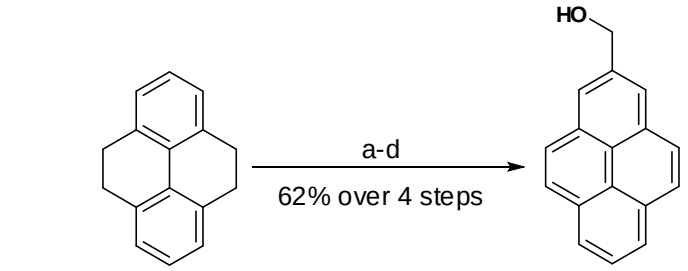
Recognition of double-stranded DNA using energetically activated duplexes with interstrand zippers of 1-, 2- or 4-pyrenyl-functionalized O2'-alkylated RNA monomers

Saswata Karmakar, Andreas S. Madsen, Dale C. Guenther, Bradley C. Gibbons and Patrick J. Hrdlicka*

hrdlicka@uidaho.edu

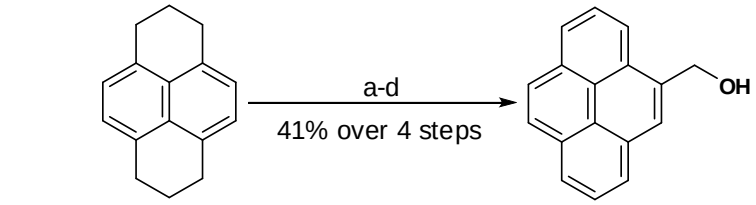
ELECTRONIC SUPPLEMENTARY INFORMATION

General experimental section. Reagents and solvents were commercially available, of analytical grade and used without further purification. Petroleum ether of the distillation range 60-80 °C was used. Solvents were dried over activated molecular sieves: THF (3Å); CH_2Cl_2, 1,2-dichloroethane, *N,N'*-diisopropylethylamine and anhydrous DMSO (4Å). Anhydrous diethyl ether, DMF and benzene were obtained from commercial suppliers (<10 ppm water). Water content of anhydrous solvents was verified on Karl-Fisher apparatus. Reactions were conducted under argon whenever anhydrous solvents were used. Reactions were monitored by TLC using silica gel coated plates with a fluorescence indicator (SiO_2-60, F-254) which were visualized a) under UV light and/or b) by dipping in 5% conc. H_2SO_4 in absolute ethanol (v/v) followed by heating. Silica gel column chromatography was performed with Silica gel 60 (particle size 0.040–0.063 mm) using moderate pressure (pressure ball). Evaporation of solvents was carried out under reduced pressure at temperatures below 45 °C. After column chromatography, appropriate fractions were pooled, evaporated and dried at high vacuum for at least 12h to give the obtained products in high purity (>95%) as ascertained by 1D NMR techniques. Chemical shifts of ^{1}H NMR (500 MHz), ^{13}C NMR (125.6 MHz), and/or ^{31}P NMR (121.5 MHz) are reported relative to deuterated solvent or other internal standards (80% phosphoric acid for ^{31}P NMR). Exchangeable (ex) protons were detected by disappearance of signals upon D_2O addition. Assignments of NMR spectra are based on 2D spectra (COSY, HSQC) and DEPT-spectra. Quaternary carbons are not assigned in ^{13}C NMR but verified from HSQC and DEPT spectra (absence of signals). MALDI-HRMS spectra of compounds were recorded on a Waters Q-TOF Premiere mass spectrometer using 2,5-dihydroxybenzoic acid as a matrix and polyethylene glycol (PEG 600) as an internal calibration standard.



4,5,9,10-tetrahydropyrene **2-pyrenemethanol**

Scheme S1. Synthesis of 2-pyrenemethanol. Reagents and conditions: (a) Br_2, DMF, rt, 6h; (b) DDQ, benzene, reflux, 4h; (c) (i) *n*-BuLi, THF/ether, -78 °C for 2h; (ii) DMF, -30 to -50 °C for 1h, then rt for 15h; (d) $NaBH_4$, THF, rt, overnight.



4,5,9,10-tetrahydropyrene **4-pyrenemethanol**

Scheme S2. Synthesis of 4-pyrenemethanol. Reagents and conditions: (a) Br_2, AcOH, rt, 1h; (b) DDQ, benzene, reflux, 4h; (c) (i) *n*-BuLi, THF/ether, -78 °C for 2h; (ii) DMF, -50 to -30 °C for 1h, then rt for 15h; (d) $NaBH_4$, THF, rt, overnight.

Table S1. MALDI-MS of ONs modified with monomers **Y** and **Z**.[a]

ONs	Sequence	Calc. *m*/*z* [M+H]	Found *m*/*z* [M+H]
Y1	5'-G**Y**G ATA TGC	2969	2969
Y2	5'-GTG A**Y**A TGC	2969	2969
Y3	5'-GTG ATA **Y**GC	2969	2969
Y4	3'-CAC **Y**AT ACG	2898	2898
Y5	3'-CAC TA**Y** ACG	2898	2898
Y6	3'-CAC **Y**A**Y** ACG	3114	3114
Z1	5'-G**Z**G ATA TGC	2969	2969
Z2	5'-GTG A**Z**A TGC	2969	2969
Z3	5'-GTG ATA **Z**GC	2969	2969
Z4	3'-CAC **Z**AT ACG	2898	2898
Z5	3'-CAC TA**Z** ACG	2898	2898
Z6	3'-CAC **Z**A**Z** ACG	3114	3114

[a] For structures of monomers **Y** and **Z**, see Figure 1 in the main manuscript.

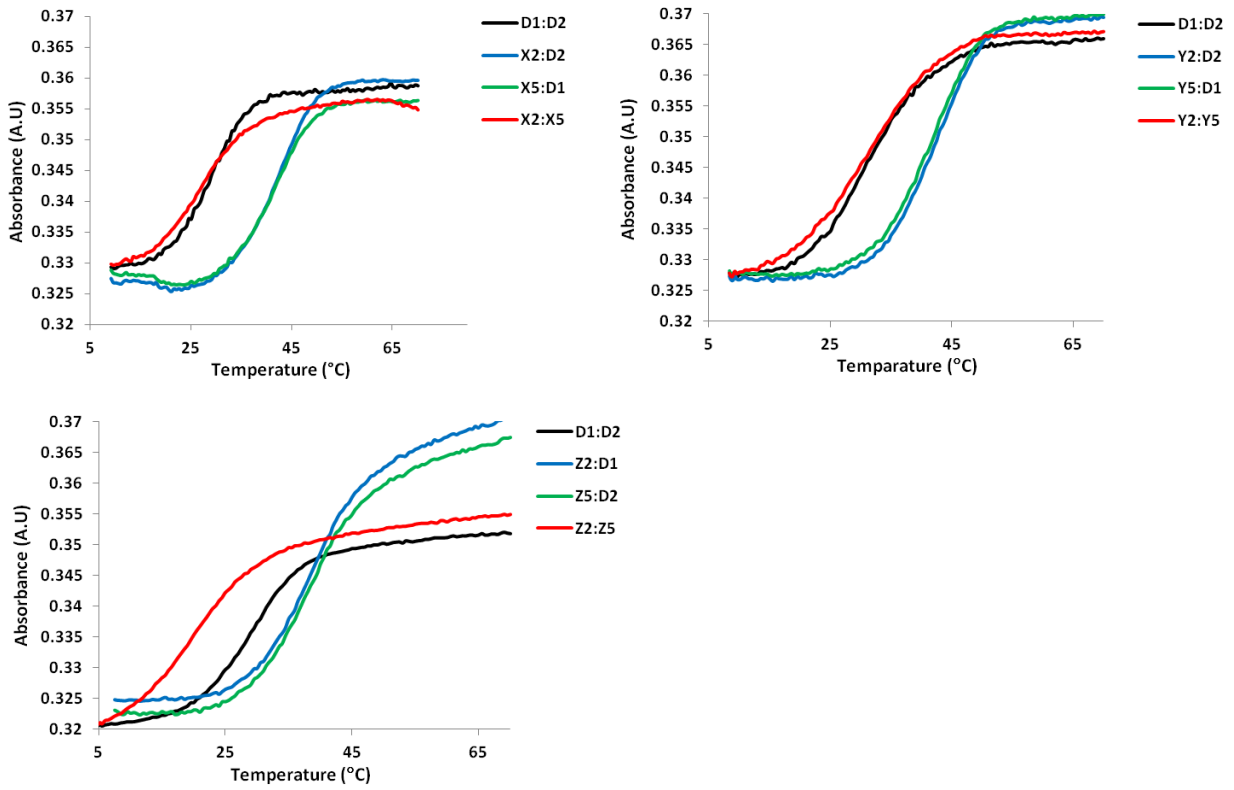


Figure S1. Representative thermal denaturation curves of **X**/**Y**/**Z**-modified duplexes and reference duplex **D1**:**D2**. For experimental conditions, see Table 1.

Table S2. DNA selectivity of **B1-B6**.[a]

			$\Delta\Delta T_m$/mod (DNA-RNA) [°C]		
ON	**Duplex**	**B =**	**X**	**Y**	**Z**
B1	5'-G**B**G ATA TGC		+7.0	+7.5	+6.0
B2	5'-GTG A**B**A TGC		+9.0	+6.5	+7.0
B3	5'-GTG ATA **B**GC		+8.0	+8.5	+9.0
B4	3'-CAC **B**AT ACG		+8.0	+7.5	+5.0
B5	3'-CAC TA**B** ACG		+10.5	+6.5	+7.0
B6	3'-CAC **B**A**B** ACG		+7.3	+6.0	+5.5

[a] DNA selectivity defined as $\Delta\Delta T_m$/mod (DNA-RNA) = ΔT_m/mod (vs cDNA) - ΔT_m/mod (vs cRNA).

Additional discussion of binding specificity. **X2**/**Y2**/**Z2** display less efficient discrimination of mismatched RNA targets compared to reference strand **D1**, except when a mismatched G is opposite of the pyrene-functionalized monomers (Table S3).

ONs with two modifications positioned as next-nearest neighbors (**B6**-series) very efficiently discriminate DNA targets with a single mismatched nucleotide opposite of the central adenosine residue (Table S4). Similar observations have been observed with analogous ONs modified with other O2'-pyrene-functionalized RNA monomers or N2'-pyrene-functionalized 2'-*N*-methyl-2'-amino-DNA,[S1] which suggests that this probe architecture may be a general strategy toward improving binding specificity. However, this hypothesis must be evaluated across different sequence contexts.

Table S3. Discrimination of mismatched RNA targets by **X2**/**Y2**/**Z2** and reference ONs.[a]

			RNA: 3'-CAC U**B**U ACG			
			T_m/°C	ΔT_m/°C		
ON	**Sequence**	**B** =	A	C	G	U
D1	5'-GTG ATA TGC		**26.5**	<-16.5	-4.5	<-16.5
X2[b]	5'-GTG A**X**A TGC		**31.0**	-17.5	-3.5	-9.5
Y2	5'-GTG A**Y**A TGC		**34.0**	-13.5	-8.5	-13.5
Z2	5'-GTG A**Z**A TGC		**27.5**	-13.0	-7.0	-8.0

[a] For conditions of thermal denaturation experiments, see Table 1. T_m's of fully matched duplexes are shown in bold. ΔT_m = change in T_m relative to fully matched DNA:RNA duplex.

[b] Data previously reported in reference S1. Included to facilitate direct comparison.

Table S4. Discrimination of mismatched DNA targets by **X6**/**Y6**/**Z6** and reference ONs.[a]

			DNA : 5'-GTG A**B**A ACG			
			T_m [°C]	ΔT_m [°C]		
ON	Sequence	**B** =	T	A	C	G
D2	3'-CAC TAT ACG		**29.5**	-17.0	-15.5	-9.0
X6	3'-CAC **X**A**X** ACG		**43.5**	-24.0	-17.0	-14.0
Y6	3'-CAC **Y**A**Y** ACG		**45.5**	-24.0	-16.0	-14.0
Z6	3'-CAC **Z**A**Z** ACG		**32.5**	-21.0	-16.0	-12.0

[a] For conditions of thermal denaturation experiments, see Table 1. T_m's of fully matched duplexes are shown in bold. ΔT_m = change in T_m relative to fully matched DNA:DNA duplex.

[b] Data previously reported in reference S1. Included to facilitate direct comparison.

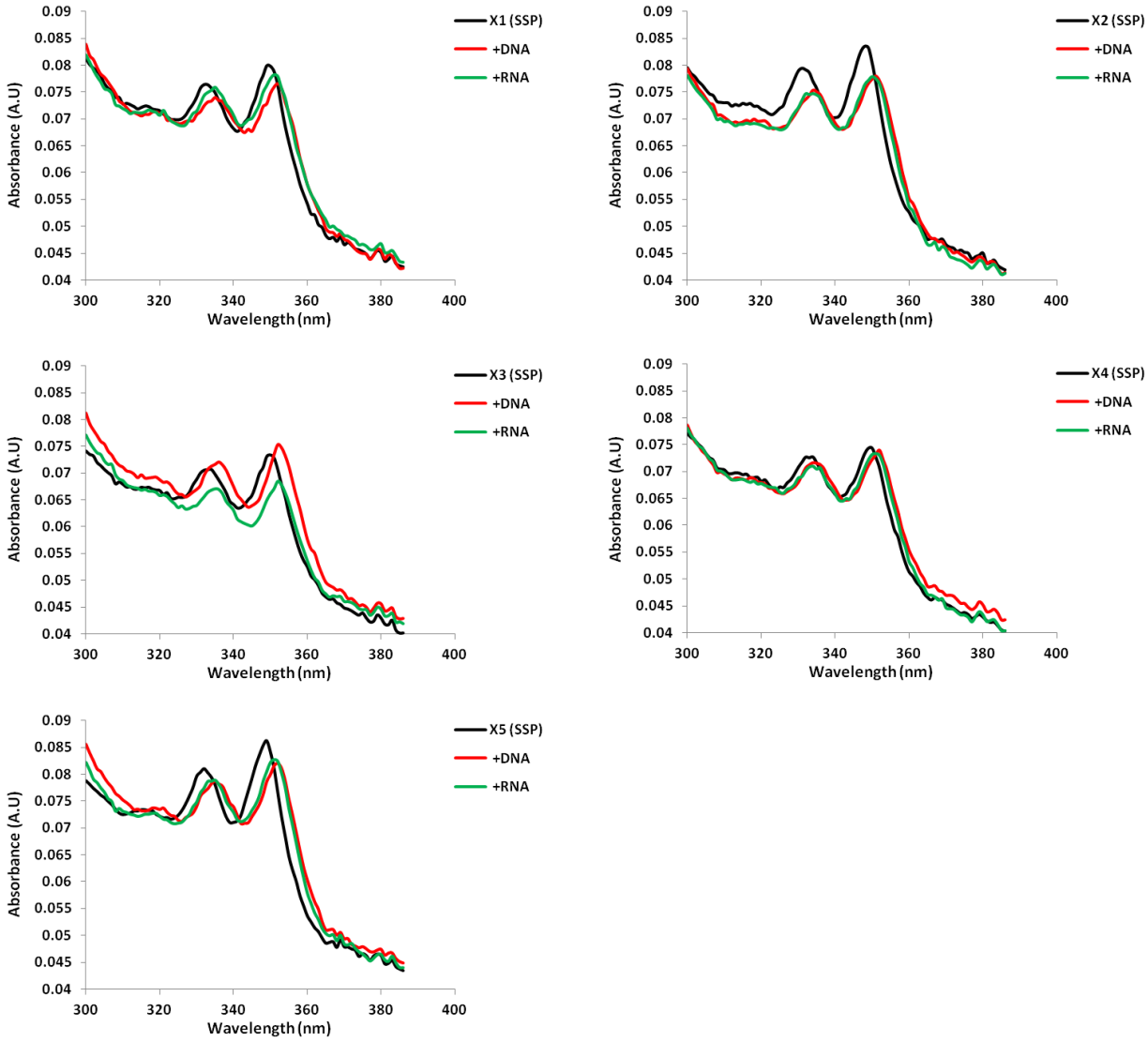


Figure S2. Absorbance spectra of single-stranded **X1**-**X5** and their corresponding duplexes with DNA/RNA targets. Spectra were recorded at T = 5 °C using each strand at 1.0 μM concentration in T_m buffer.

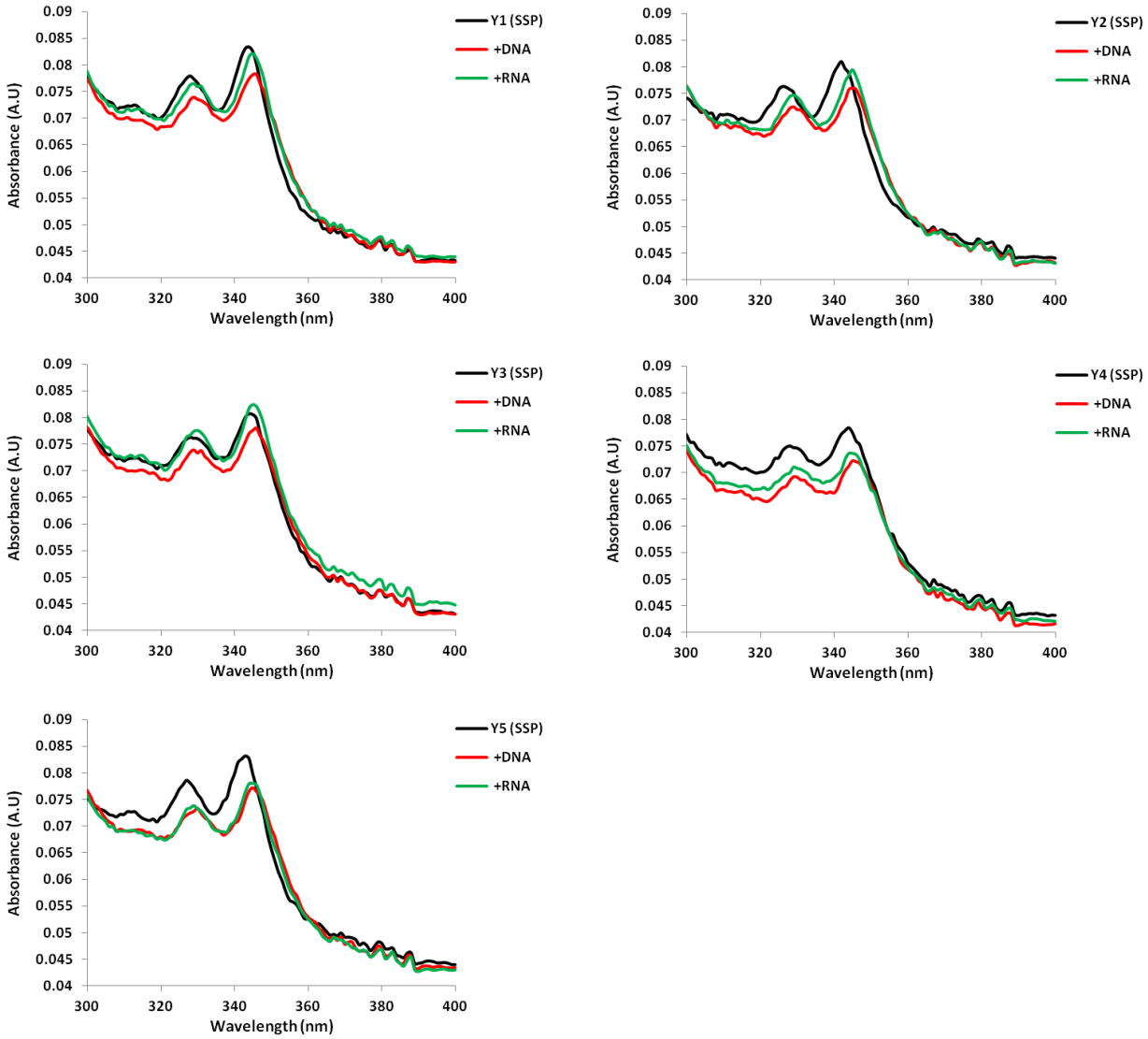


Figure S3. Absorbance spectra of single-stranded **Y1-Y5** and their corresponding duplexes with DNA/RNA targets. For conditions, see Fig. S2.

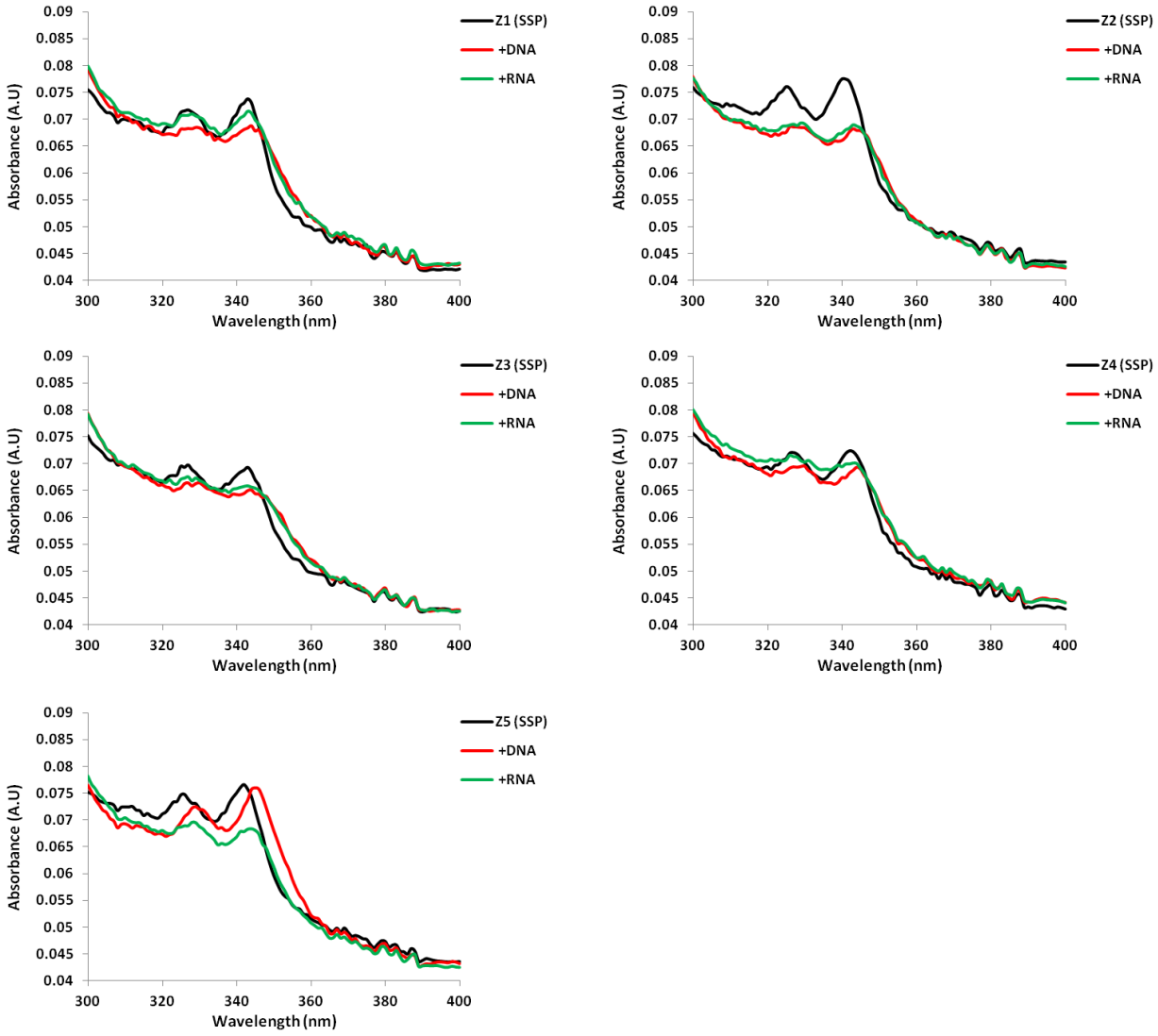


Figure S4. Absorbance spectra of single-stranded **Z1**-**Z5** and their corresponding duplexes with DNA/RNA targets. For conditions, see Fig. S2.

