad (3)$$

Combining Equation (2) and (3) leads to (4) and (5).

$$[PIP-H] = \frac{[PIP_{total}][H^{+}]}{K_a + [H^{+}]} \quad (4)$$

$$[PIP^{-}] = \frac{[PIP_{total}]K_a}{K_a + [H^{+}]} \quad (5)$$

From the Beer-Lambert law, the UV absorbance of **3d** and deprotonated form of **3d** at 268 nm and 272 nm can be written as Equation (6) and (7),

$$Abs_{268} = \varepsilon_{268}[PIP-H]\ell + \varepsilon'_{268}[PIP^{-}]\ell \quad (6)$$

$$Abs_{272} = \varepsilon_{272}[PIP-H]\ell + \varepsilon'_{272}[PIP^{-}]\ell \quad (7)$$

where ε_{268} and ε_{272} represent the molar attenuation coefficient of compound **3d** at 268 nm and 272 nm, and ε'_{268} and ε'_{272} represent that of deprotonated form of **3d** at 268 nm and 272 nm, respectively, and ℓ represents the path length of UV/Vis absorption measurement. By combining the equation (4), (5), (6) and (7), relative absorbance (Abs_{268}/Abs_{272}) can be written as Equation (8).

$$\frac{Abs_{268}}{Abs_{272}} = \frac{\varepsilon_{268}[H^{+}] + \varepsilon'_{268}K_a}{\varepsilon_{272}[H^{+}] + \varepsilon'_{272}K_a} = \frac{10^{-\mathrm{pH}} + \frac{\varepsilon'_{268}}{\varepsilon_{268}}10^{-\mathrm{p}K\mathrm{a}}}{\frac{\varepsilon_{272}}{\varepsilon_{268}}10^{-\mathrm{pH}} + \frac{\varepsilon'_{272}}{\varepsilon_{268}}10^{-\mathrm{p}K\mathrm{a}}} \quad (8)$$

The Equation (8) was fitted to the experimental data of Abs_{268}/Abs_{272} (Figure3a) as a function of pH, using KaleidaGraph 4.1.4 (Figure 3b). pK_a, $\varepsilon'_{268}/\varepsilon_{268}$, $\varepsilon_{272}/\varepsilon_{268}$, $\varepsilon'_{272}/\varepsilon_{268}$ were obtained as following; pK_a = 5.0, $\varepsilon'_{268}/\varepsilon_{268}$ = 0.68, $\varepsilon_{272}/\varepsilon_{268}$ = 0.87, $\varepsilon'_{272}/\varepsilon_{268}$ =0.89. By using the Equation (8), the concentration error of [PIP$_{total}$] did not affect the result of curve fitting.