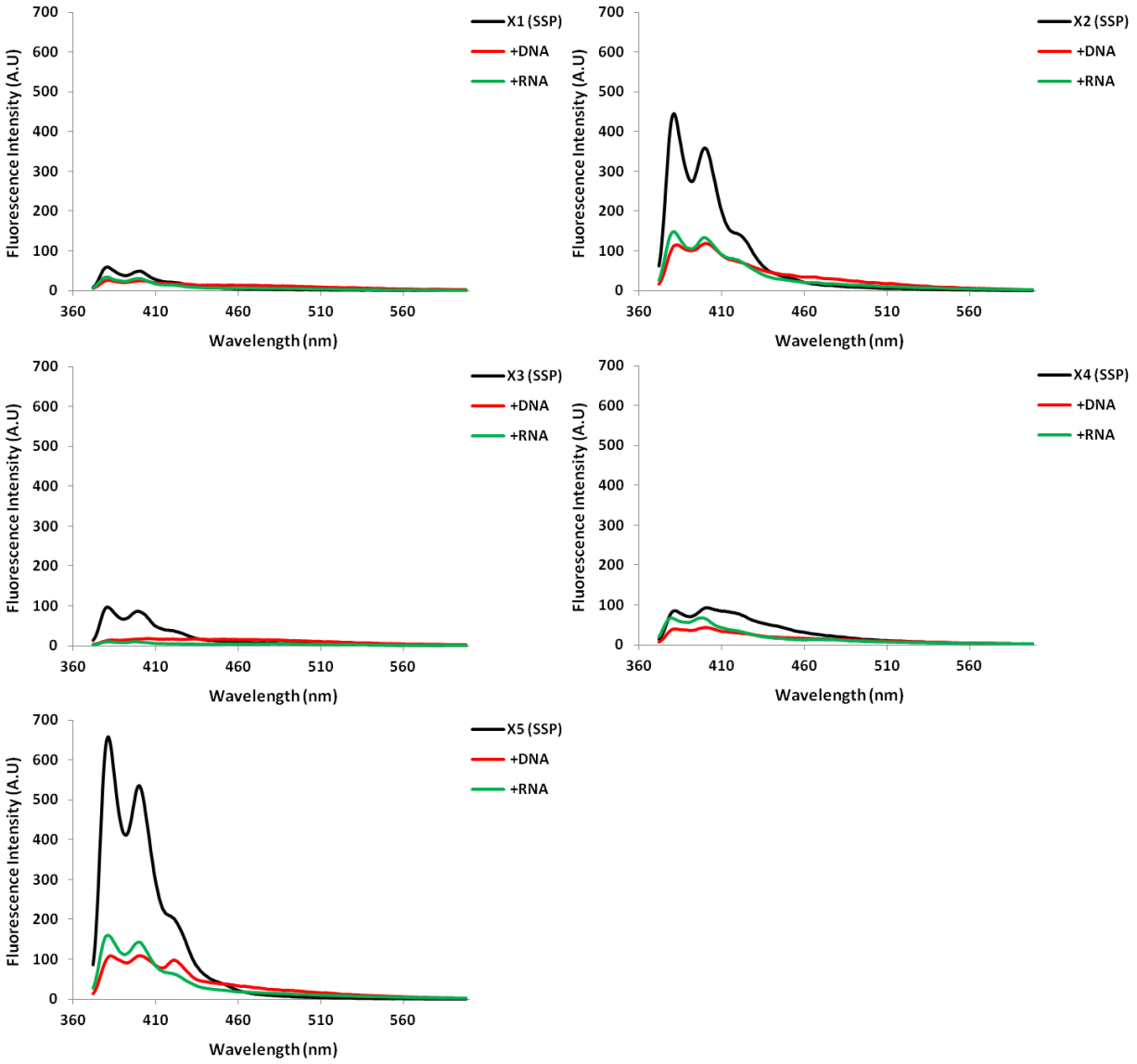


Figure S5. Steady-state fluorescence emission spectra of **X**-modified ONs and their corresponding duplexes with DNA/RNA targets. Spectra were recorded at T = 5 °C using λ_{ex} = 350 nm and each strand at 1.0 µM concentration in T_m buffer. Some, but not all, of these spectra have been previously published in reference S1. Spectra are included to facilitate direct comparison.

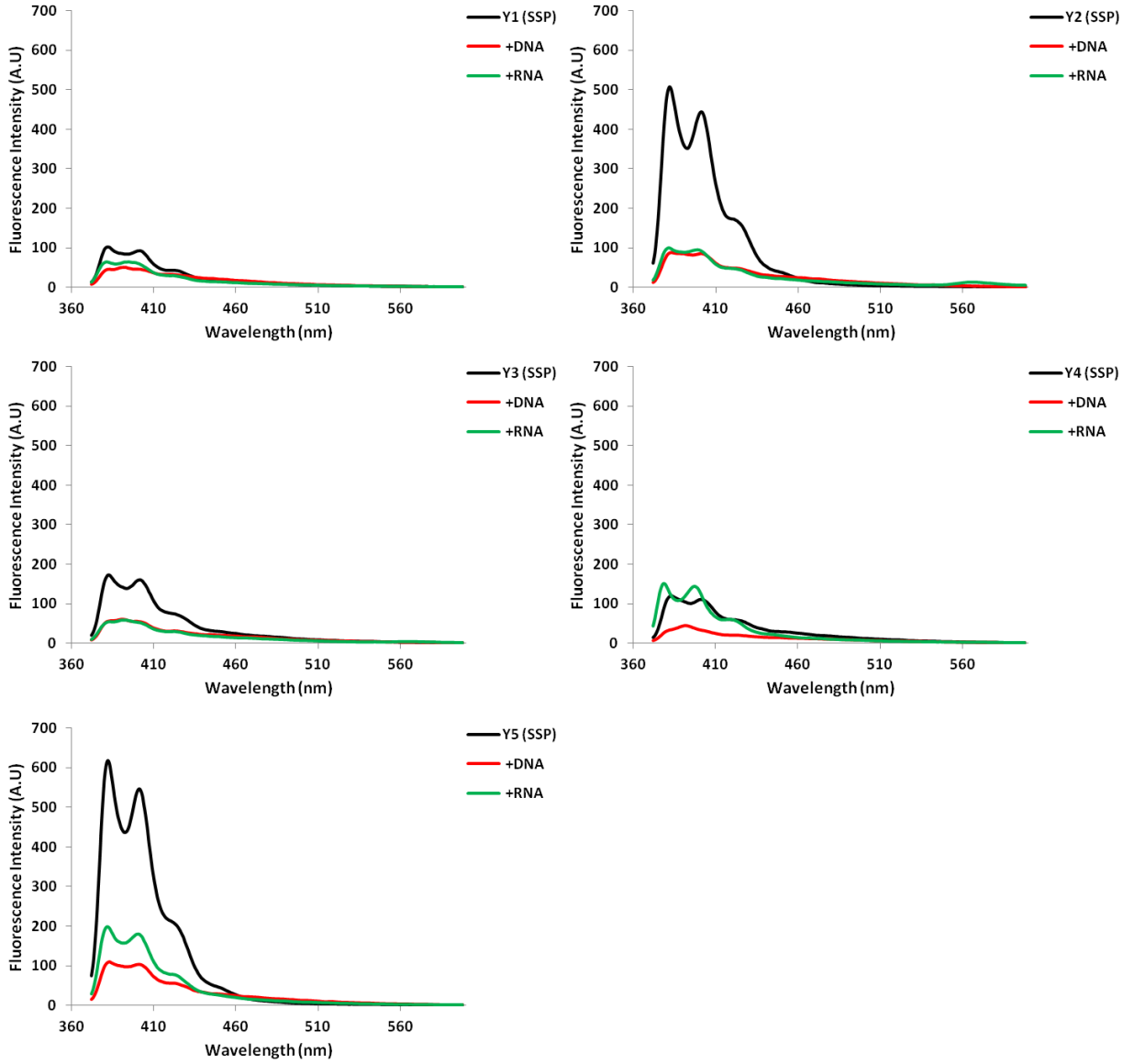


Figure S6. Steady-state fluorescence emission spectra of **Y**-modified ONs and their corresponding duplexes with DNA/RNA targets. λ_{ex} = 345 nm. For other conditions, see Fig. S5.

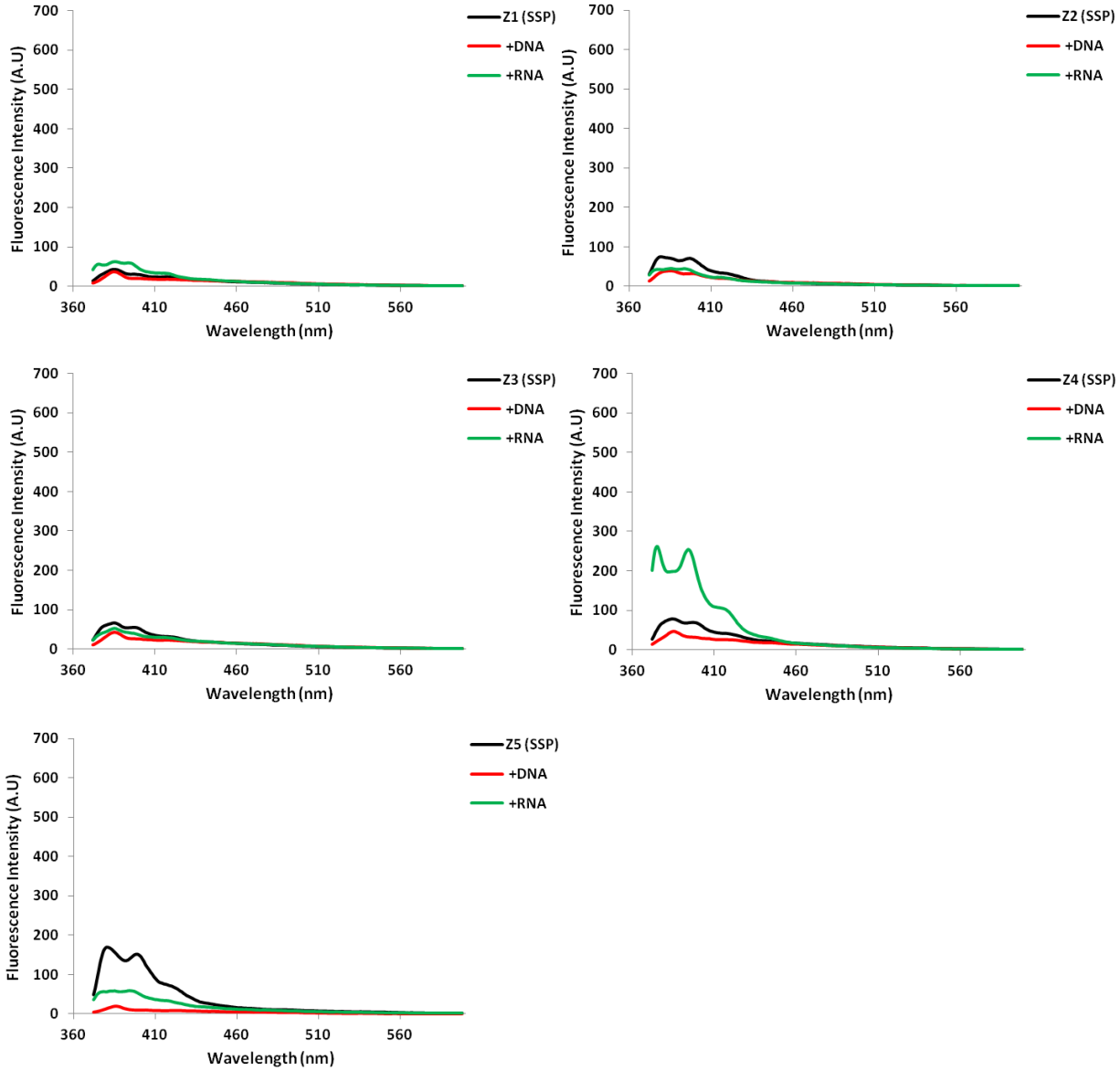


Figure S7. Steady-state fluorescence emission spectra of **Z**-modified ONs and their corresponding duplexes with DNA/RNA targets. λ_{ex} = 340 nm. For other conditions, see Fig. S5.

Table S5. Change in enthalpy upon duplex formation (ΔH) and change in enthalpy upon probe recognition of iso-sequential dsDNA target **D1**:**D4** (ΔH_{rec}).[a]

ON	ZP	Sequence	$\Delta H^{293}[\Delta\Delta H^{293}]$ (kJ/mol) upper ON vs cDNA	lower ON vs cDNA	probe duplex	ΔH_{rec} (kJ/mol)
X1 X5	**+4**	5'-G**X**G ATA TGC 3'-CAC TA**X** ACG	-281±4 [+25]	-299±2 [+7]	-279±1 [+27]	+5
X1 X4	**+2**	5'-G**X**G ATA TGC 3'-CAC **X**AT ACG	-281±4 [+25]	-300±7 [+6]	-248±8 [+58]	-27
X2 X5	**+1**	5'-GTG A**X**A TGC 3'-CAC TA**X** ACG	**-305±1 [+1]**	**-299±2 [+7]**	**-244±3 [+62]**	**-54**
X2 X4	**-1**	5'-GTG A**X**A TGC 3'-CAC **X**AT ACG	-305±1 [+1]	-300±7 [+6]	-296±6 [+10]	-3
X3 X5	**-1**	5'-GTG ATA **X**GC 3'-CAC TA**X** ACG	-270±7 [+36]	-299±2 [+7]	-280±5 [+26]	+17
X3 X4	**-3**	5'-GTG ATA **X**GC 3'-CAC **X**AT ACG	-270±7 [+36]	-300±7 [+6]	-309±4 [-3]	+45
Y1 Y5	**+4**	5'-G**Y**G ATA TGC 3'-CAC TA**Y** ACG	-292±14 [+14]	-306±15 [±0]	-277±2 [+29]	-15
Y1 Y4	**+2**	5'-G**Y**G ATA TGC 3'-CAC **Y**AT ACG	-292±14 [+14]	-271±20 [+35]	-238±3 [+68]	-19
Y2 Y5	**+1**	5'-GTG A**Y**A TGC 3'-CAC TA**Y** ACG	**-309±10 [-3]**	**-306±15 [±0]**	**-257±5 [+49]**	**-52**
Y2 Y4	**-1**	5'-GTG A**Y**A TGC 3'-CAC **Y**AT ACG	-309±10 [-3]	-271±20 [+35]	-307±6 [+1]	+31
Y3 Y5	**-1**	5'-GTG ATA **Y**GC 3'-CAC TA**Y** ACG	-317±10 [-11]	-306±15 [±0]	-288±2 [+18]	-29
Y3 Y4	**-3**	5'-GTG ATA **Y**GC 3'-CAC **Y**AT ACG	-317±10 [-11]	-271±20 [+35]	-276±4 [+30]	-6
Z1 Z5	**+4**	5'-G**Z**G ATA TGC 3'-CAC TA**Z** ACG	-309±8 [-3]	-253±26 [+53]	-252±4 [+54]	-4
Z1 Z4	**+2**	5'-G**Z**G ATA TGC 3'-CAC **Z**AT ACG	-309±8 [-3]	-332±7 [-26]	-251±5 [+55]	-84
Z2 Z5	**+1**	5'-GTG A**Z**A TGC 3'-CAC TA**Z** ACG	**-260±8 [+46]**	**-253±26 [+53]**	**N/A**	-
Z2 Z4	**-1**	5'-GTG A**Z**A TGC 3'-CAC **Z**AT ACG	-260±8 [+46]	-332±7 [-26]	-283±9 [+23]	-3
Z3 Z5	**-1**	5'-GTG ATA **Z**GC 3'-CAC TA**Z** ACG	-280±4 [+26]	-253±26 [+53]	-251±2 [+55]	+24
Z3 Z4	**-3**	5'-GTG ATA **Z**GC 3'-CAC **Z**AT ACG	-280±4 [+26]	-332±7 [-26]	-247±1 [+59]	-59

[a] $\Delta\Delta H$ is measured relative to ΔH for **D1**:**D4 =** -306 kJ/mol. $\Delta H_{rec} = \Delta H$ (upper strand vs cDNA) + ΔH (lower strand vs cDNA) - ΔH (probe duplex) - ΔH (dsDNA target). "±" denotes standard deviation. N/A = the lack of a clear lower base line prevented determination of this value.

Table S6. Change in entropy at 293K upon duplex formation ($-T^{293}\Delta S$) and change in entropy upon probe recognition of iso-sequential dsDNA target **D1**:**D4** ($-T^{293}\Delta S_{rec}$).[a]

Duplex	Zipper	Sequence	$-T^{293}\Delta S$ [$\Delta(T^{293}\Delta S)$] (kJ/mol)			$-T^{293}\Delta S_{rec}$ (kJ/mol)
			upper strand vs cDNA	lower strand vs cDNA	probe duplex	
X1 **X5**	**+4**	5'-G**X**G ATA TGC 3'-CAC TA**X** ACG	235±3 [-30]	247±6 [-18]	224±1 [-41]	-7
X1 **X4**	**+2**	5'-G**X**G ATA TGC 3'-CAC **X**AT ACG	235±3 [-30]	254±6 [-11]	203±8 [-62]	+21
X2 **X5**	**+1**	5'-GTG A**X**A TGC 3'-CAC TA**X** ACG	**251±1 [-14]**	**247±6 [-18]**	**204±3 [-61]**	**+29**
X2 **X4**	**-1**	5'-GTG A**X**A TGC 3'-CAC **X**AT ACG	251±1 [-14]	254±6 [-11]	245±5 [-20]	-5
X3 **X5**	**-1**	5'-GTG ATA **X**GC 3'-CAC TA**X** ACG	220±7 [-45]	247±6 [-18]	225±5 [-40]	-23
X3 **X4**	**-3**	5'-GTG ATA **X**GC 3'-CAC **X**AT ACG	220±7 [-45]	254±6 [-11]	256±3 [-9]	-47
Y1 **Y5**	**+4**	5'-G**Y**G ATA TGC 3'-CAC TA**Y** ACG	244±14 [-21]	251±14 [-14]	221±2 [-44]	+9
Y1 **Y4**	**+2**	5'-G**Y**G ATA TGC 3'-CAC **Y**AT ACG	244±14 [-21]	225±20 [-40]	193±3 [-72]	-3
Y2 **Y5**	**+1**	5'-GTG A**Y**A TGC 3'-CAC TA**Y** ACG	**253±10 [-12]**	**251±14 [-14]**	**214±5 [-51]**	**+25**
Y2 **Y4**	**-1**	5'-GTG A**Y**A TGC 3'-CAC **Y**AT ACG	253±10 [-12]	225±20 [-40]	252±5 [-13]	-39
Y3 **Y5**	**-1**	5'-GTG ATA **Y**GC 3'-CAC TA**Y** ACG	266±11 [+1]	251±14 [-14]	230±2 [-35]	+22
Y3 **Y4**	**-3**	5'-GTG ATA **Y**GC 3'-CAC **Y**AT ACG	266±11 [+1]	225±20 [-40]	223±4 [-42]	+3
Z1 **Z5**	**+4**	5'-G**Z**G ATA TGC 3'-CAC TA**Z** ACG	265±8 [±0]	205±24 [-60]	203±3 [-62]	+2
Z1 **Z4**	**+2**	5'-G**Z**G ATA TGC 3'-CAC **Z**AT ACG	265±8 [±0]	291±7 [+26]	210±5 [-55]	+81
Z2 **Z5**	**+1**	5'-GTG A**Z**A TGC 3'-CAC TA**Z** ACG	**212±8 [-53]**	**205±24 [-60]**	**N/A**	**N/A**
Z2 **Z4**	**-1**	5'-GTG A**Z**A TGC 3'-CAC **Z**AT ACG	212±8 [-53]	291±7 [+26]	242±11 [-23]	-4
Z3 **Z5**	**-1**	5'-GTG ATA **Z**GC 3'-CAC TA**Z** ACG	234±3 [-31]	205±24 [-60]	205±2 [-60]	-31
Z3 **Z4**	**-3**	5'-GTG ATA **Z**GC 3'-CAC **Z**AT ACG	234±3 [-31]	291±7 [+26]	205±1 [-60]	+55

[a] $\Delta(T^{293}\Delta S)$ is measured relative to $-T^{293}\Delta S$ for **D1**:**D4** = 265 kJ/mol. $-T^{293}\Delta S_{rec} = T^{293}\Delta S$ (upper strand vs cDNA) + $T^{293}\Delta S$ (lower strand vs cDNA) - $T^{293}\Delta S$ (probe duplex) - $T^{293}\Delta S$ (dsDNA target). "±" denotes standard deviation. N/A = the lack of a clear lower base line prevented determination of this value.