T_m experiments

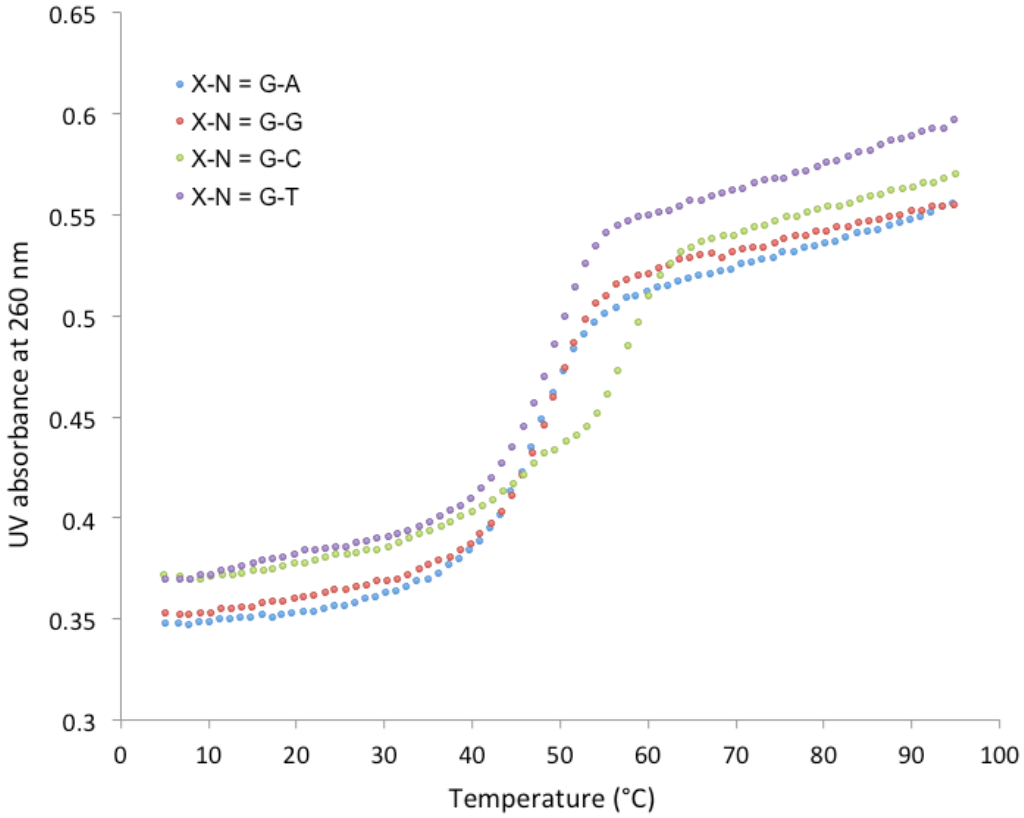
Protocol for T_m experiments

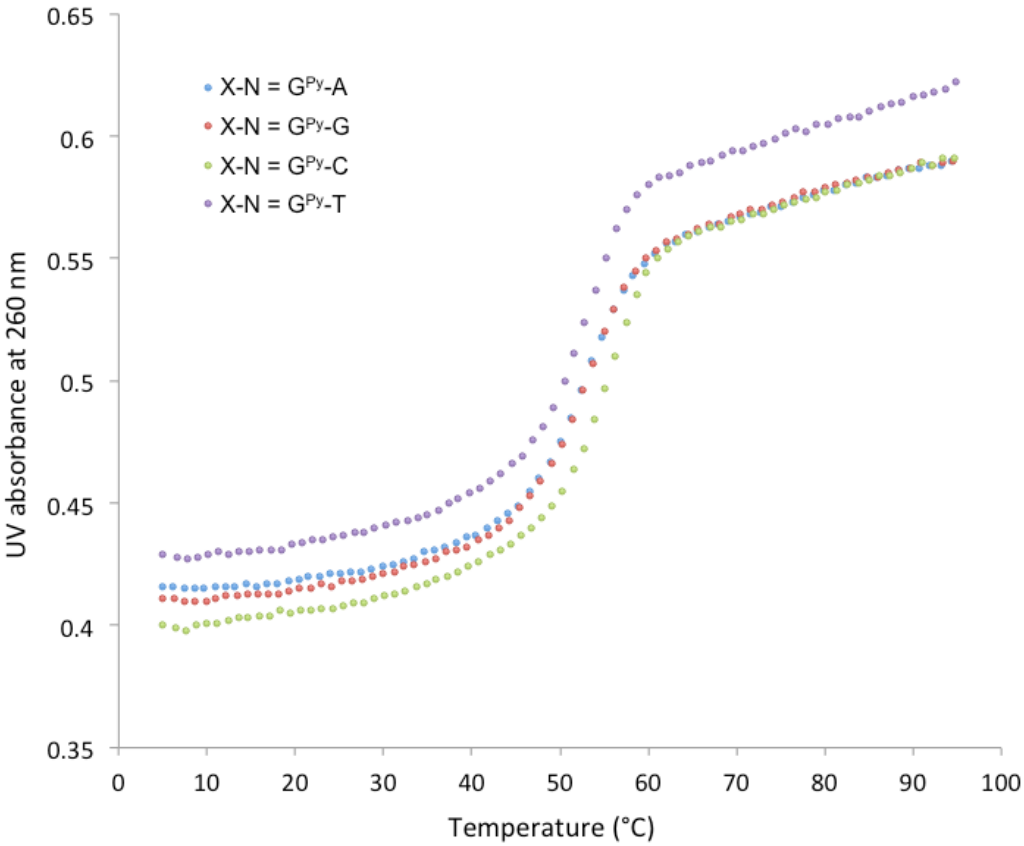
Oligodeoxynucleotides (ODNs) were dissolved in deionized distilled water and their concentration was determined from absorbance measurements at 260 nm. The absorption coefficients of ODNs were calculated according to the nearest-neighbor method using the Oligo Analyzer 3.1 (http://sg.idtdna.com/calc/analyzer) by assuming that **ODN1-4** were identical to **ODN0** by replacing G^{Py}, G^{Pym}, G^{Pyra} and PIP to G.

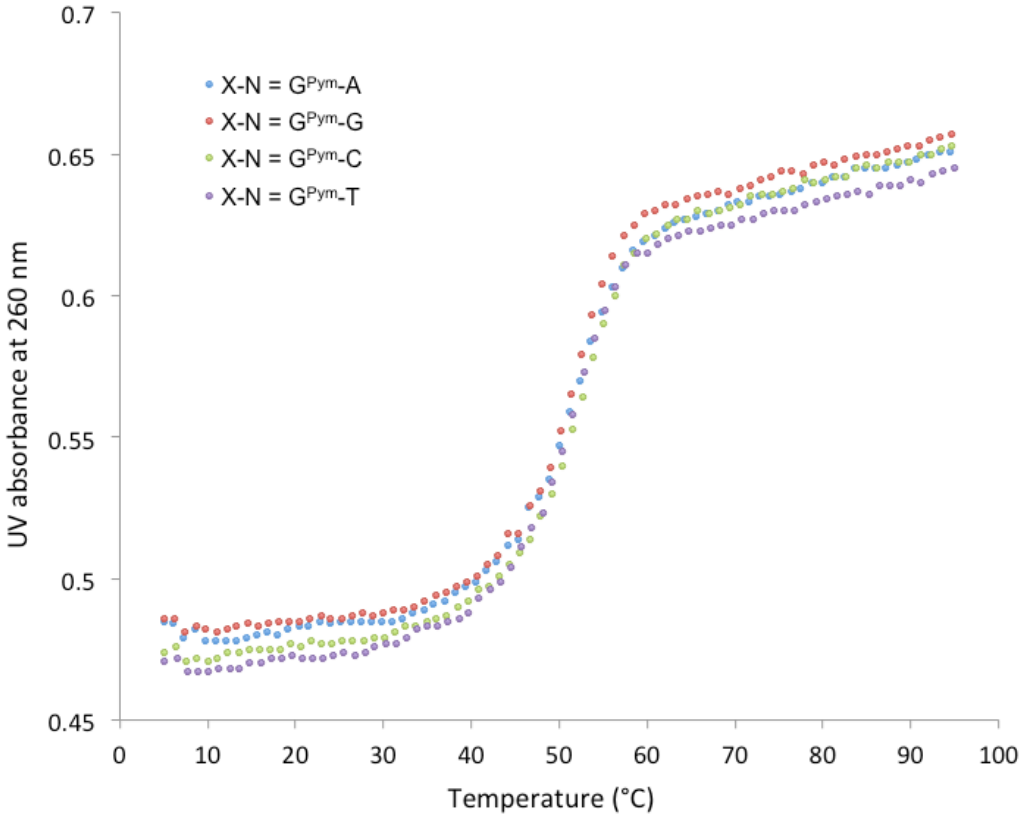
The oligonucleotides (300 pmol each) were dissolved in 200 μL of 10 mM sodium cacodylate buffer (pH 7.0 or 5.5, 100 mM NaCl, 10 mM $MgCl_2$, 0.5 mM spermine). The final concentration of the duplex was 1.5 μM. The solution was placed in a quartz cells (10 mm) and incubated at 90 °C. After 10 min, the solution was cooled to 5 °C then heated to 90 °C at a rate of 0.5 °C/min. The absorption at 260 nm was recorded and used to draw UV-melting curves. The measurement was carried out three times independently. The UV-melting curve was smoothed by the Savitzky-Golay method. Melting temperatures were calculated as the temperature that gave the maximum of the first derivation of the UV-melting curves. The average of three T_m values was used to determine the final T_m value.

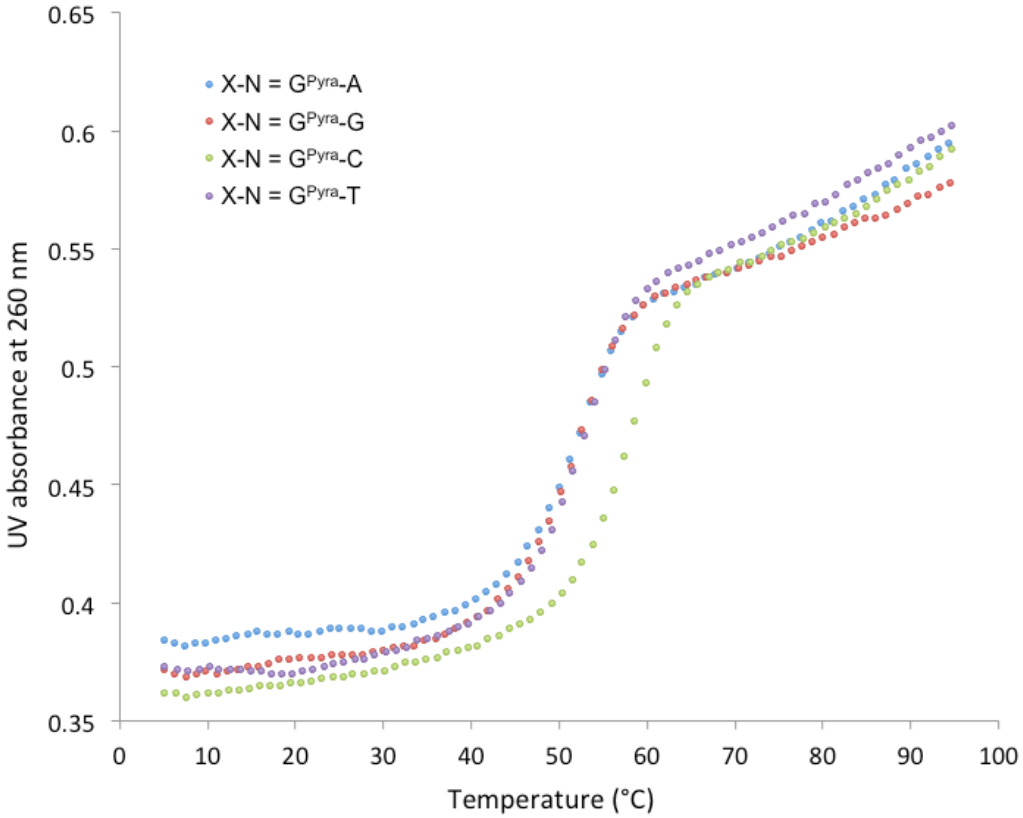
Representative melting curves of the duplexes (pH 7.0)