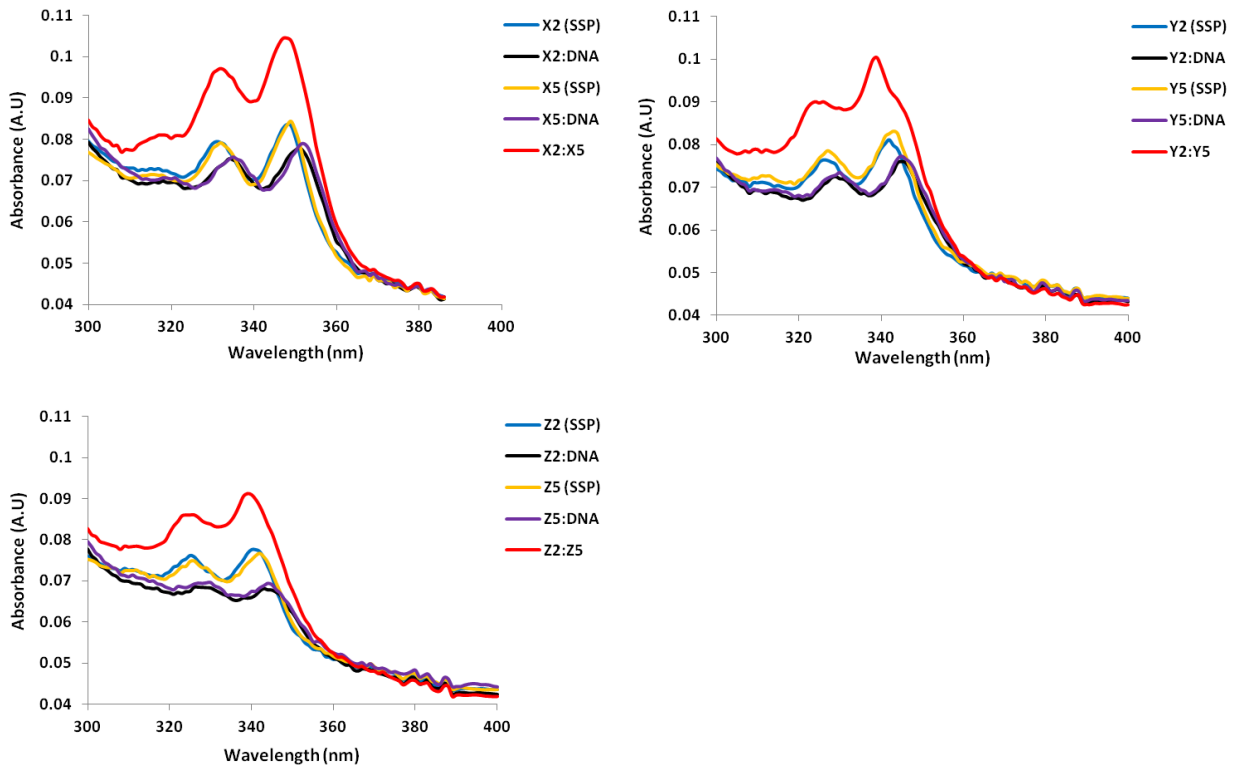


Figure S8. Absorbance spectra of representative Invaders, duplexes between individual probe strands and cDNA, and single-stranded probes (SSP). Spectra were recorded at T = 5 °C using each strand at 1.0 μM concentration in T_m buffer.

Table S7. Pseudorotational phase angles *P* of selected nucleotides in **B2**:**D4** duplexes.

P	**D1**:**D4**			**X2**:**D4**			**Y2**:**D4**			**Z2**:**D4**	
A_4:T_{15}	93	112		96	116		105	110		102	106
B_5:A_{14}	129	111		142	98		148	103		149	175
A_6:B_{13}	102	109		124	97		155	113		171	141
T_7:A_{12}	121	123		90	100		106	102		111	98

Table S8. Pseudorotational phase angles *P* of selected nucleotides in **B2**:**B5** duplexes.

P	**D1**:**D4**			**X2**:**X5**					**Y2**:**Y5**			**Z2**:**Z5**			
				Type A		Type B			Type A			Type A		Type B	
A_4:T_{15}	93	112		70	103	100	104		101	106		98	105	100	106
B_5:A_{14}	129	111		161[a]	122	149	168		148	104		147	107	150	180
A_6:B_{13}	102	109		123	160	176	148		104	147		107	146	173	148[a]
T_7:A_{12}	121	123		104	71	106	116		109	117		105	110	106	111

[a] Not intercalated.

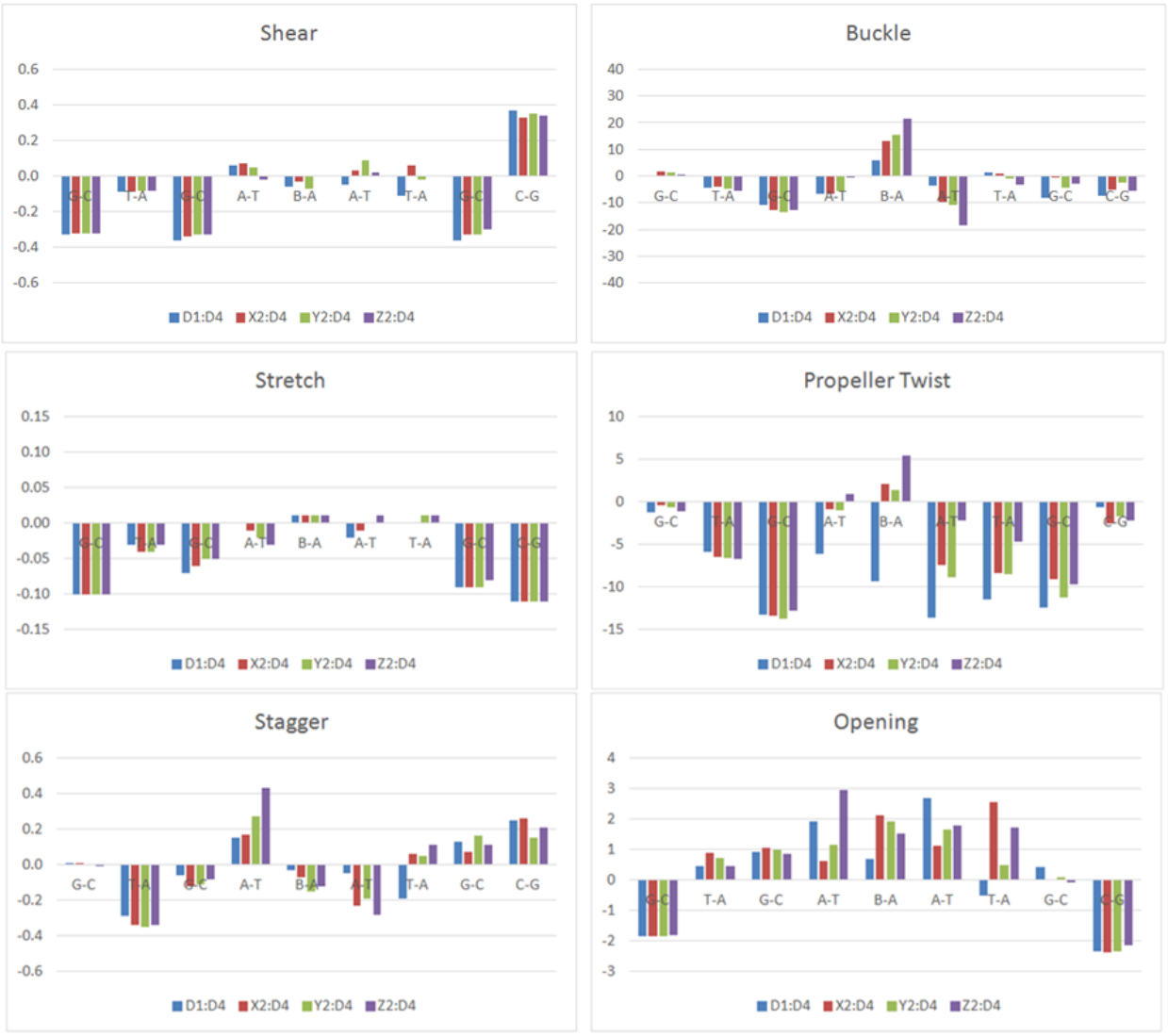


Figure S9. Structural base pair parameters for duplexes **D1**:**D4** and **B2**:**D4**.

Figure S10. Structural dinucleotide step parameters for duplexes **D1**:**D4** and **B2**:**D4**.

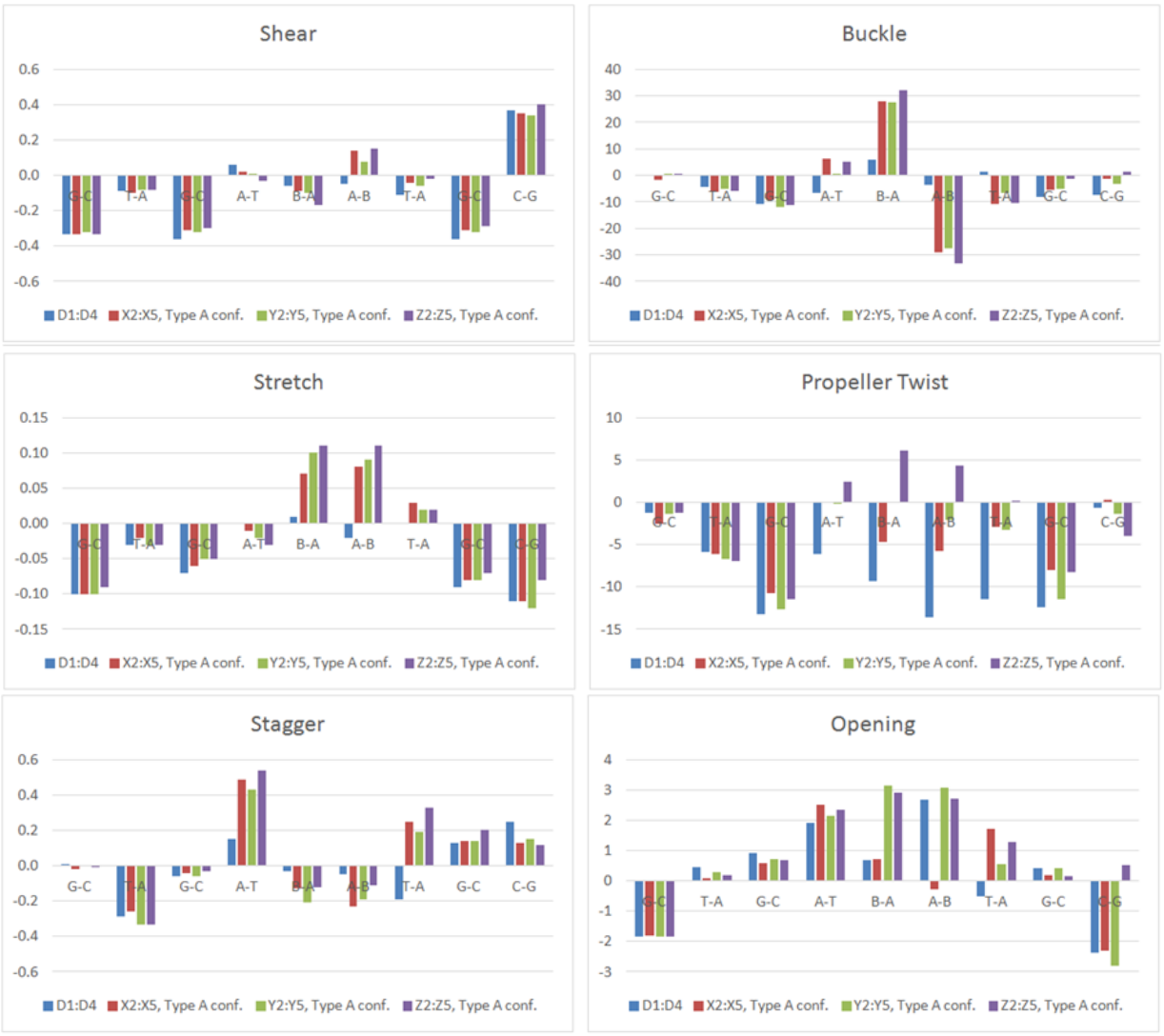


Figure S11. Structural base pair parameters for duplexes **D1**:**D4** and **B2**:**B5** (Type A).

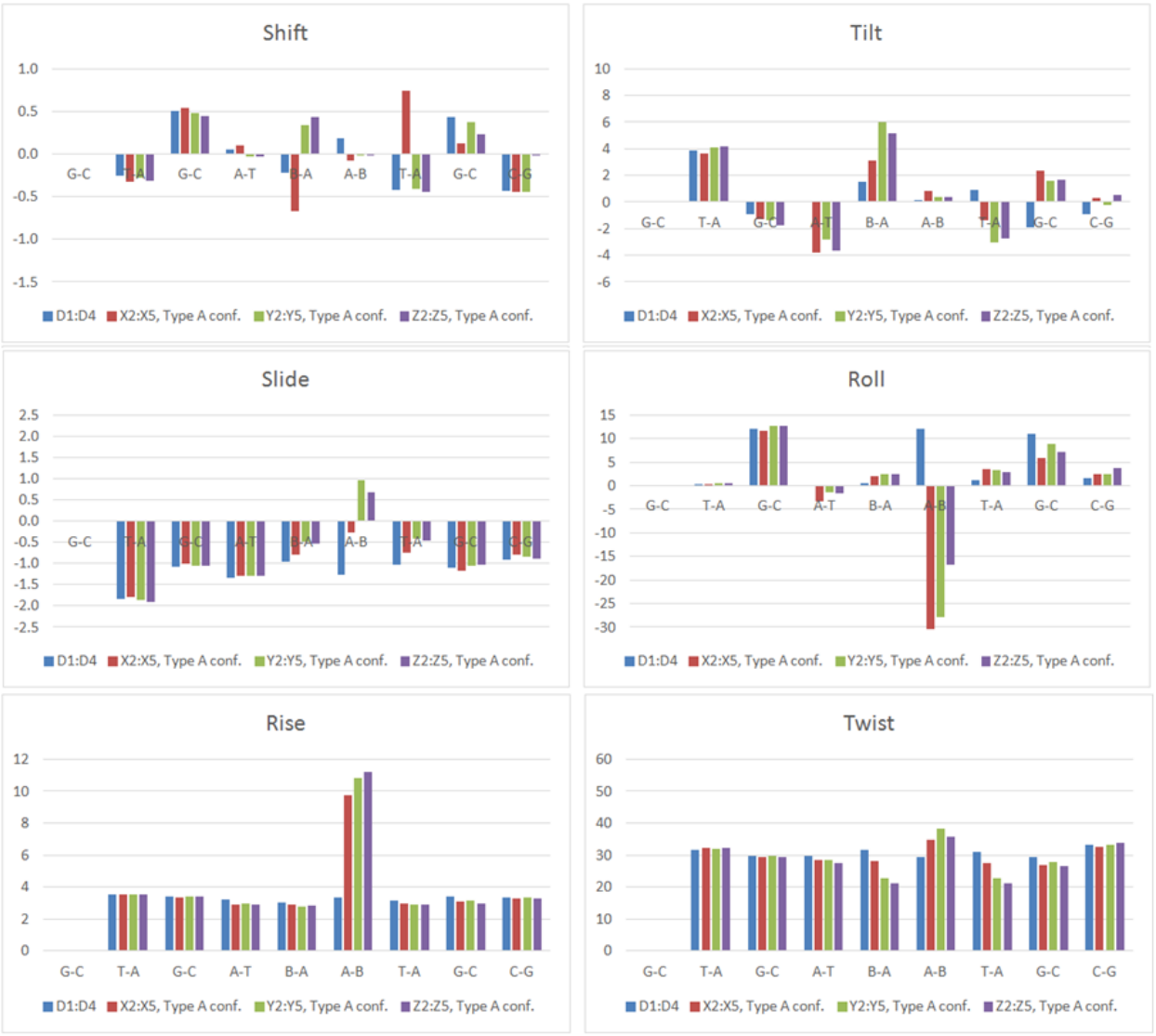


Figure S12. Structural dinucleotide step parameters for duplexes **D1**:**D4** and **B2**:**B5** (Type A).

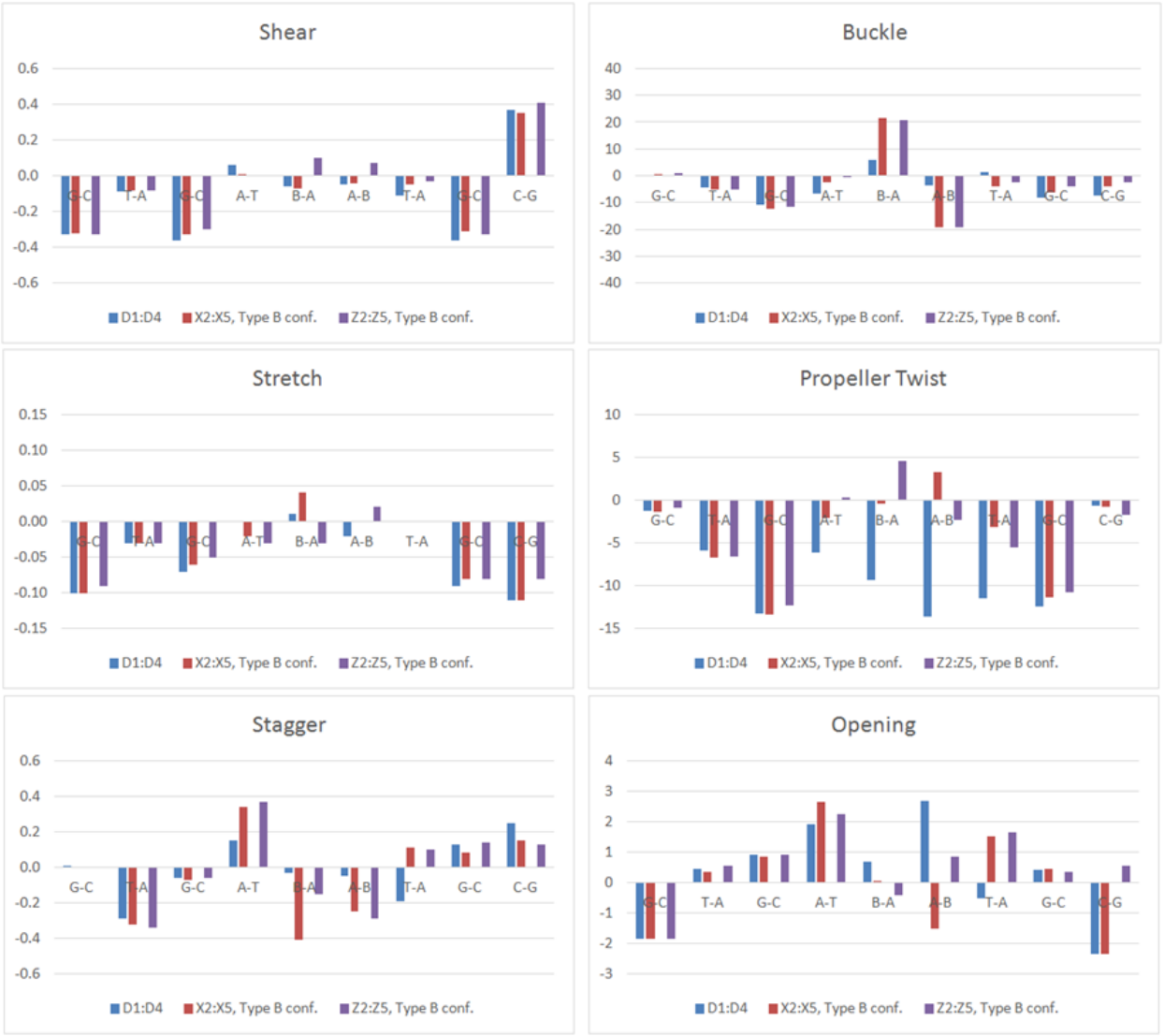


Figure S13. Structural base pair parameters for duplexes **D1**:**D4** and **B2**:**B5** (Type B).

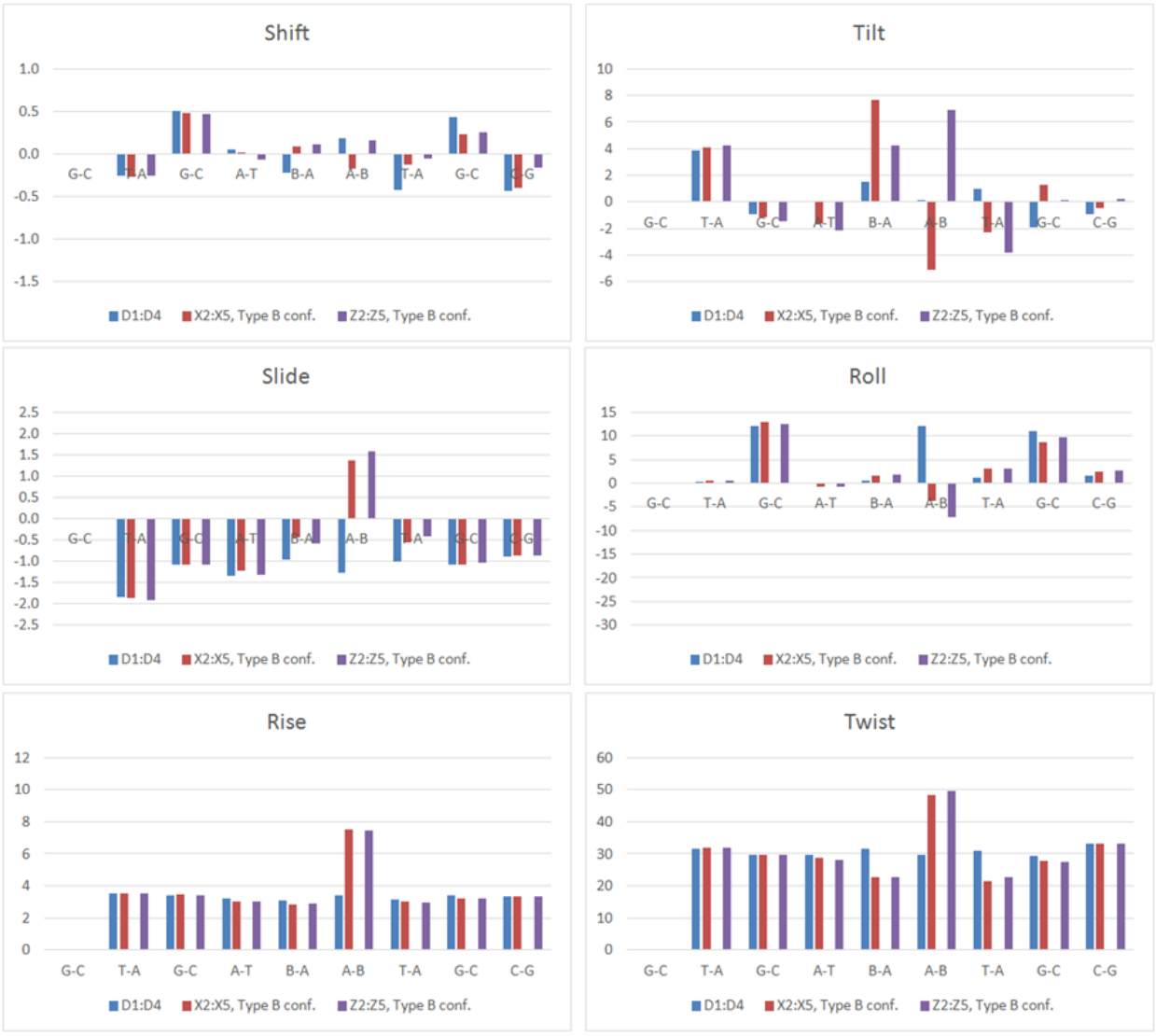


Figure S14. Structural dinucleotide step parameters for duplexes **D1**:**D4** and **B2**:**B5** (Type B).