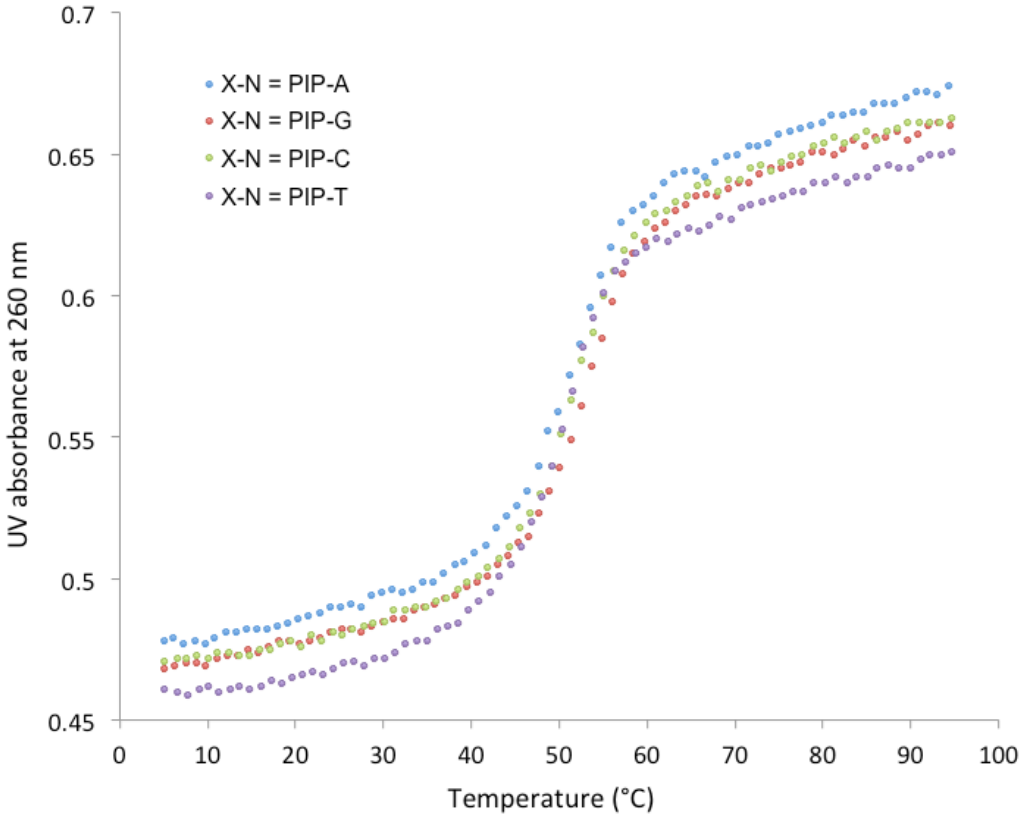
ODN0-4	5´-TTTTCTTCTCTT **X** TTCTT-3´
DNA(C, A, G or T)	3´-AAAAGAAGAGAA **N** AAGAA-5´


X-N = G-A
X-N = G-G
X-N = G-C
X-N = G-T
UV absorbance at 260 nm
Temperature (°C)
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0
10
20
30
40
50
60
70
80
90
100


X-N = GPy-A
X-N = GPy-G
X-N = GPy-C
X-N = GPy-T
UV absorbance at 260 nm
Temperature (°C)
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0
10
20
30
40
50
60
70
80
90
100


X-N = G^Pym-A
X-N = G^Pym-G
X-N = G^Pym-C
X-N = G^Pym-T
UV absorbance at 260 nm
Temperature (°C)
0.7
0.65
0.6
0.55
0.5
0.45
0
10
20
30
40
50
60
70
80
90
100