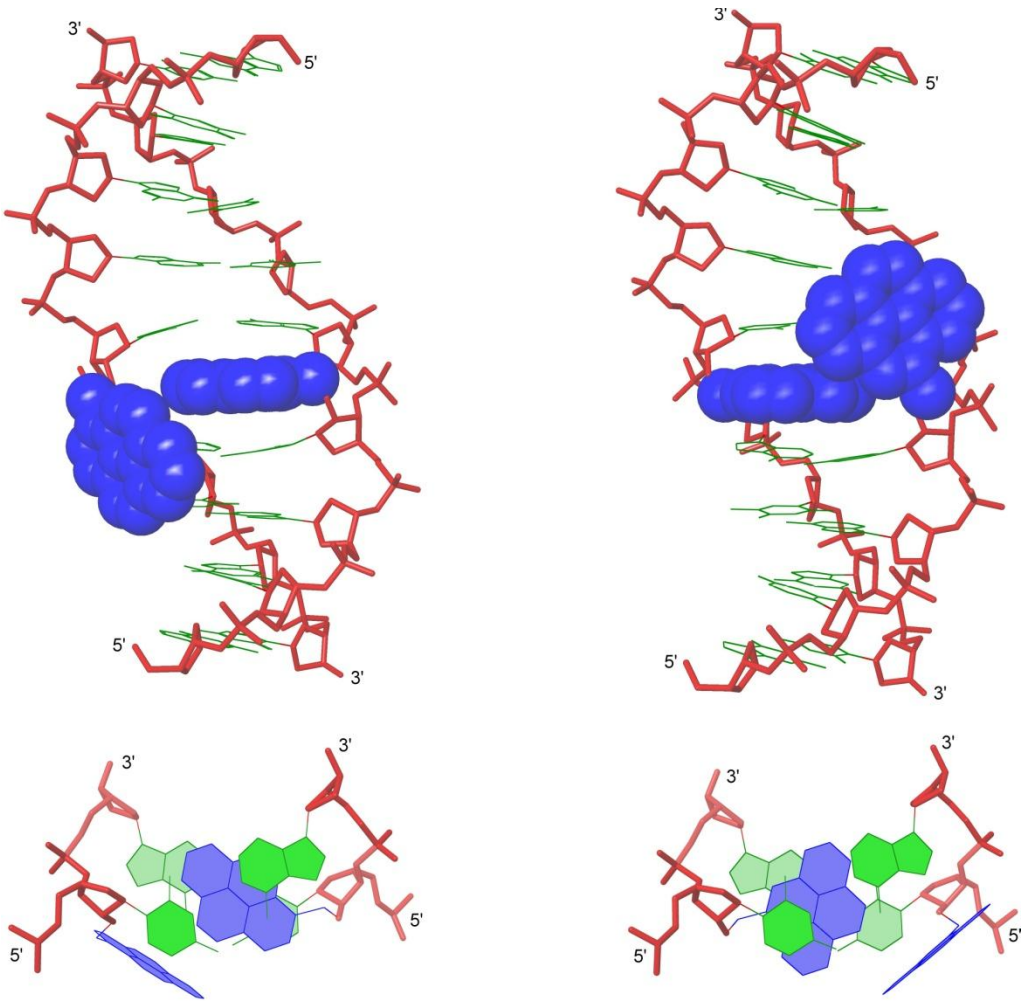
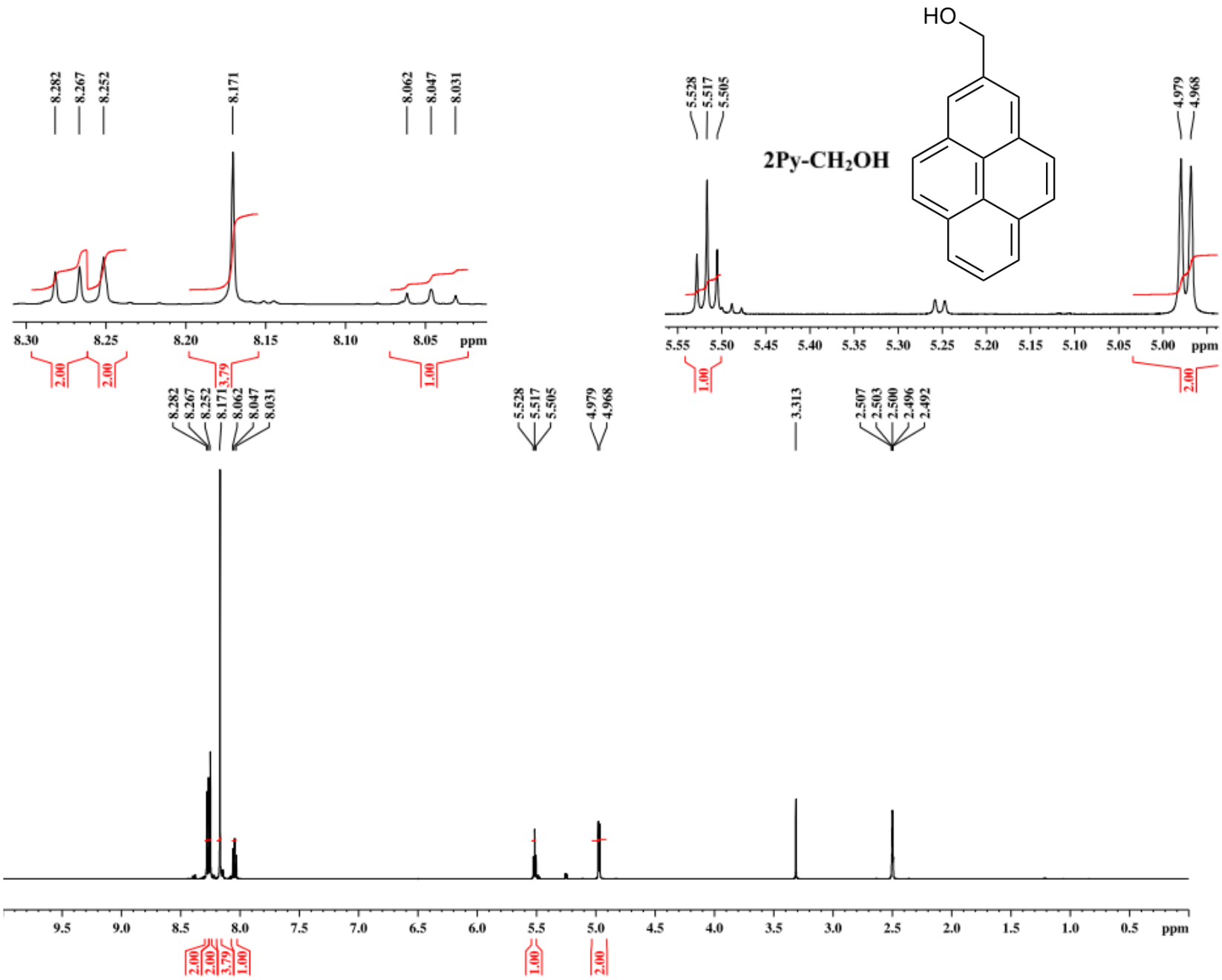
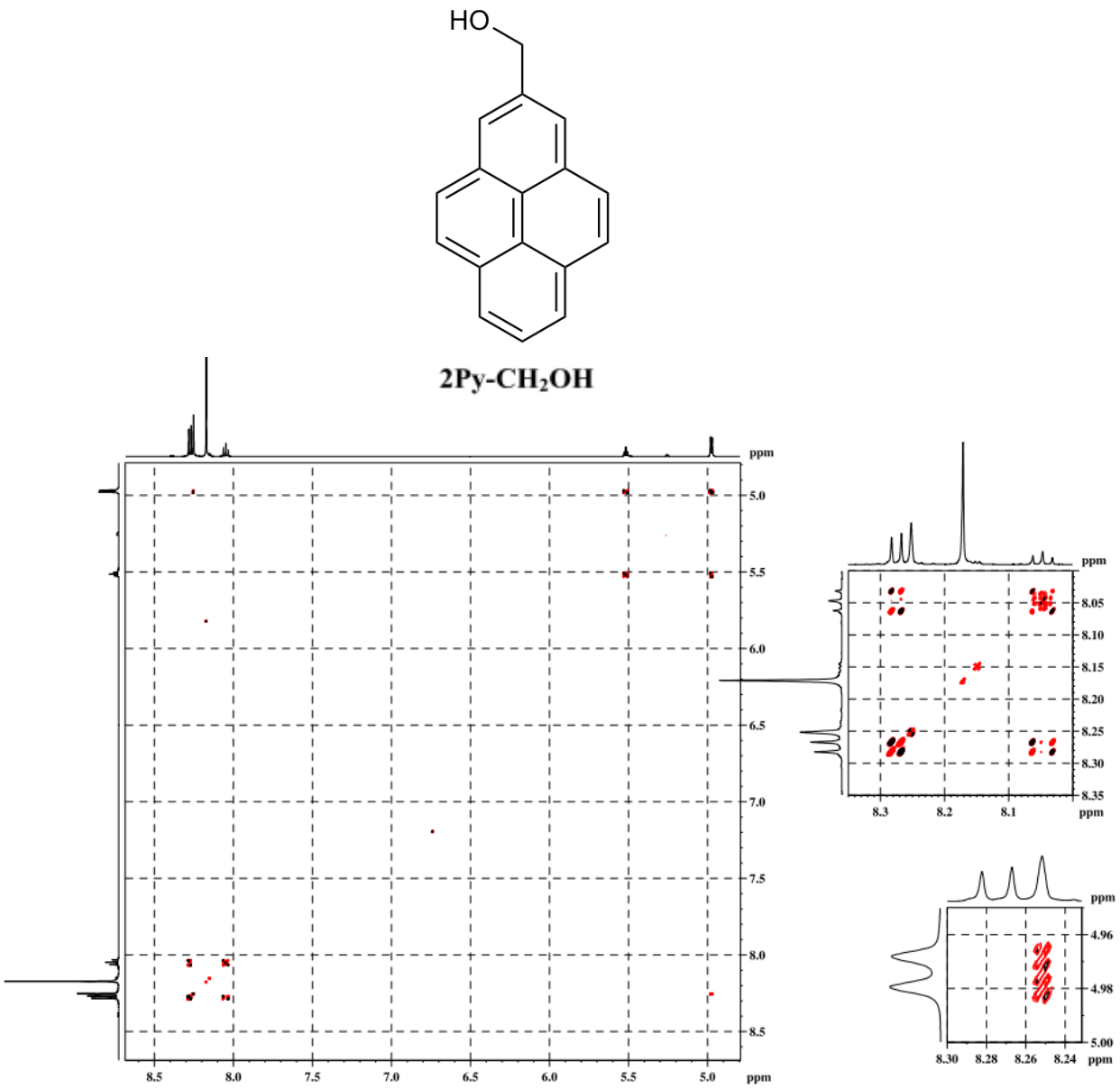


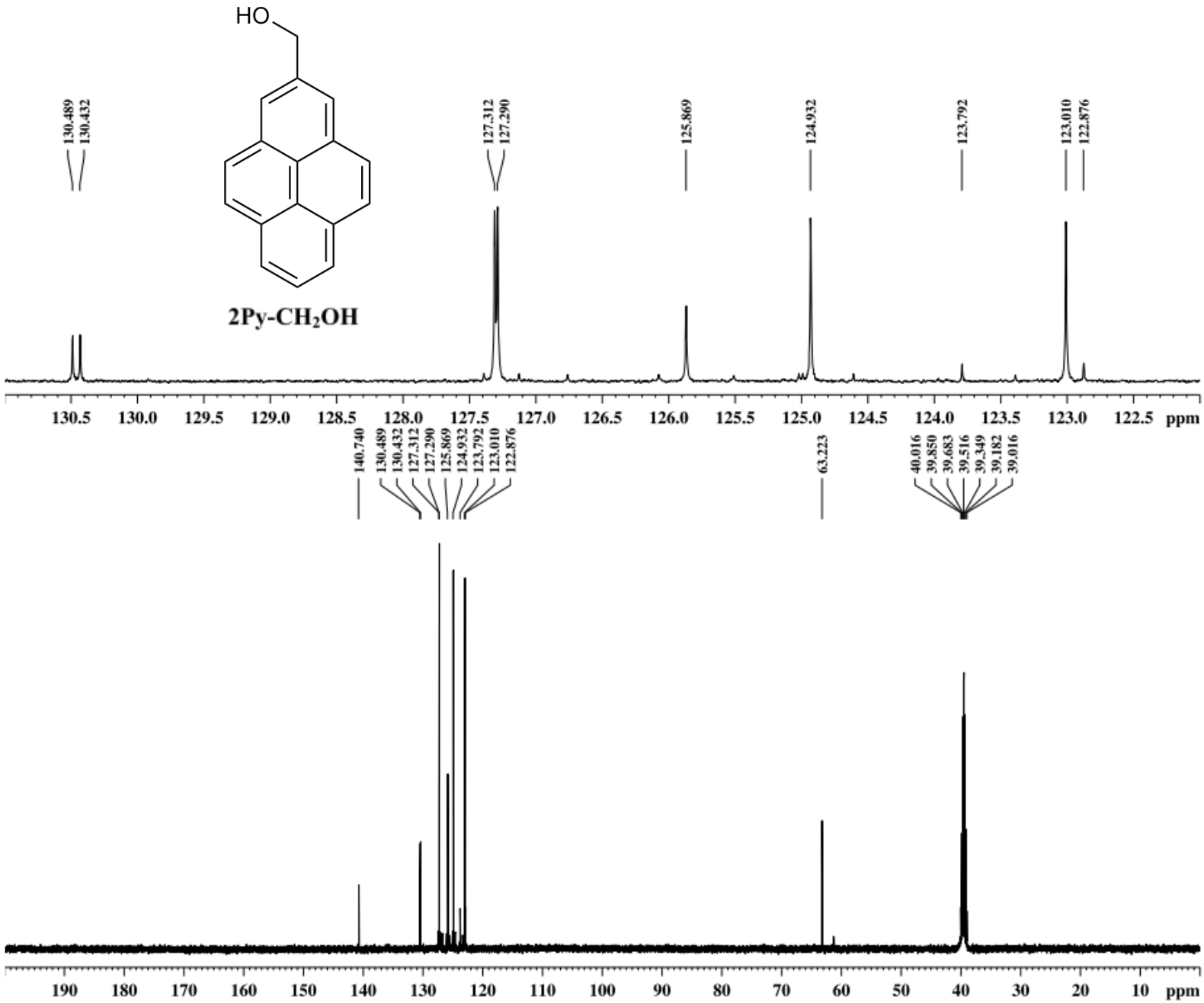
Figure S15. Lowest energy structures of **X2**:**X5** (left) and **Z2**:**Z5** (right) in Type B conformation (**Y2**:**Y5** maintained a Type A conformation throughout the simulation protocol). Top: side view of duplex; bottom: top view of the central duplex region. Color code: sugar phosphate backbone (red); pyrene moieties (blue) and nucleobases (green). Hydrogen atoms, sodium ions and bond orders are omitted for clarity.

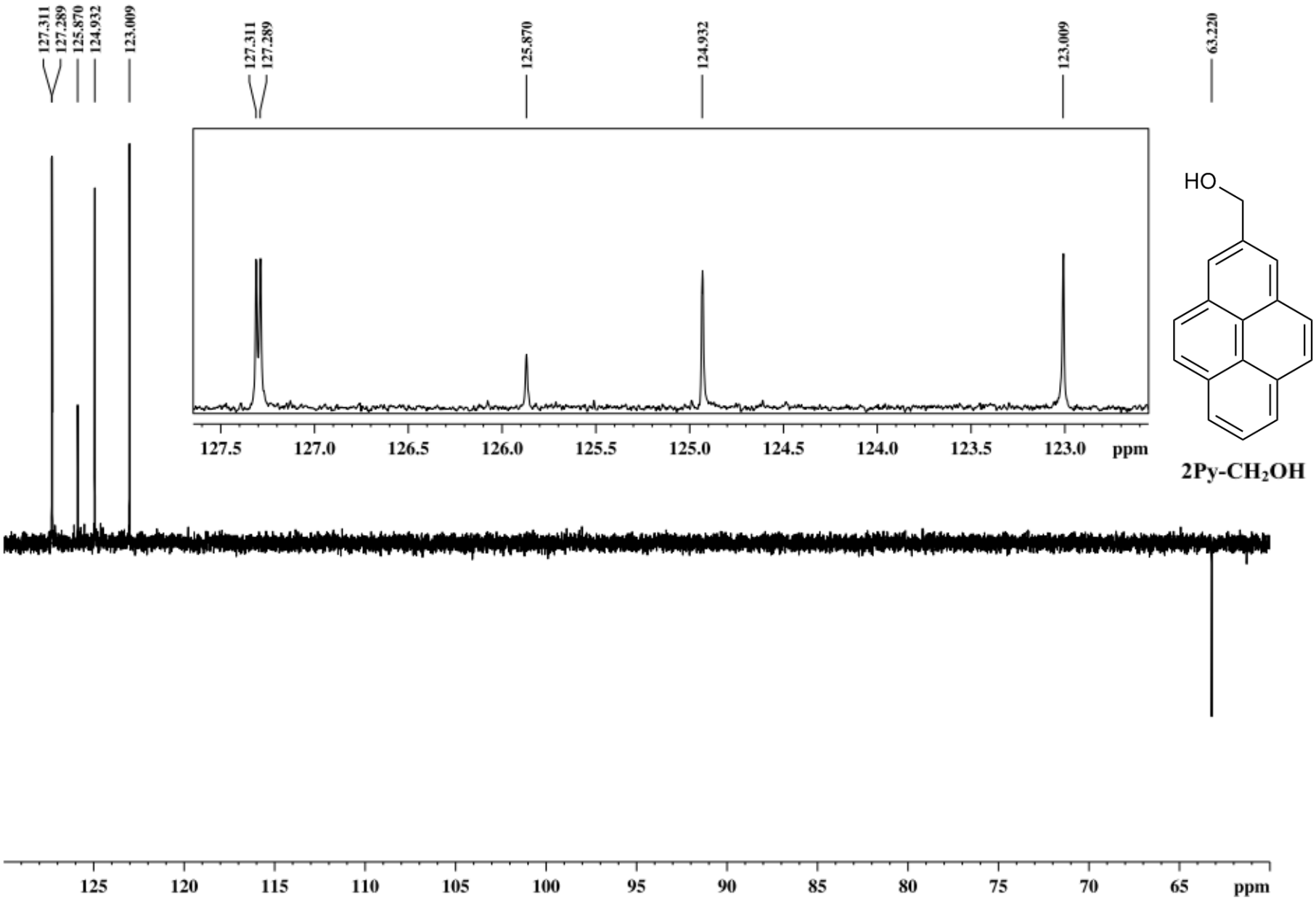
References.

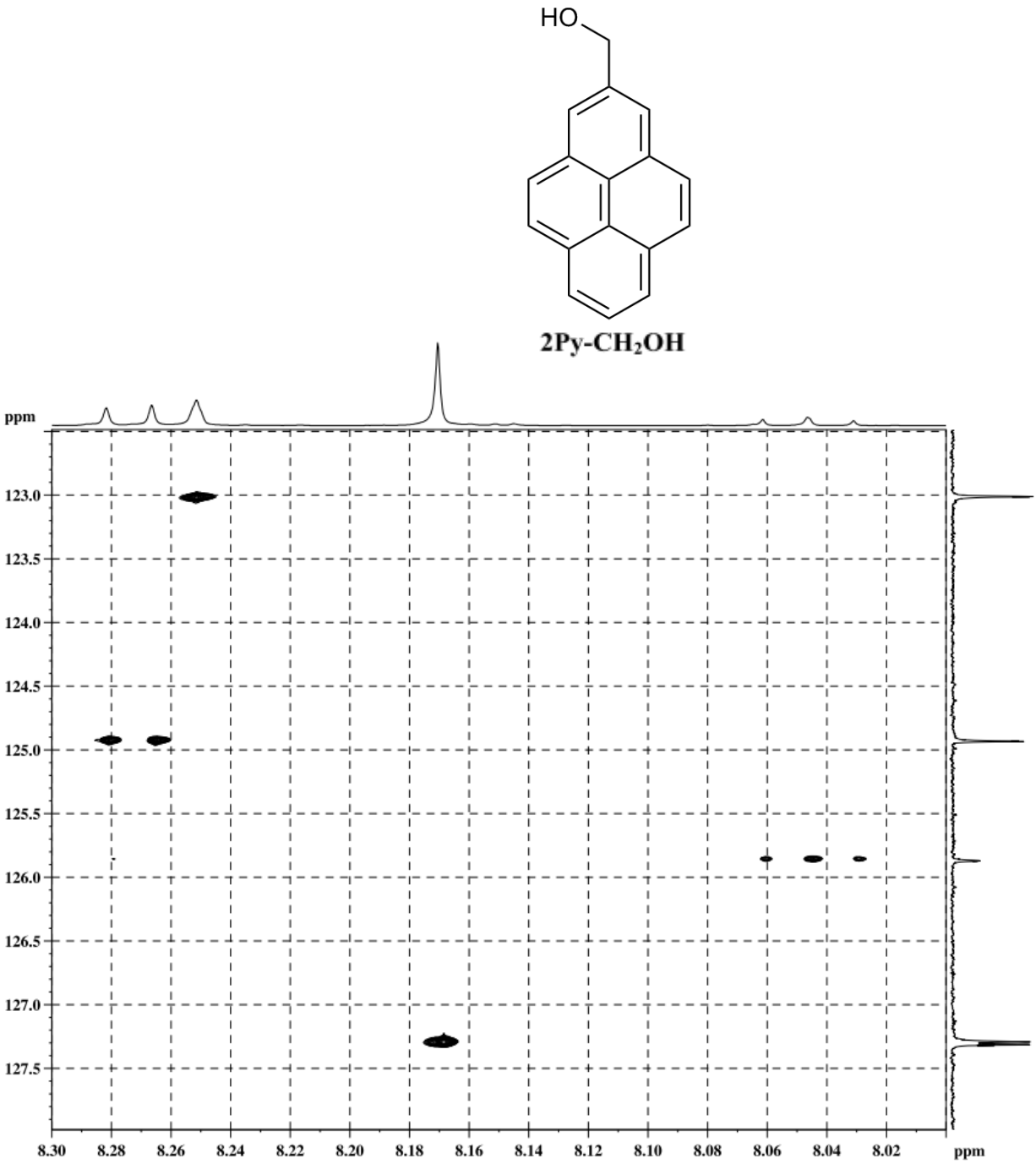
(S1) S. Karmakar, B. A. Anderson, R. L. Rathje, S. Andersen, T. Jensen, P. Nielsen and P. J. Hrdlicka, *J. Org. Chem*., 2011, **76**, 7119-7131.