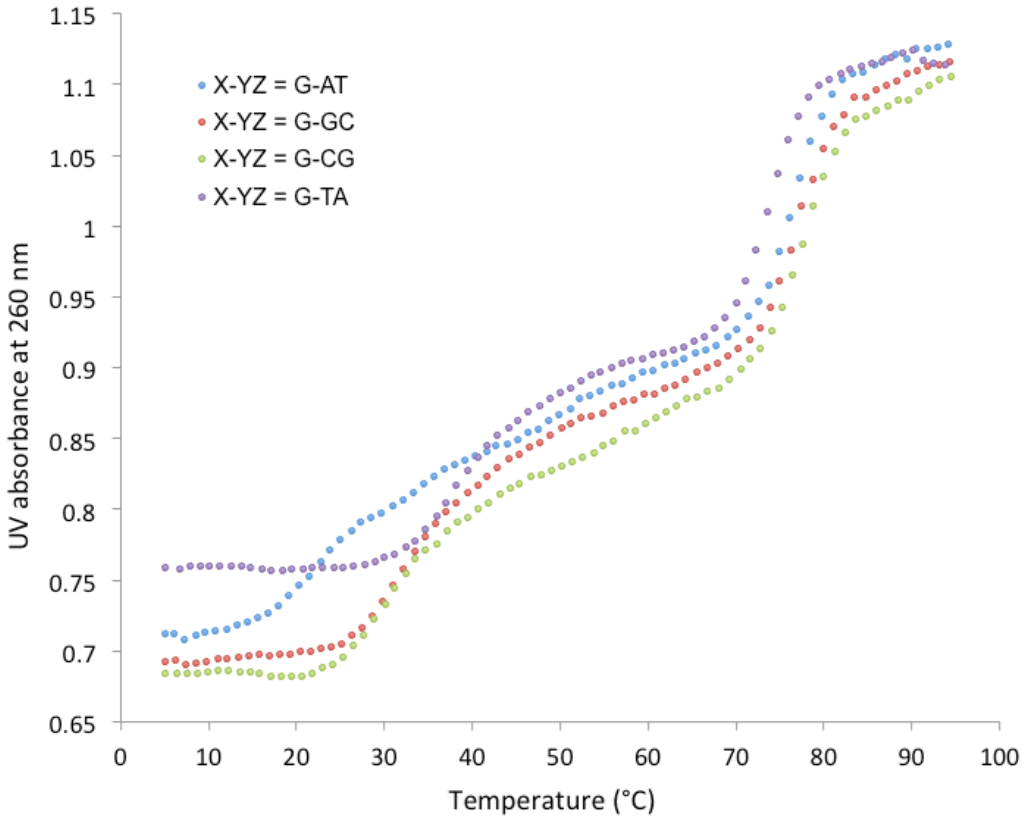

X-N = G^Pyra-A
X-N = G^Pyra-G
X-N = G^Pyra-C
X-N = G^Pyra-T
UV absorbance at 260 nm
Temperature (°C)
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0
10
20
30
40
50
60
70
80
90
100