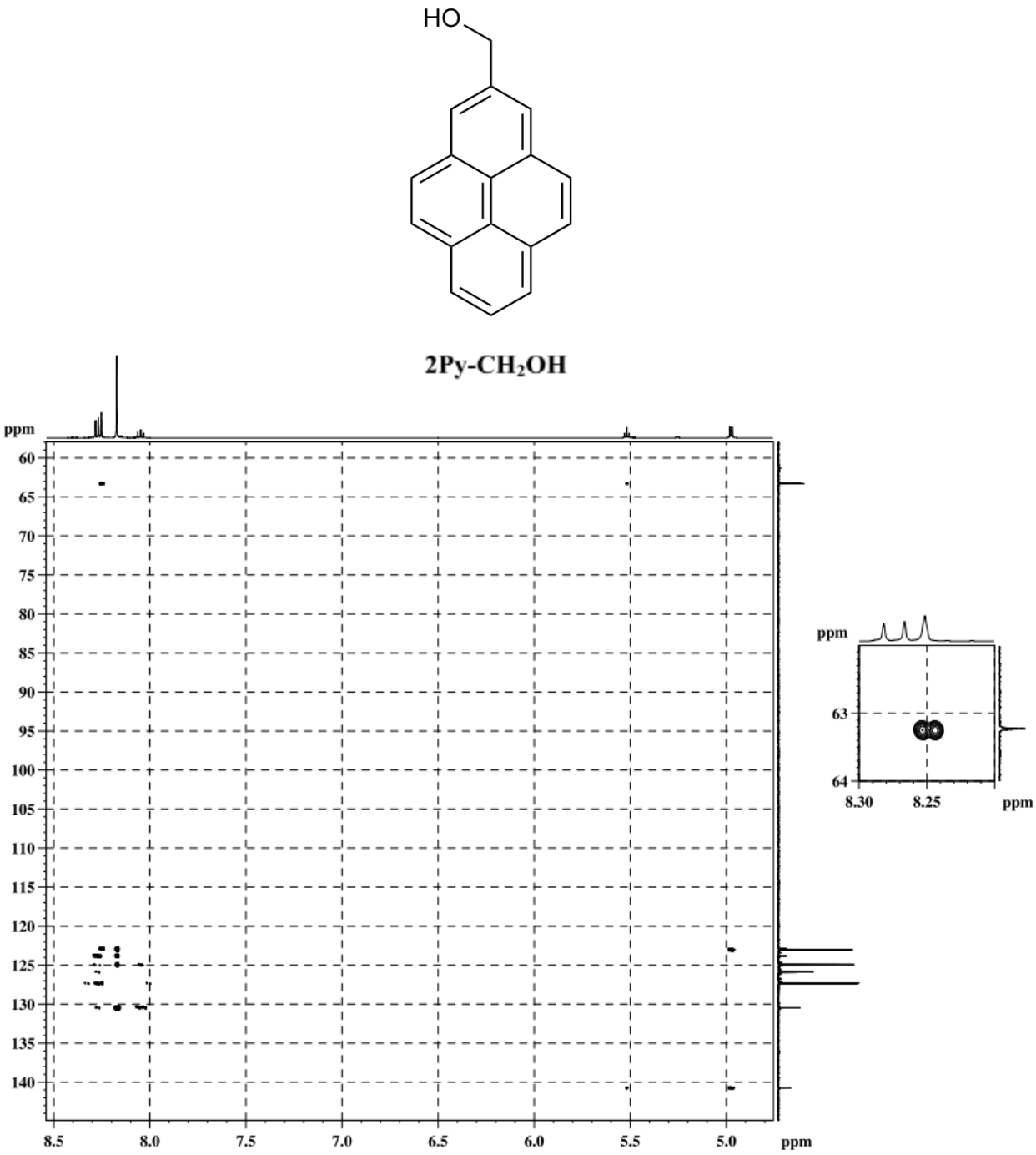

2Py-CH2OH
HO
8.282
8.267
8.252
8.171
8.062
8.047
8.031
5.528
5.517
5.505
4.979
4.968
3.313
2.507
2.503
2.500
2.496
2.492
ppm


HO
2Py-CH2OH
ppm
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
ppm
ppm
8.05
8.10
8.15
8.20
8.25
8.30
8.35
8.3
8.2
8.1
ppm
ppm
4.96
4.98
5.00
8.30
8.28
8.26
8.24
ppm


HO
2Py-CH2OH
130.489
130.432
127.312
127.290
125.869
124.932
123.792
123.010
122.876
130.5 130.0 129.5 129.0 128.5 128.0 127.5 127.0 126.5 126.0 125.5 125.0 124.5 124.0 123.5 123.0 122.5 ppm
140.740
130.489
130.432
127.312
127.290
125.869
124.932
123.792
123.010
122.876
63.223
40.016
39.850
39.683
39.516
39.349
39.182
39.016
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


127.311
127.289
125.870
124.932
123.009
63.220
HO
127.5
127.0
126.5
126.0
125.5
125.0
124.5
124.0
123.5
123.0
ppm
2Py-CH2OH
125
120
115
110
105
100
95
90
85
80
75
70
65
ppm


HO
2Py-CH2OH
ppm
123.0
123.5
124.0
124.5
125.0
125.5
126.0
126.5
127.0
127.5
8.30
8.28
8.26
8.24
8.22
8.20
8.18
8.16
8.14
8.12
8.10
8.08
8.06
8.04
8.02
ppm


HO
2Py-CH2OH
ppm
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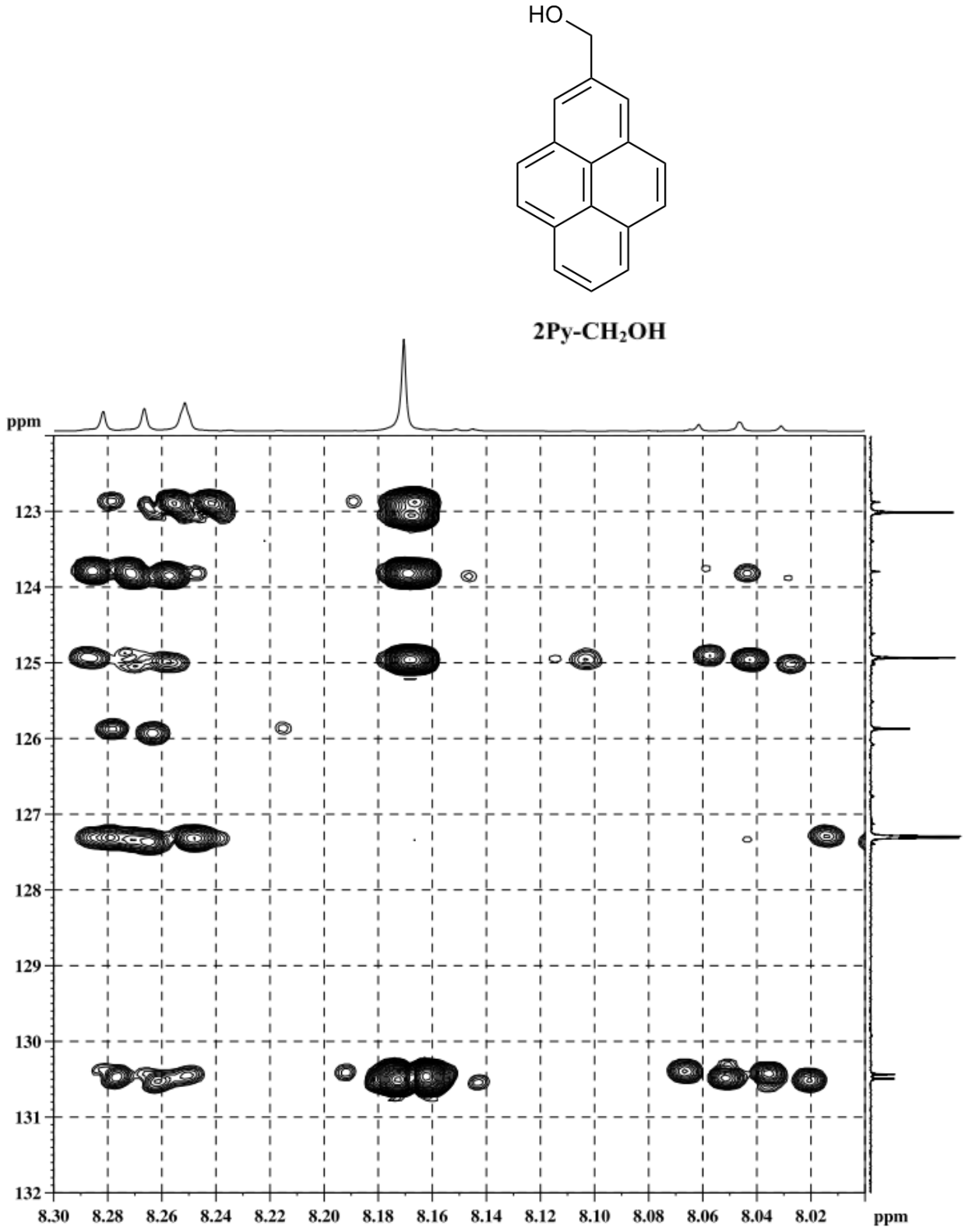
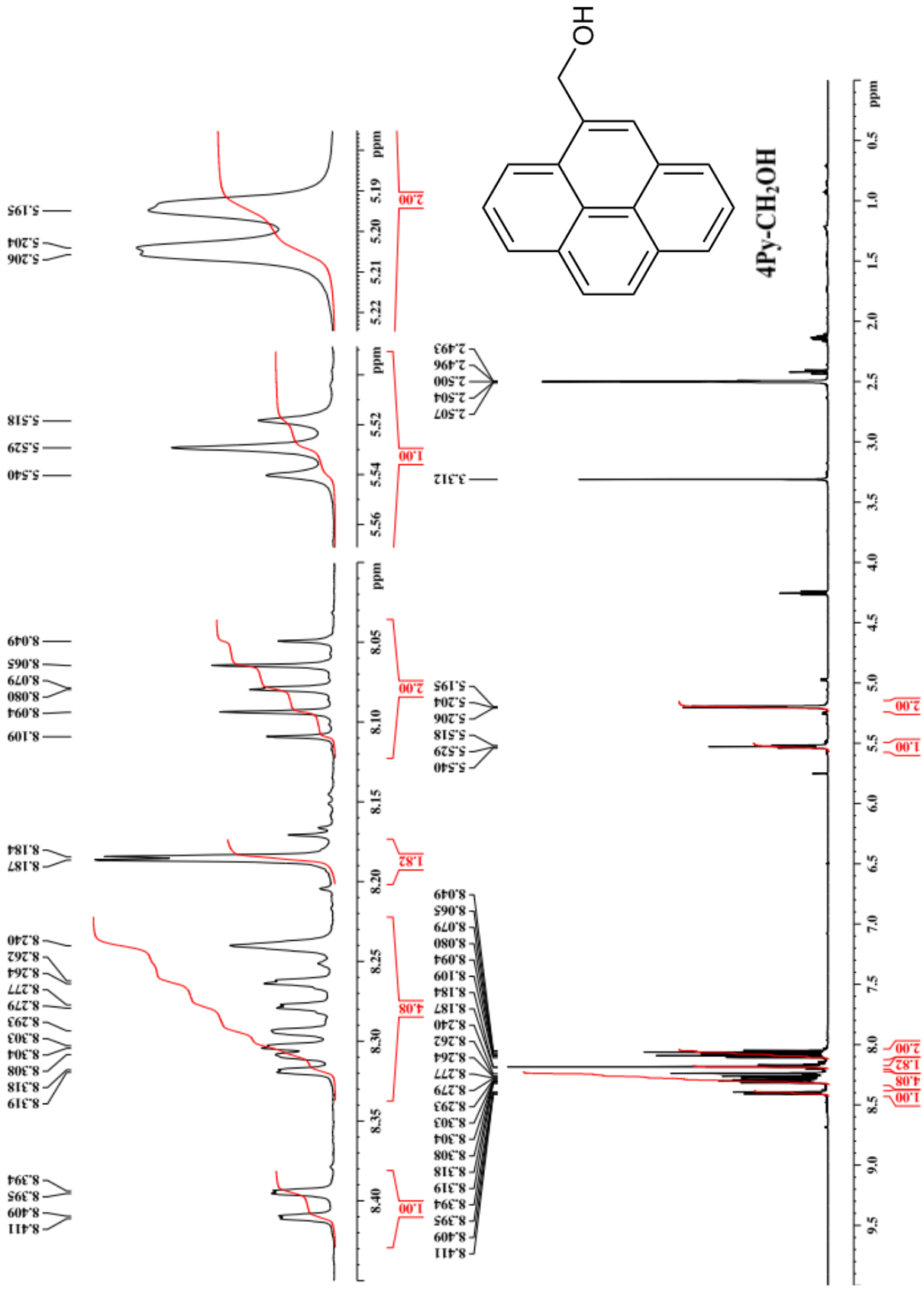
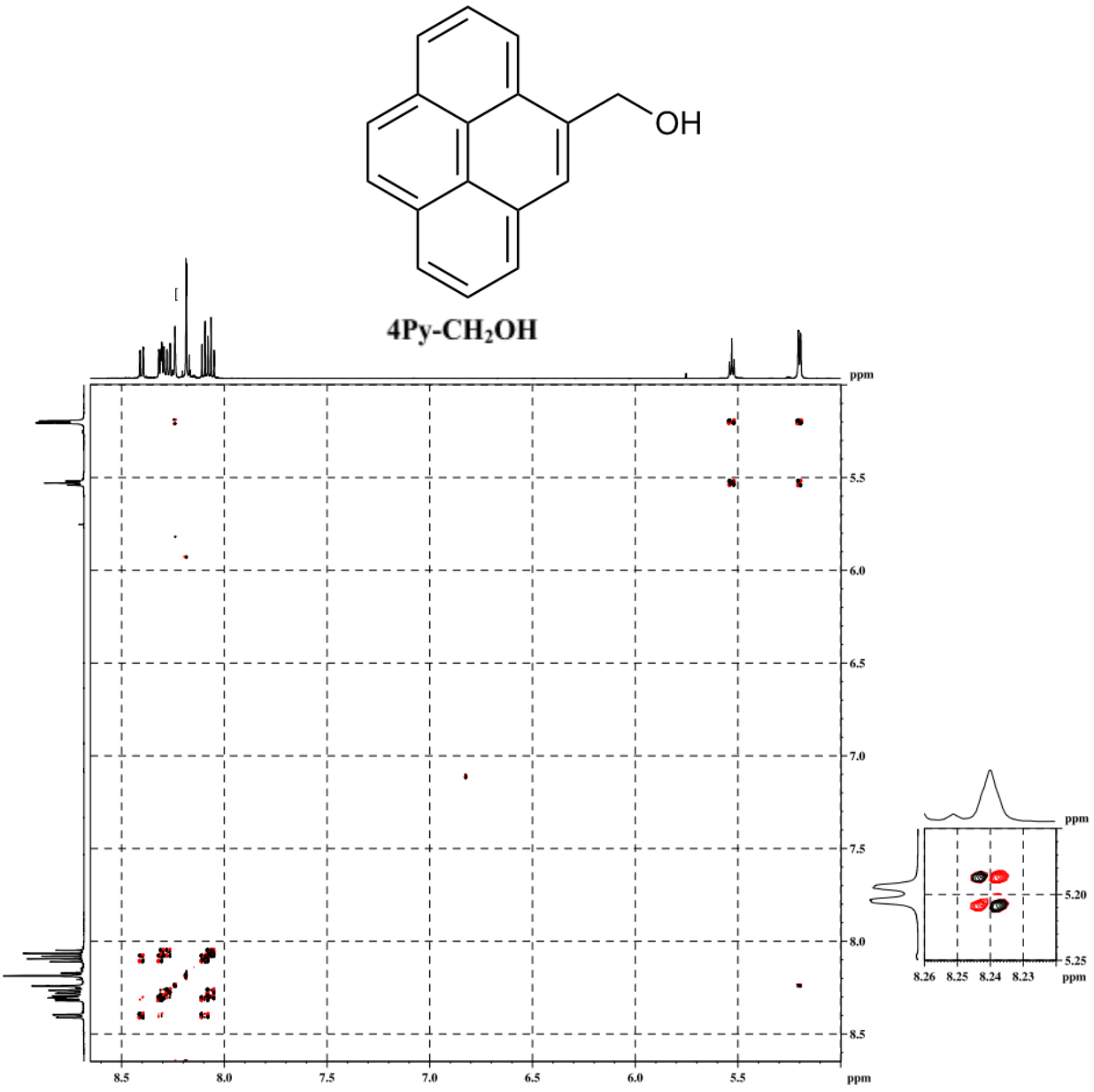
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
ppm
ppm
63
64
8.30
8.25
ppm


HO
2Py-CH2OH
ppm
123
124
125
126
127
128
129
130
131
132
8.30
8.28
8.26
8.24
8.22
8.20
8.18
8.16
8.14
8.12
8.10
8.08
8.06
8.04
8.02
ppm


4Py-CH2OH
OH
ppm


OH
4Py-CH2OH
ppm
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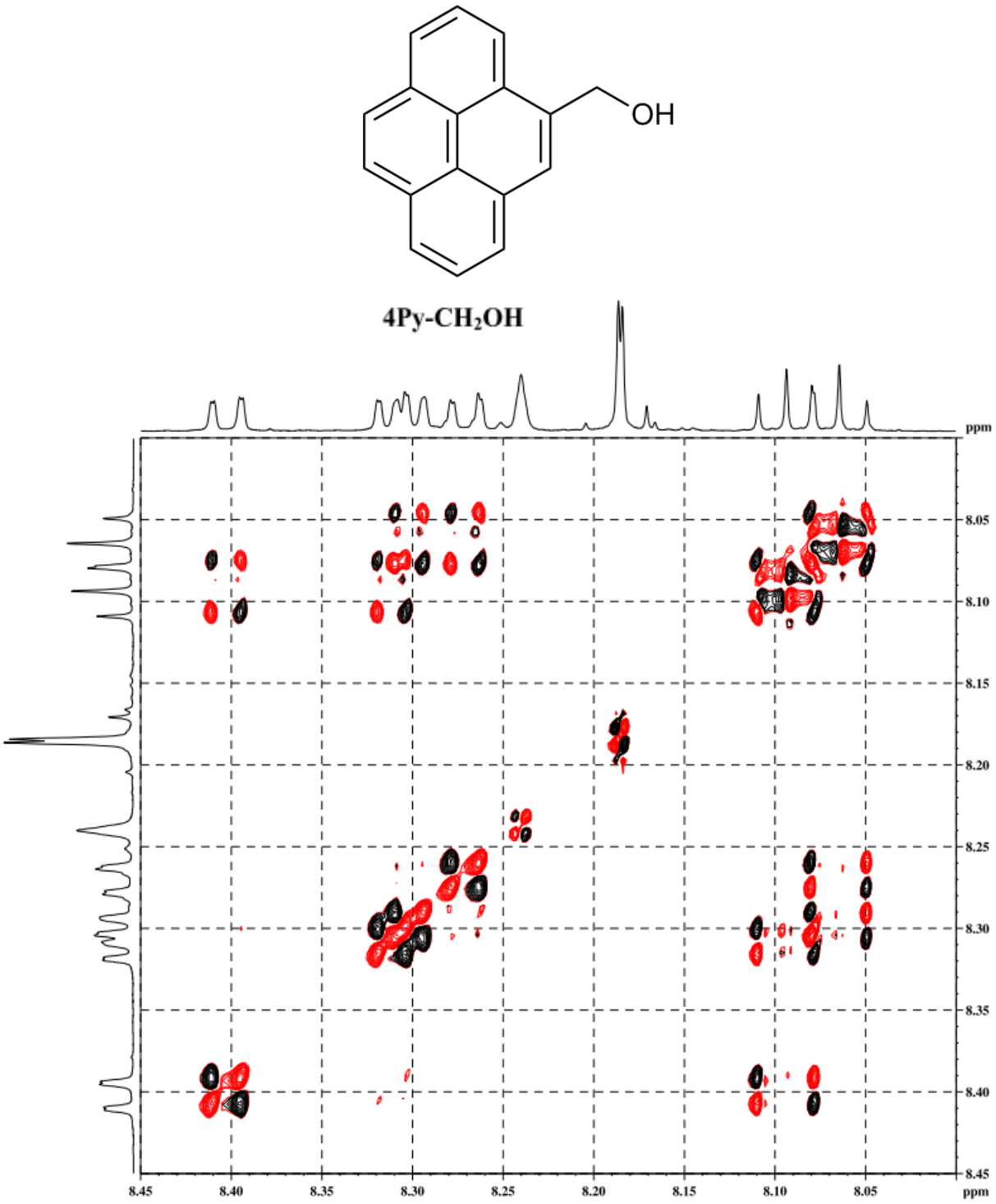
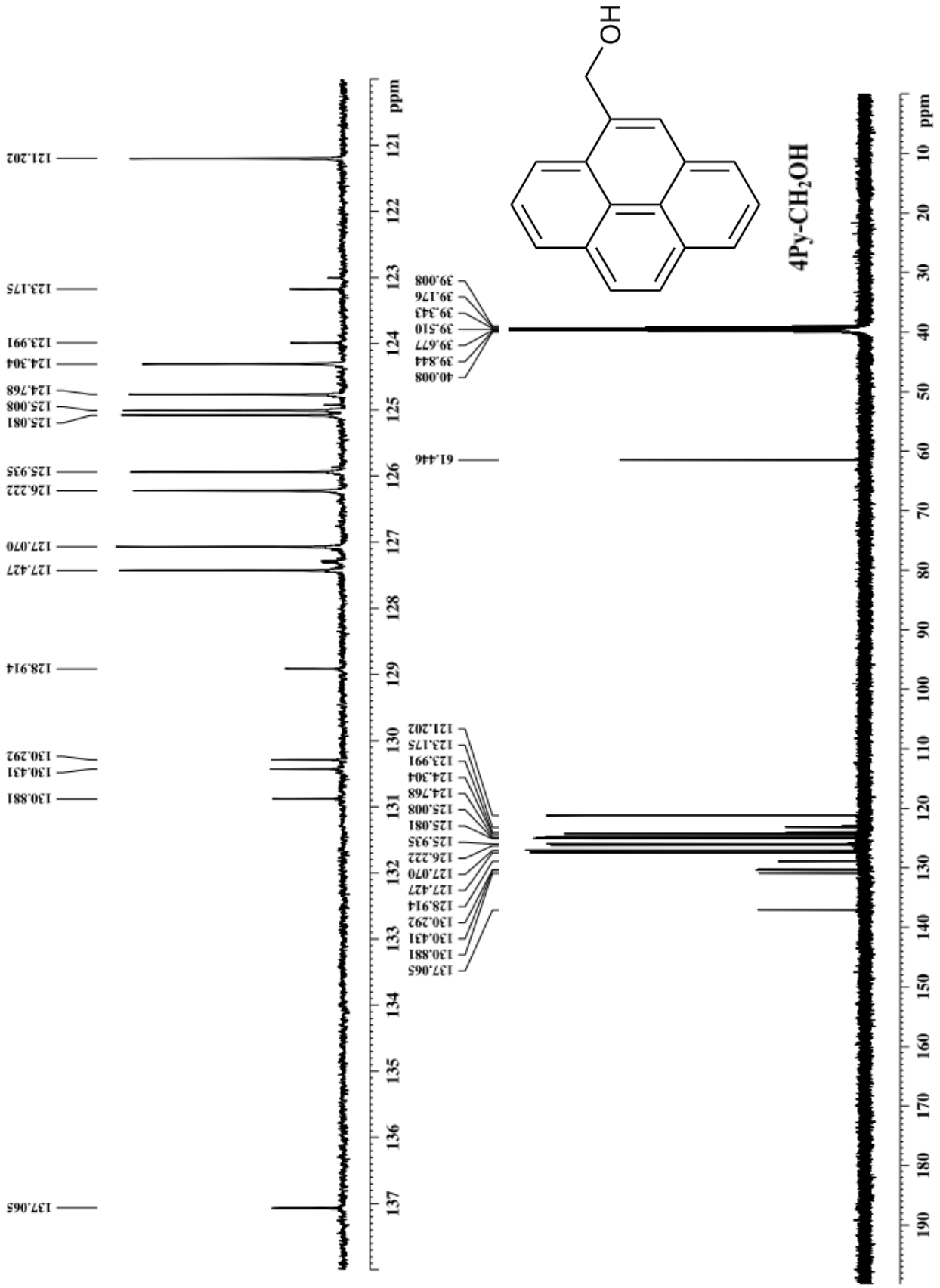
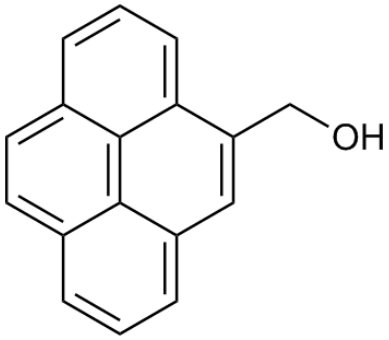
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
ppm
ppm
5.20
5.25
8.26
8.25
8.24
8.23
ppm