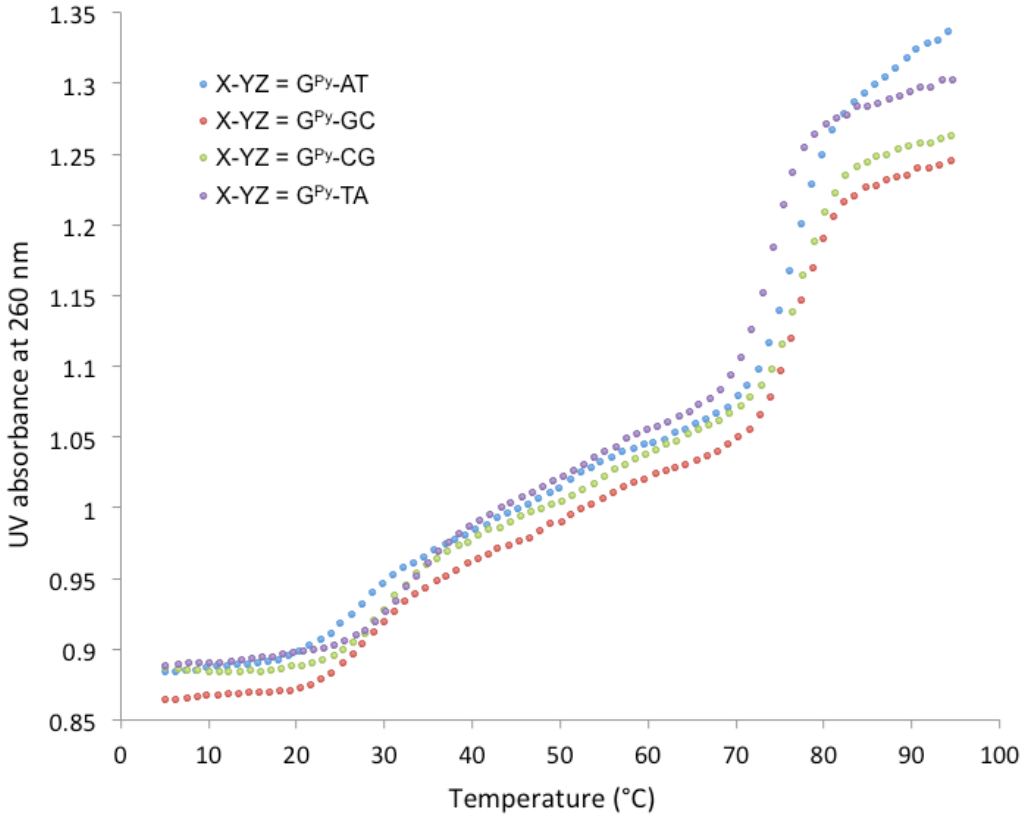

0.7
0.65
0.6
0.55
0.5
0.45
UV absorbance at 260 nm
X-N = PIP-A
X-N = PIP-G
X-N = PIP-C
X-N = PIP-T
0
10
20
30
40
50
60
70
80
90
100
Temperature (°C)

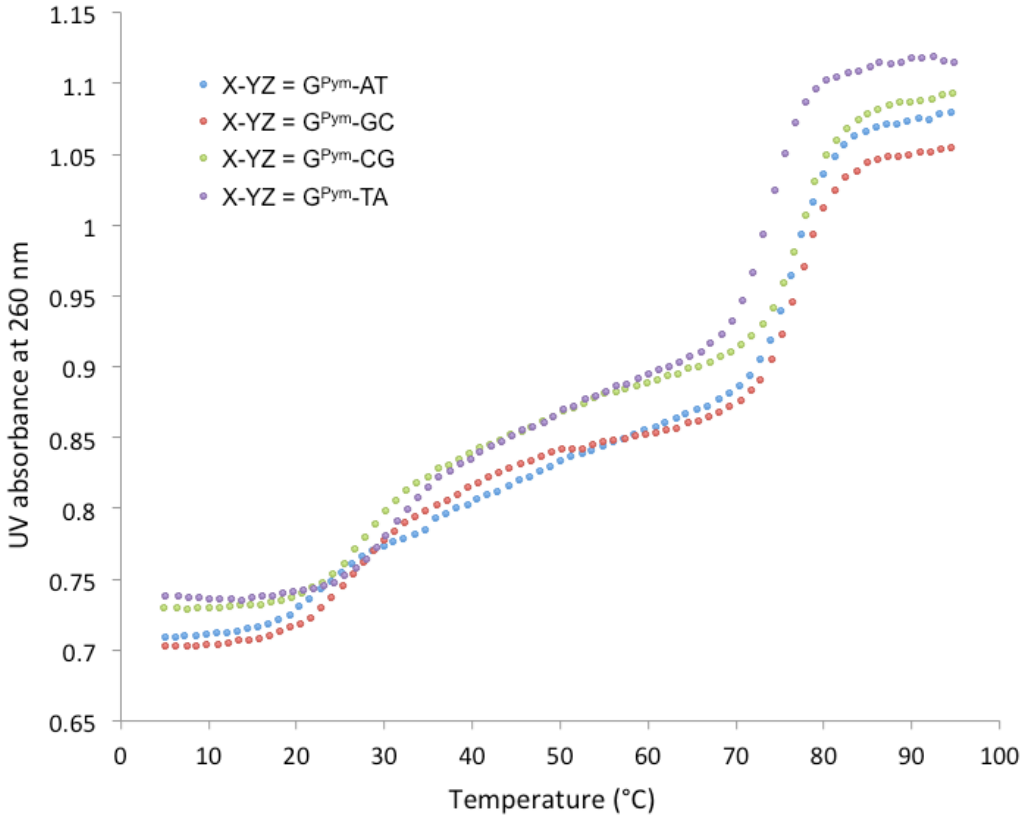
Representative melting curves of the triplexes (pH 7.0)

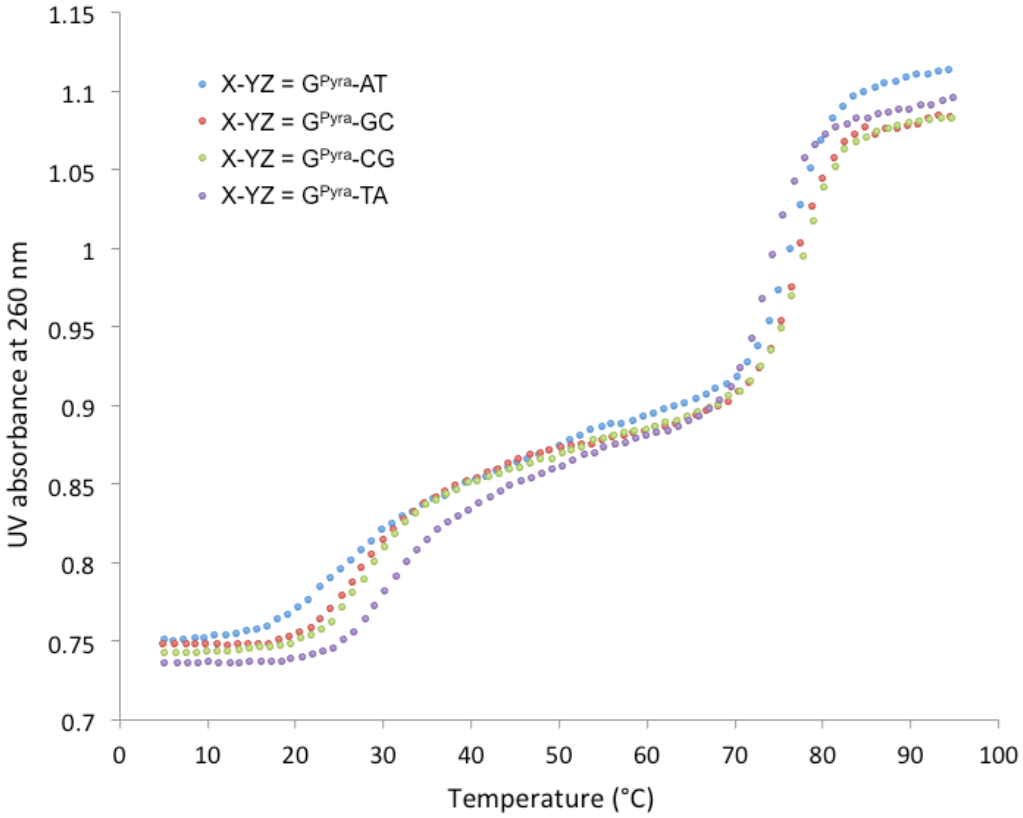
ODN0-4 (TFO) 5´–TTTTCTTCTCTT **X** TTCTT–3´

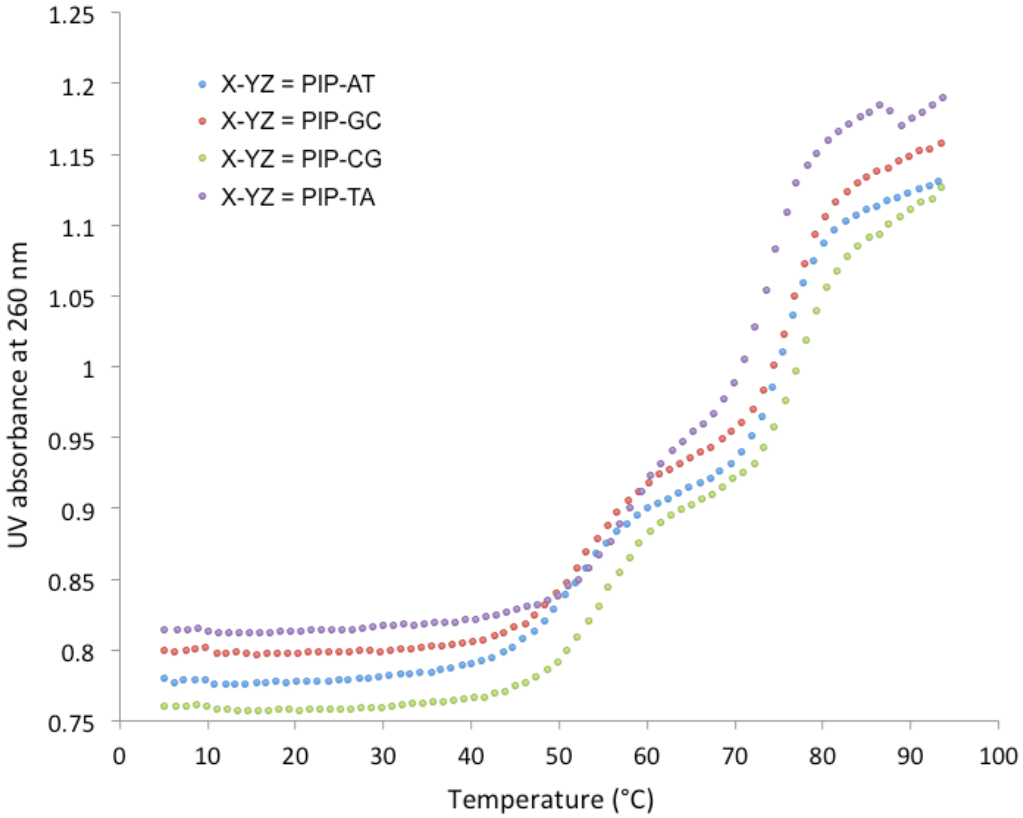
HP (T, A, G or C)
5´–AAAAGAAGAGAA **Y** AAGAA ^{T}T
3´–TTTTCTTCTCTT **Z** TTCTT $_{T}$T