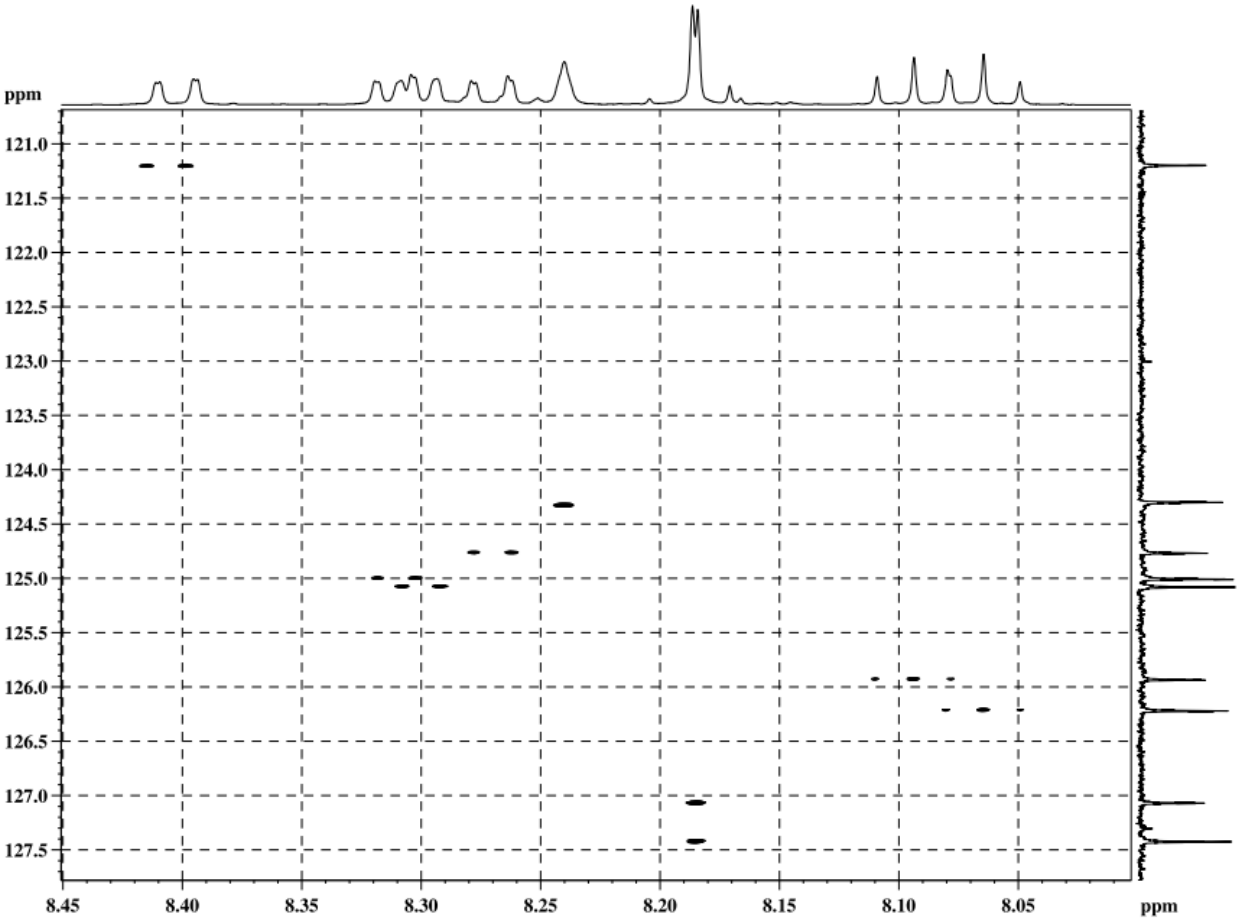

OH
4Py-CH2OH
ppm
8.05
8.10
8.15
8.20
8.25
8.30
8.35
8.40
8.45
8.45
8.40
8.35
8.30
8.25
8.20
8.15
8.10
8.05
ppm


4Py-CH2OH
OH
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
61.446
39.008
39.176
39.343
39.510
39.677
39.844
40.008
121.202
123.175
123.991
124.304
124.768
125.008
125.081
125.935
126.222
127.070
127.427
128.914
130.292
130.431
130.881
137.065

61.443
121.201
124.300
124.768
125.009
125.082
125.936
126.222
127.070
127.426
OH
4Py-CH2OH
125
120
115
110
105
100
95
90
85
80
75
70
65
ppm


OH

4Py-CH_2OH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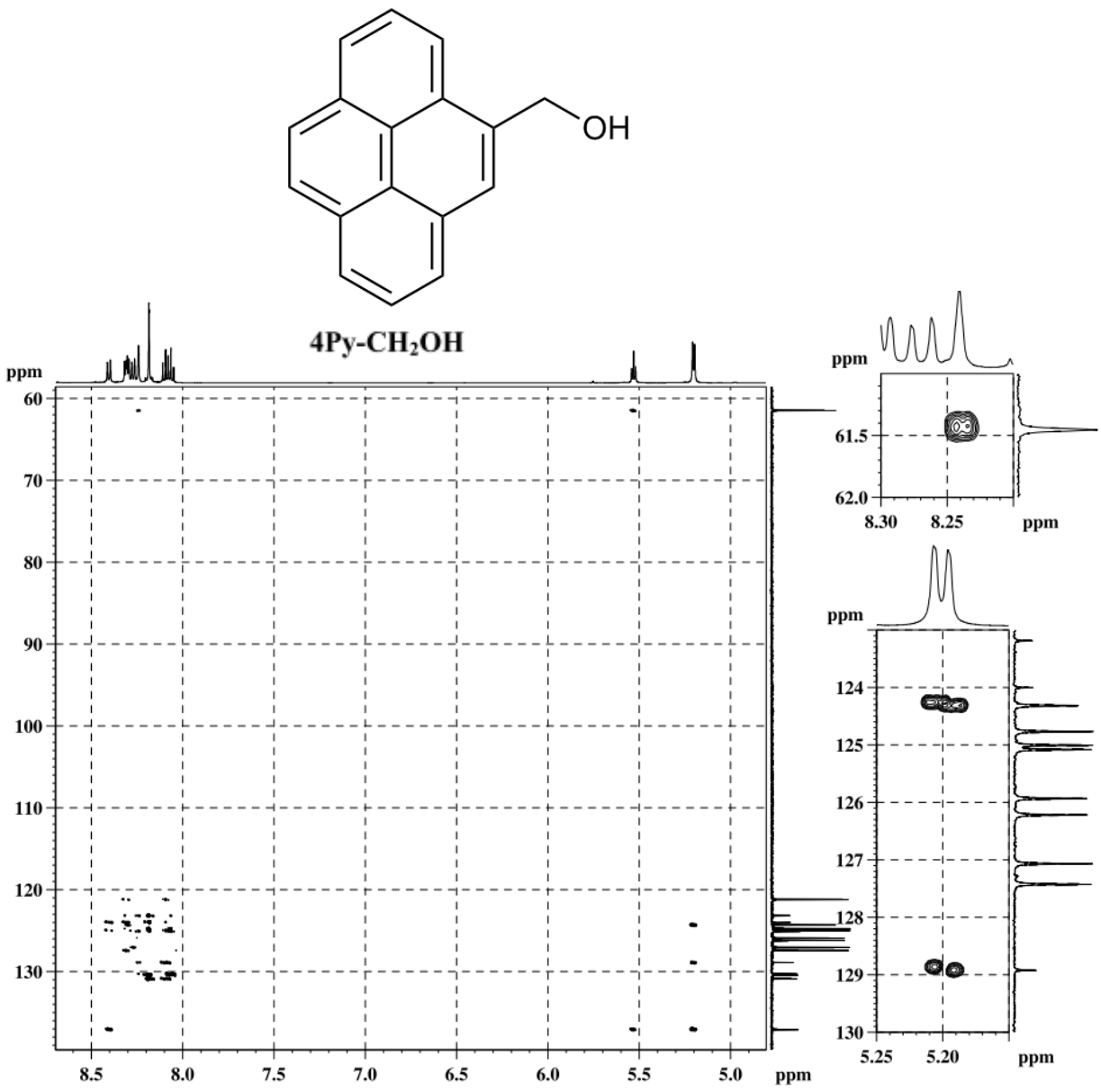
122.0
122.5
123.0
123.5
124.0
124.5
125.0
125.5
126.0
126.5
127.0
127.5
8.45
8.40
8.35
8.30
8.25
8.20
8.15
8.10
8.05
ppm


OH
4Py-CH2OH
ppm
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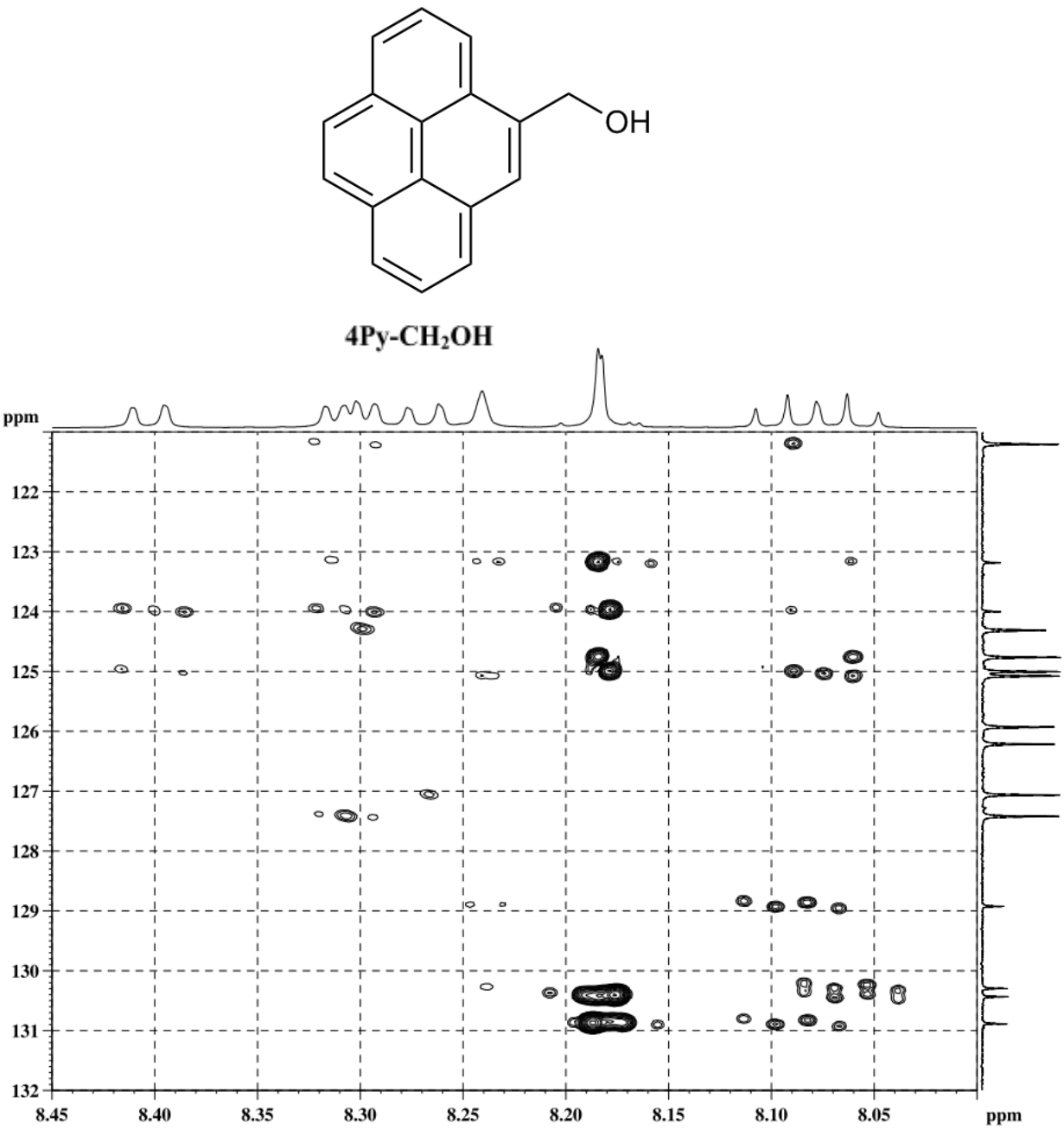
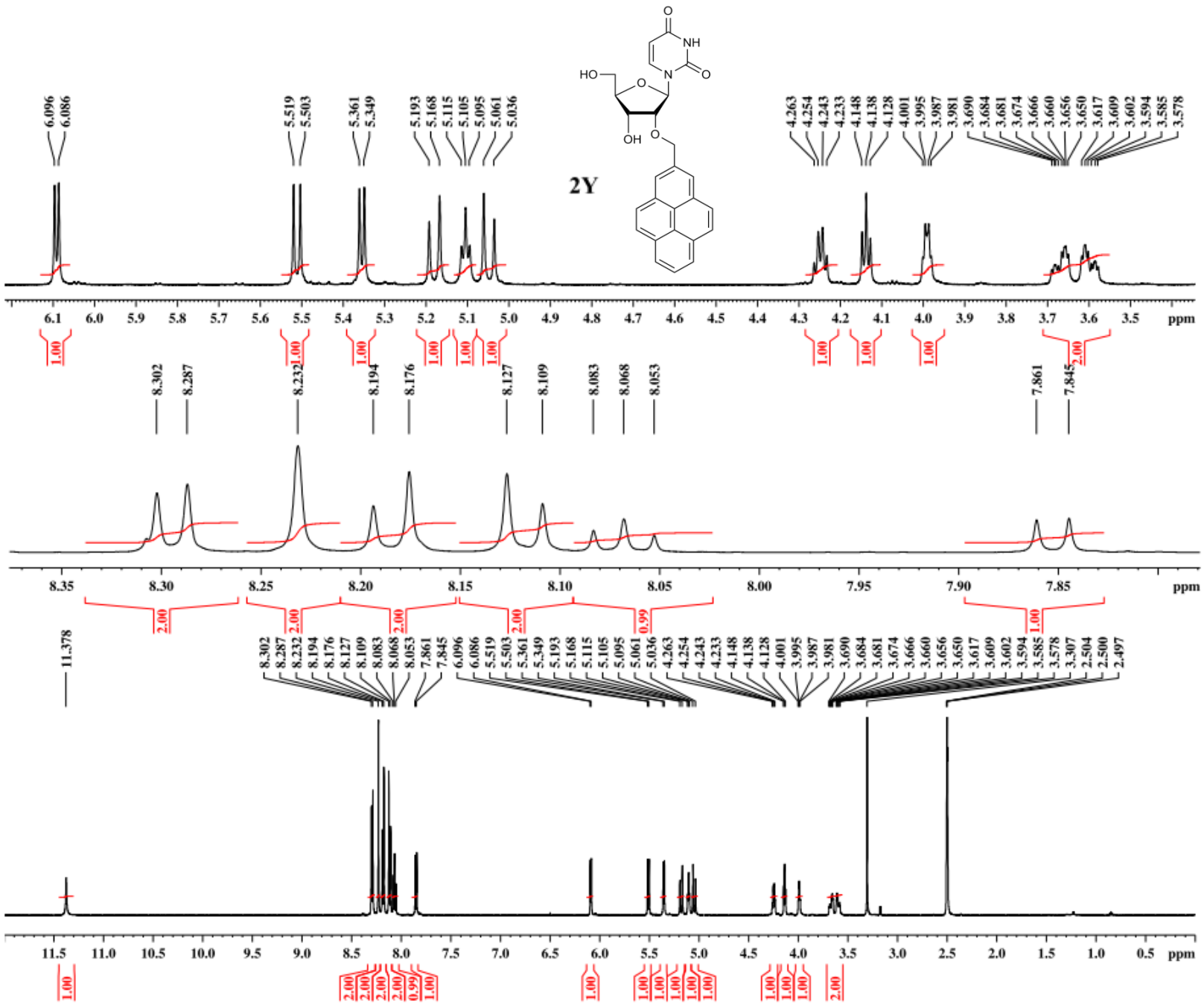
70
80
90
100
110
120
130
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
ppm
ppm
61.5
62.0
8.30
8.25
ppm
ppm
124
125
126
127
128
129
130
5.25
5.20
ppm


OH
4Py-CH2OH
ppm
122
123
124
125
126
127
128
129
130
131
132
8.45
8.40
8.35
8.30
8.25
8.20
8.15
8.10
8.05
ppm


2Y
HO
OH
NH
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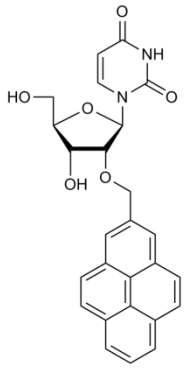
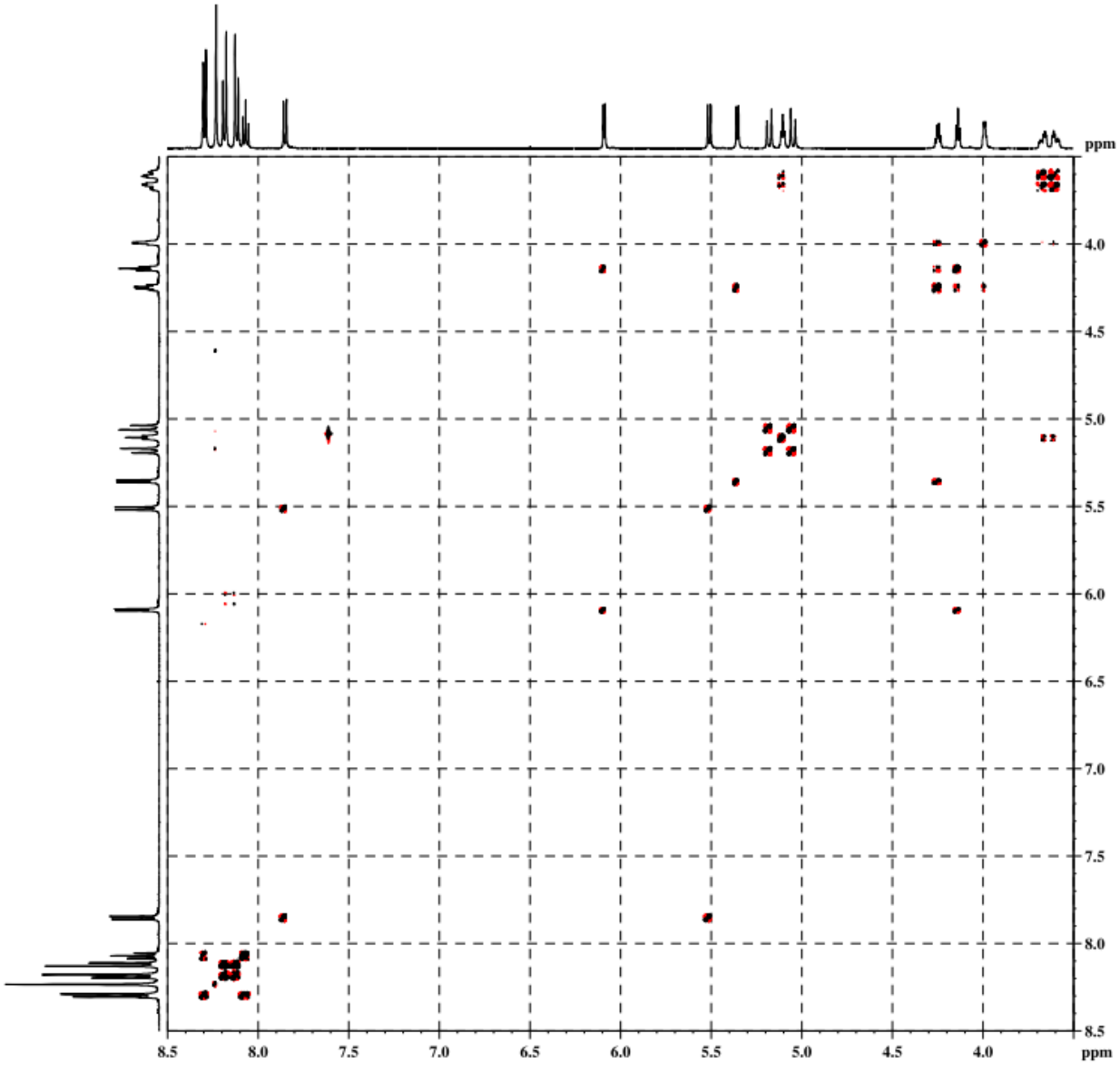
6.086
5.519
5.503
5.361
5.349
5.193
5.168
5.115
5.105
5.095
5.061
5.036
4.263
4.254
4.243
4.233
4.148
4.138
4.128
4.001
3.995
3.987
3.981
3.690
3.684
3.681
3.674
3.666
3.660
3.656
3.650
3.617
3.609
3.602
3.594
3.585
3.578
8.302
8.287
8.232
8.194
8.176
8.127
8.109
8.083
8.068
8.053
7.861
7.845
11.378
2.504
2.500
2.497
ppm