X-YZ = GPy-AT
X-YZ = GPy-GC
X-YZ = GPy-CG
X-YZ = GPy-TA
UV absorbance at 260 nm
Temperature (°C)
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0
10
20
30
40
50
60
70
80
90
100

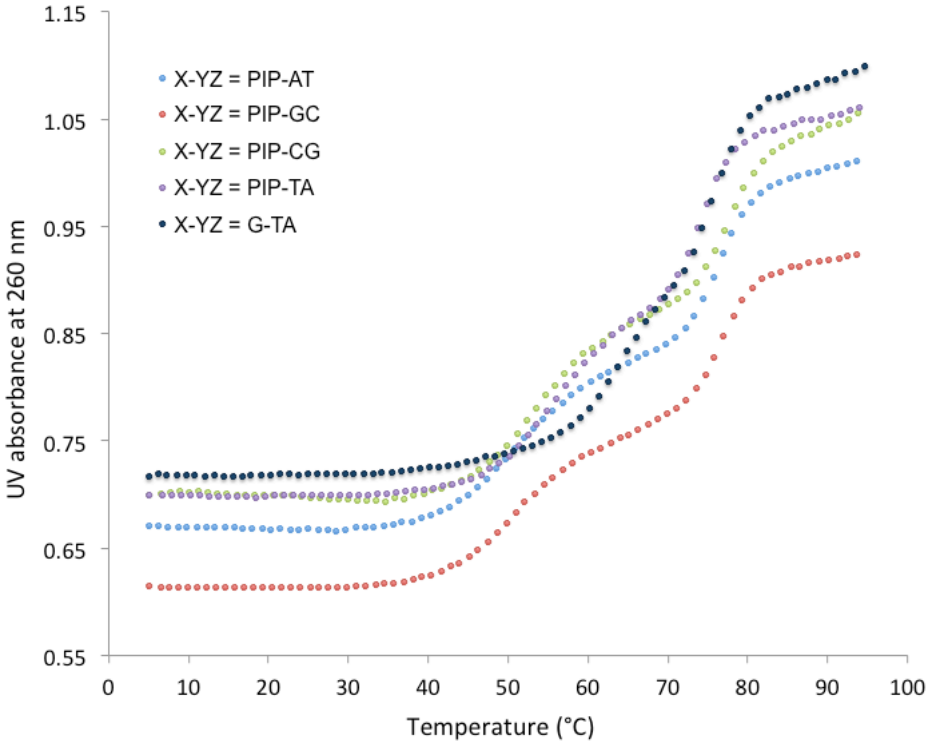

X-YZ = GPym-AT
X-YZ = GPym-GC
X-YZ = GPym-CG
X-YZ = GPym-TA
UV absorbance at 260 nm
Temperature (°C)
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0
10
20
30
40
50
60
70
80
90
100


X-YZ = G^Pyra-AT
X-YZ = G^Pyra-GC
X-YZ = G^Pyra-CG
X-YZ = G^Pyra-TA
UV absorbance at 260 nm
Temperature (°C)


X-YZ = PIP-AT
X-YZ = PIP-GC
X-YZ = PIP-CG
X-YZ = PIP-TA
UV absorbance at 260 nm
Temperature (°C)

Representative melting curves of the triplexes (pH 5.5)

ODN0 or 4 (TFO)	5´–TTTTCTTCTCTT **X** TTCTT–3´
HP (T, A, G or C)	5´–AAAAGAAGAGAA **Y** AAGAA ^{T}T 3´–TTTTCTTCTCTT **Z** TTCTT $_{T}$T



We were unable to calculate the exact T_m of the triplex made of **ODN0** and **HP(T)** (represented as X-YZ = G-TA, shown in orange), because the melting curves of triplex and that of **HP(T)** as a single-strand DNA duplex merged. We assumed that the apparent inflection point of the melting curve is approximately 60 °C or higher.