O
NH
HO
N
O
O
OH
O

2Y


ppm
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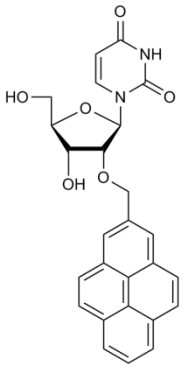
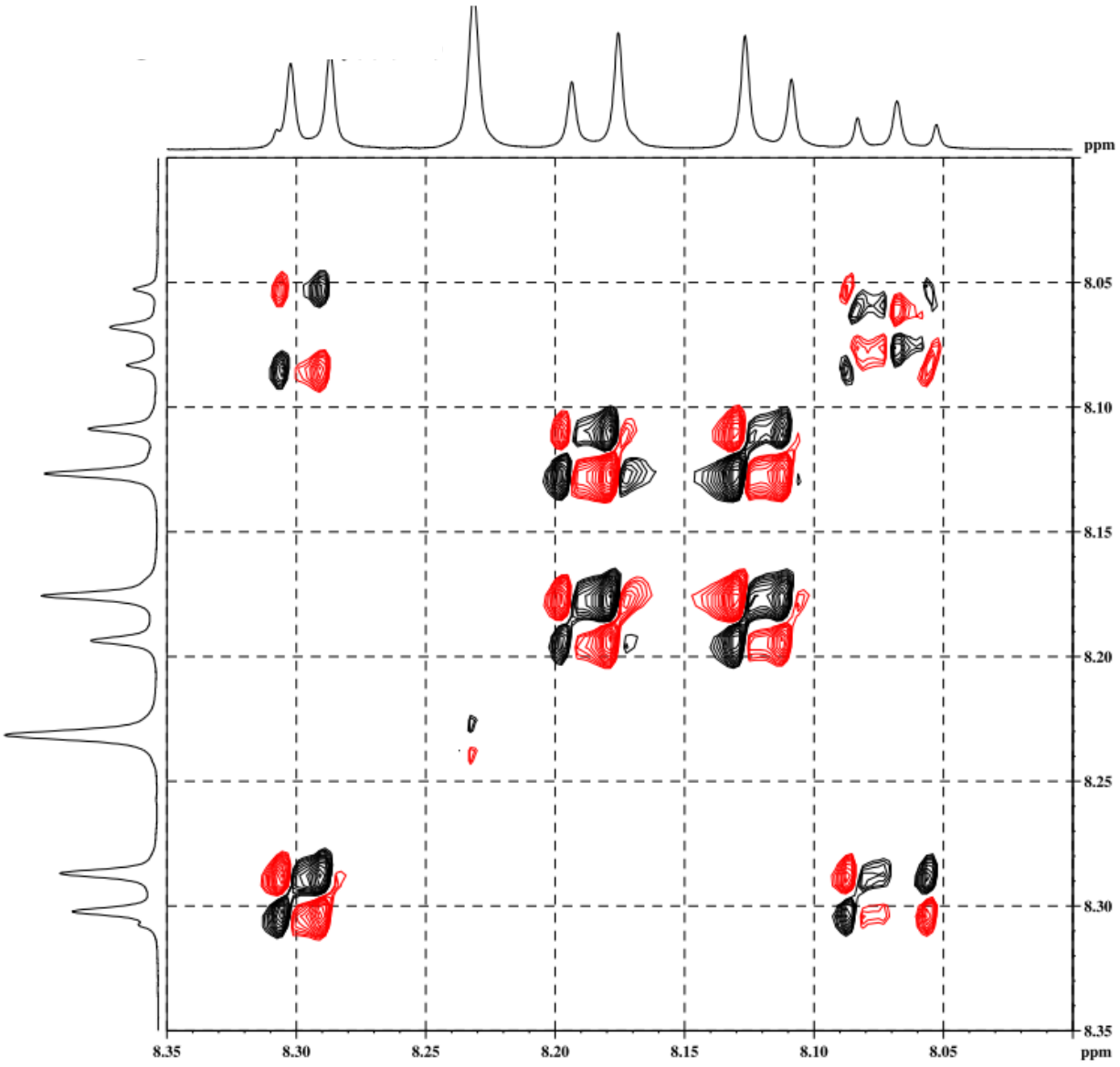
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm


O
NH
HO
N
O
O
OH
O

2Y


ppm
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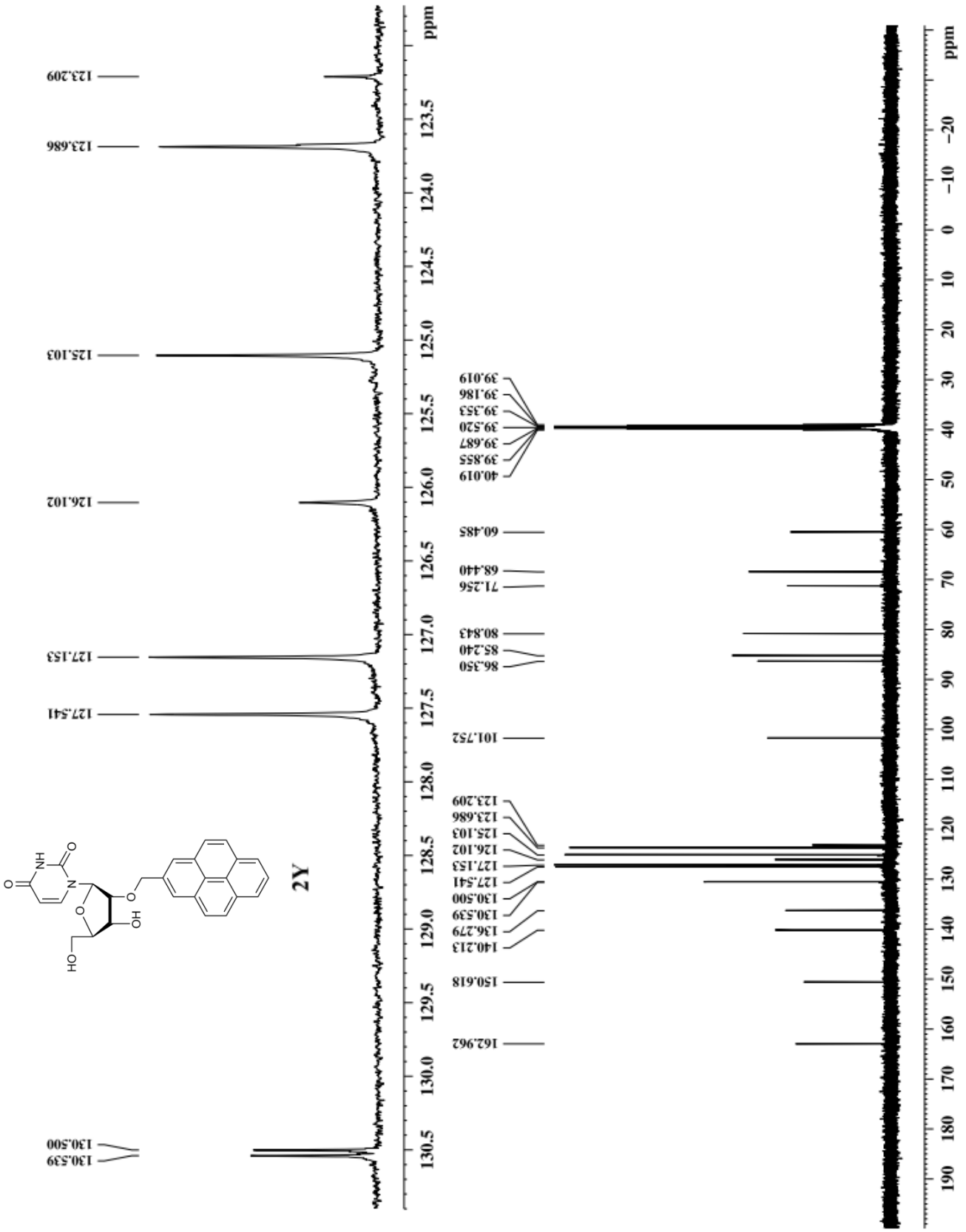
8.10
8.15
8.20
8.25
8.30
8.35
8.35
8.30
8.25
8.20
8.15
8.10
8.05
ppm


2Y
ppm
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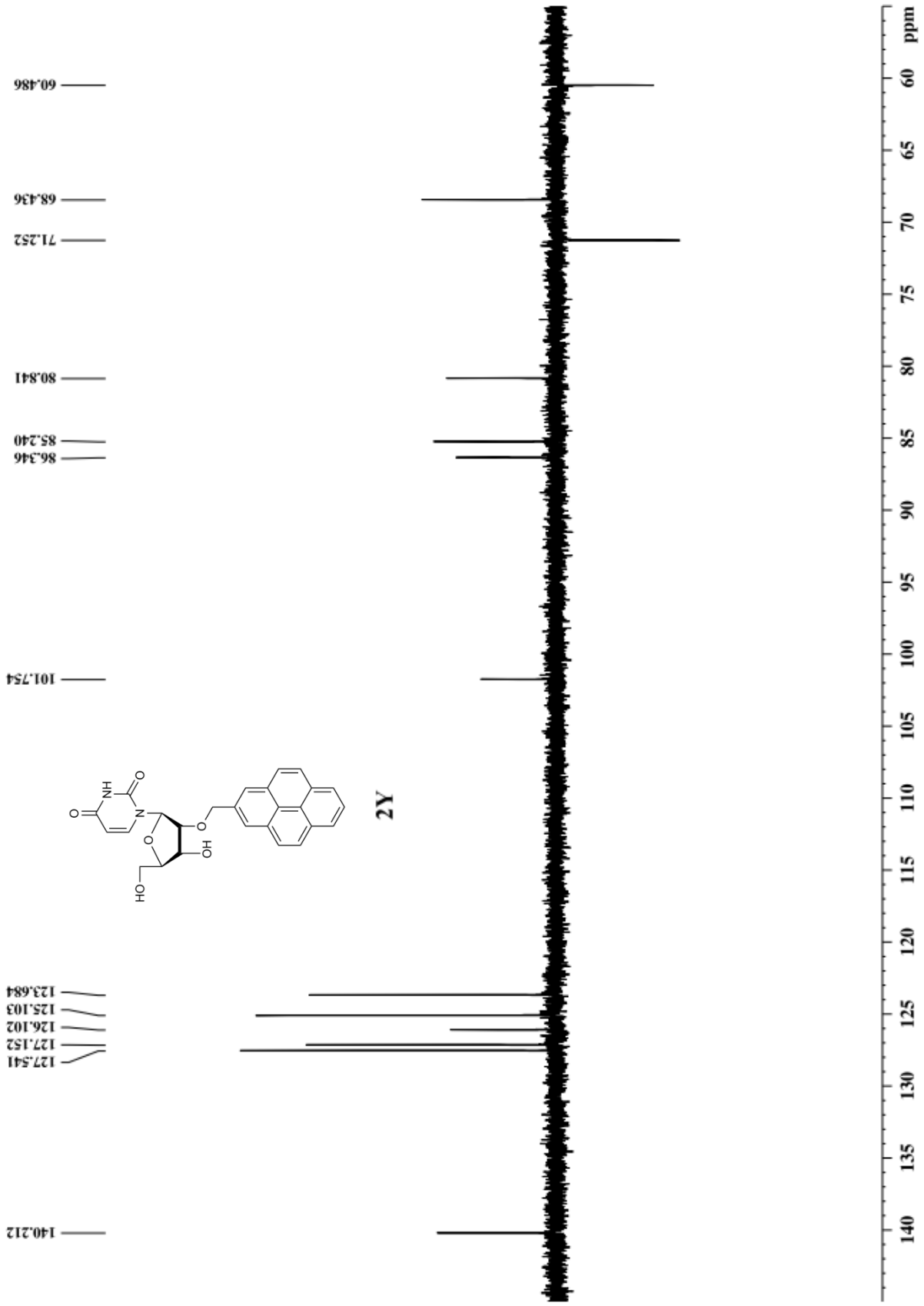
130.500
127.541
127.153
126.102
125.103
123.686
123.209
162.962
150.618
140.213
136.279
130.539
130.500
127.541
127.153
126.102
125.103
123.686
123.209
101.752
86.350
85.240
80.843
71.256
68.440
60.485
40.019
39.855
39.687
39.520
39.353
39.186
39.019


60.486
68.436
71.252
80.841
85.240
86.346
101.754
123.684
125.103
126.102
127.152
127.541
140.212
2Y
ppm
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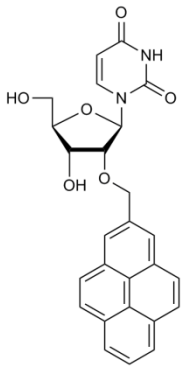
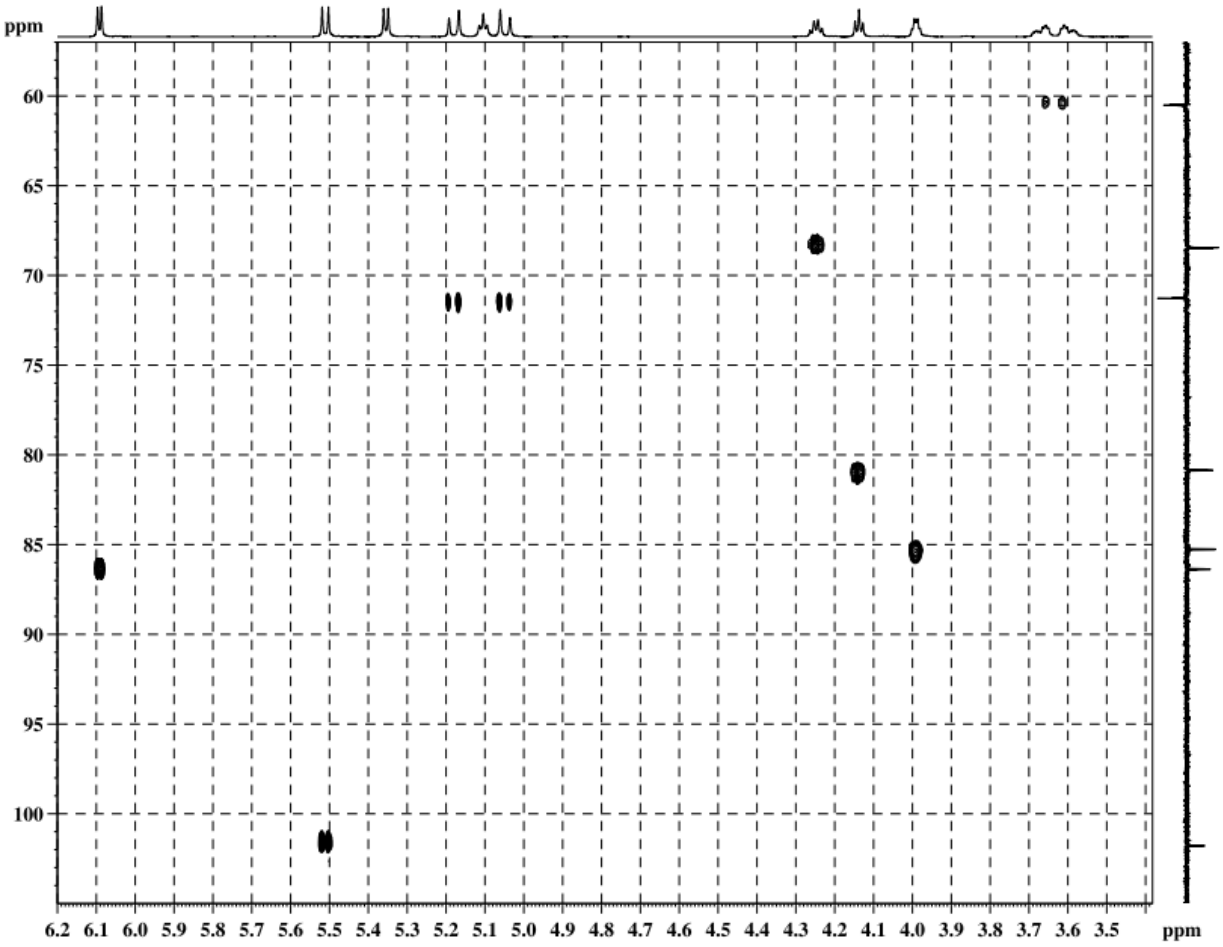
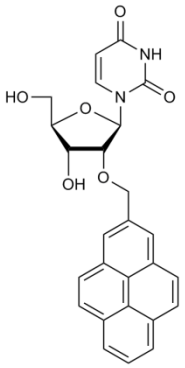
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140

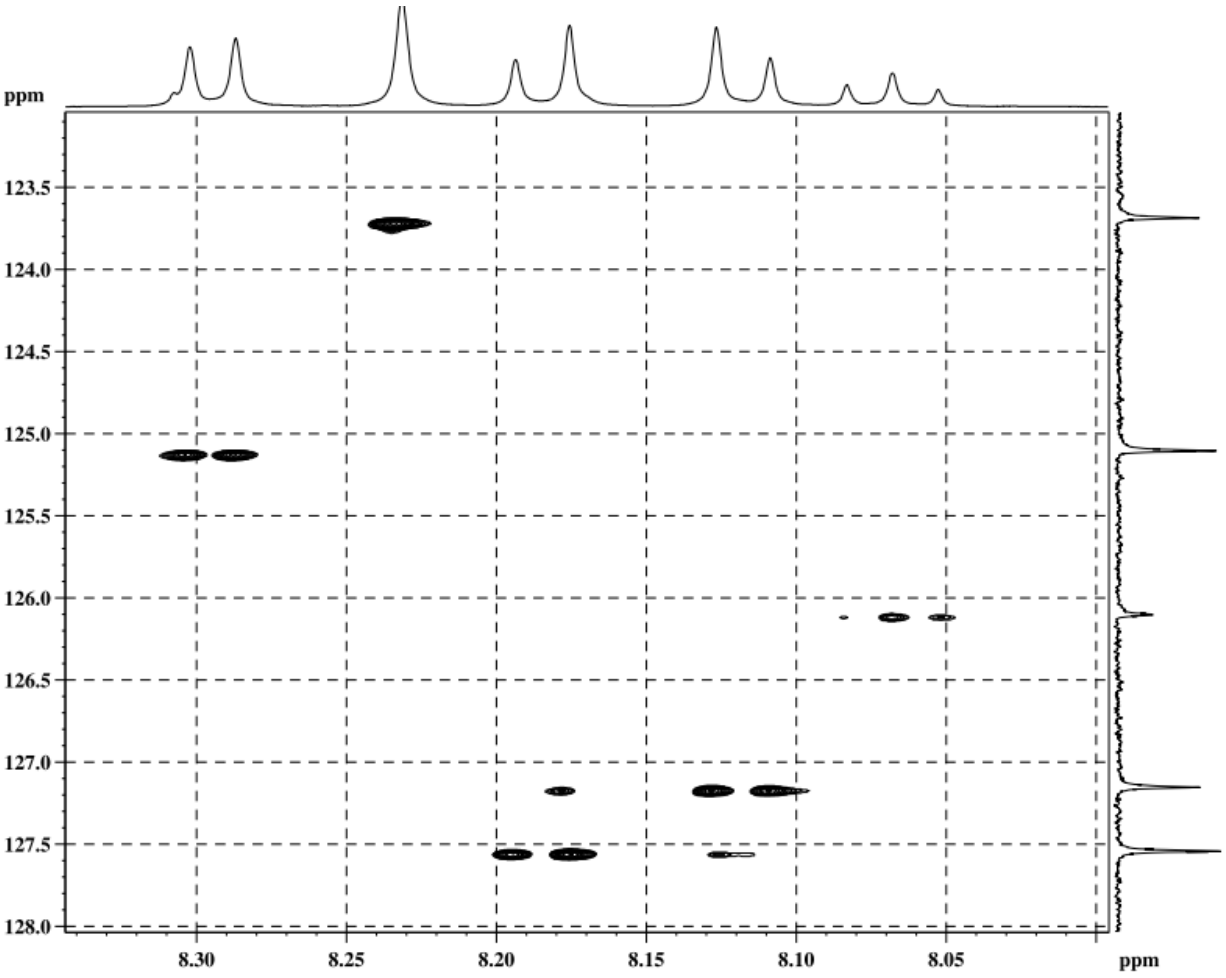

O
NH
N
O
HO
O
OH
O

2Y


ppm
60
65
70
75
80
85
90
95
100
6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5
ppm


O
NH
HO
O
N
O
OH
O

2Y


ppm
123.5
124.0
124.5
125.0
125.5
126.0
126.5
127.0
127.5
128.0
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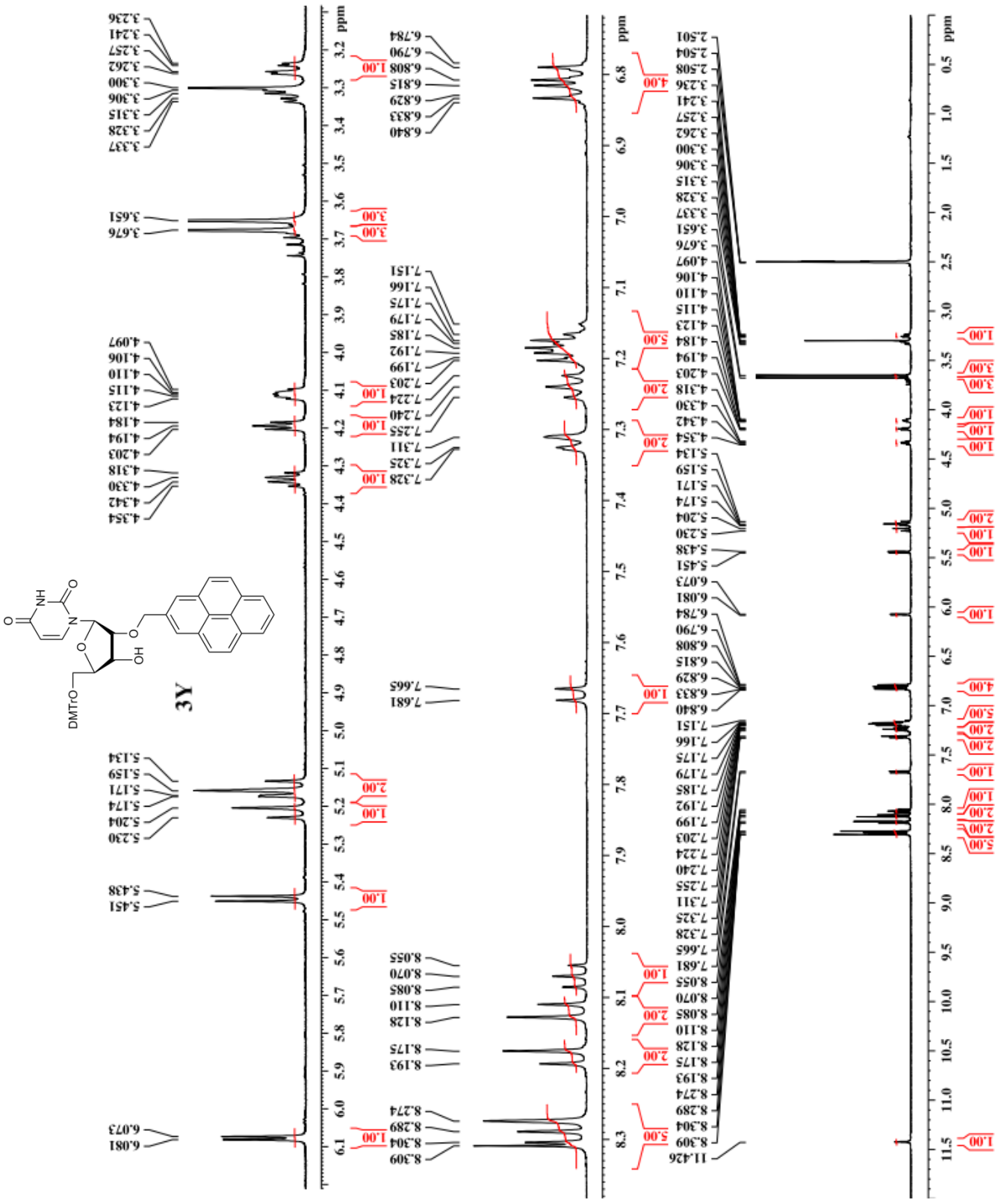
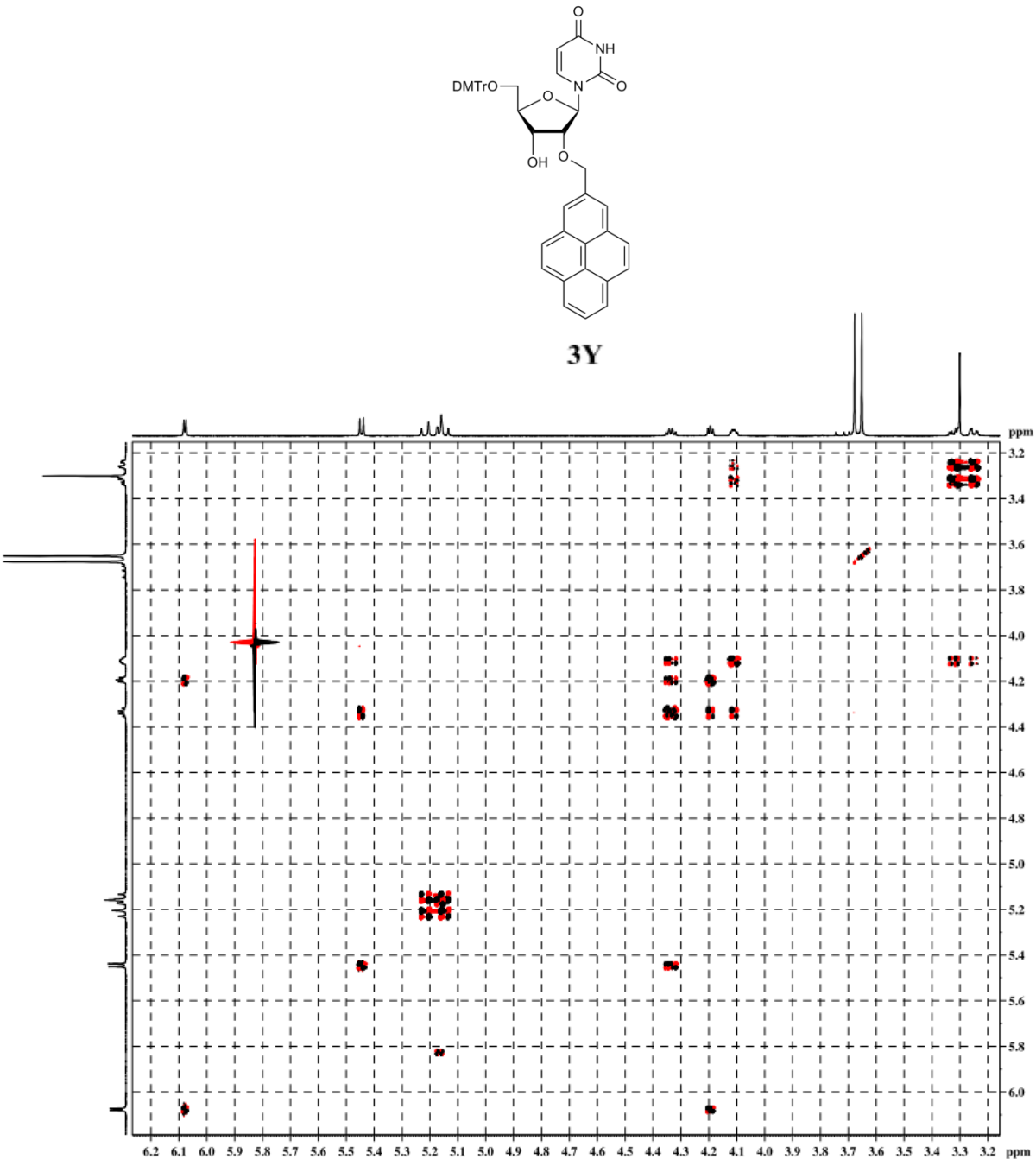
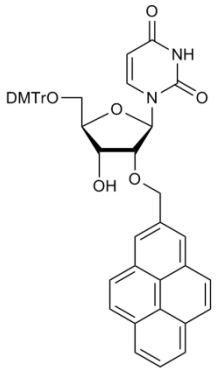
8.25
8.20
8.15
8.10
8.05
ppm

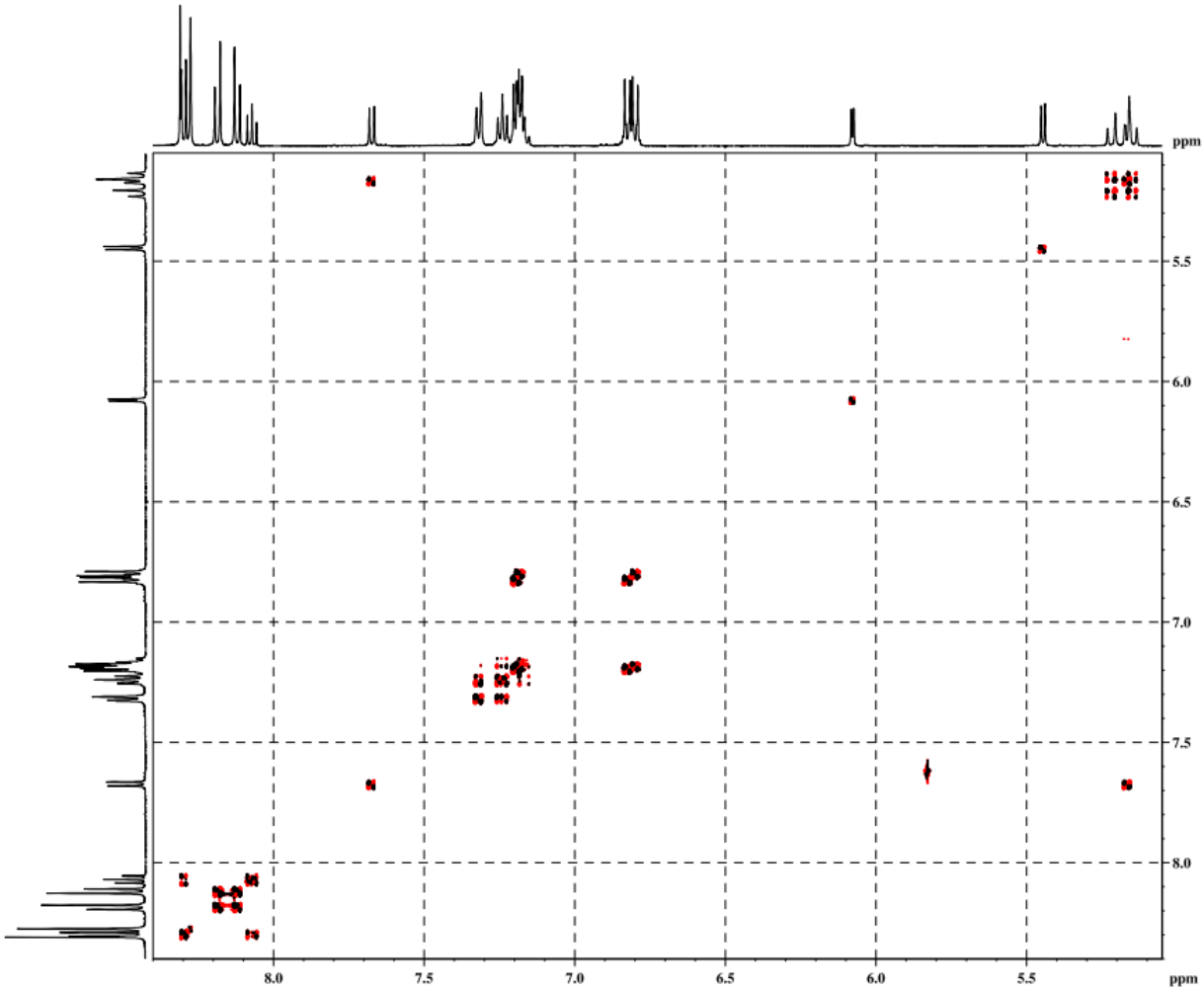

3Y
DMTrO
NH
O
N
O
OH

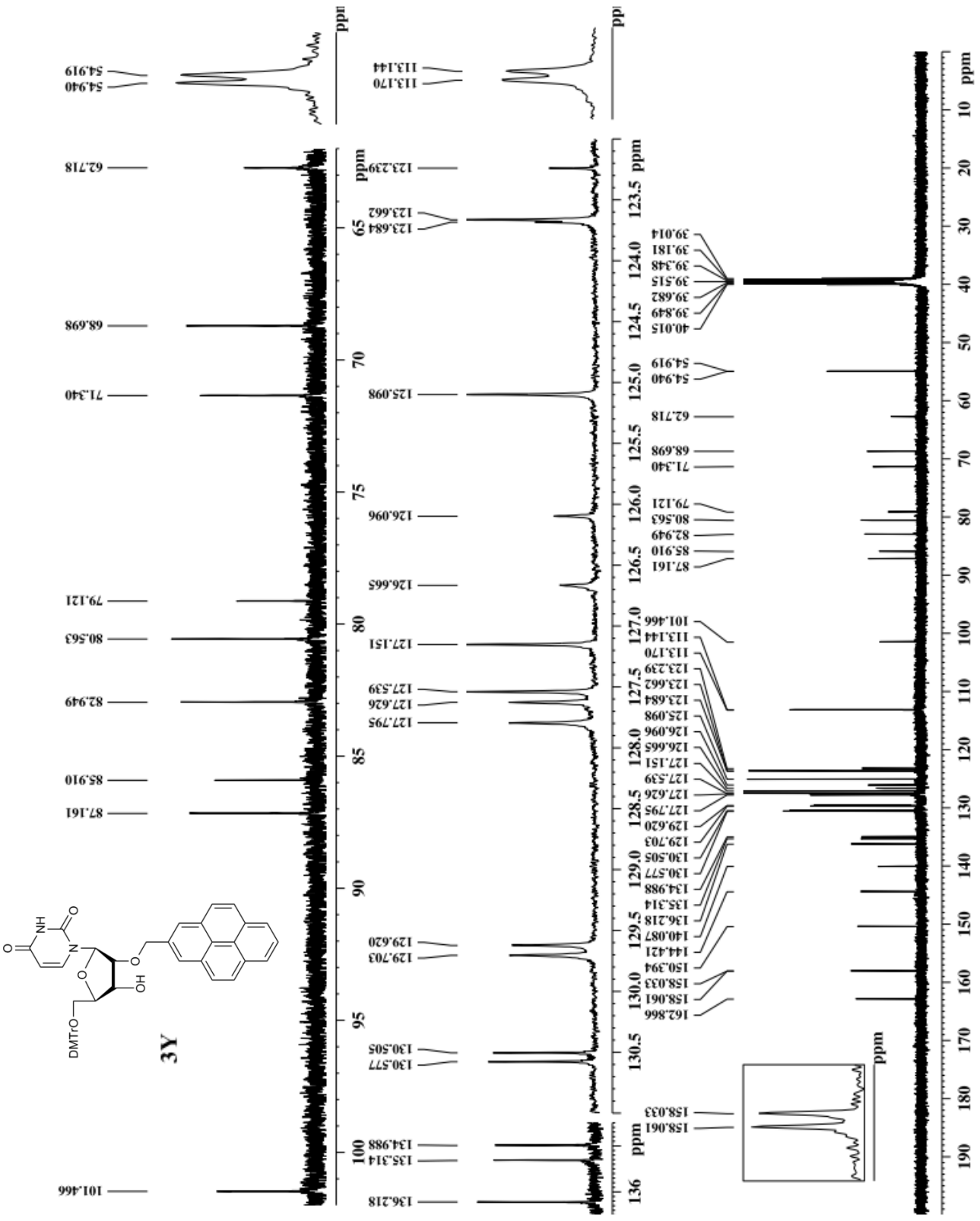

O
NH
N
O
DMTrO
O
OH
O
ppm
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
6.0
6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 ppm

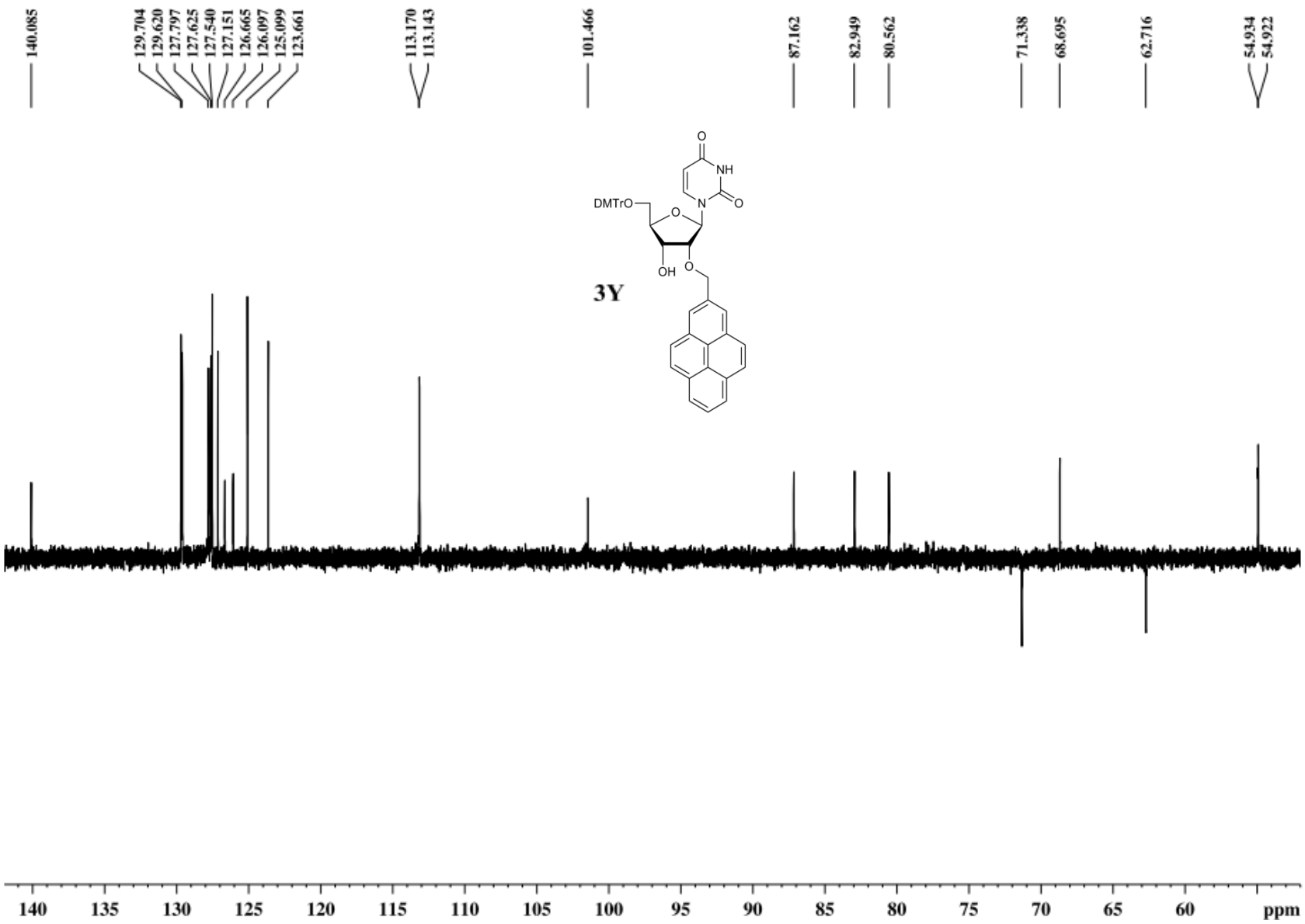
3Y



3Y




3Y
DMTrO
OH
NH
O
N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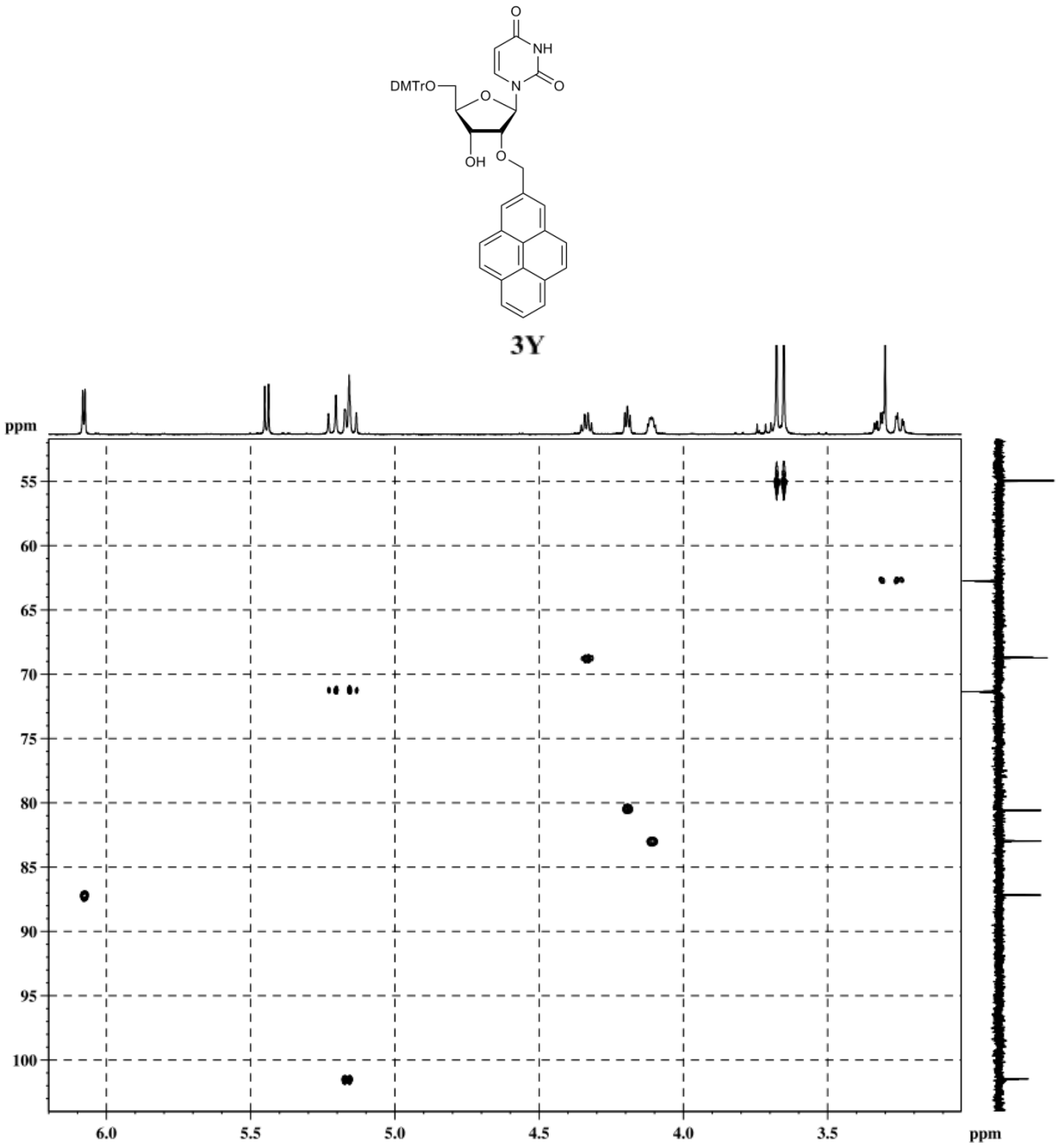
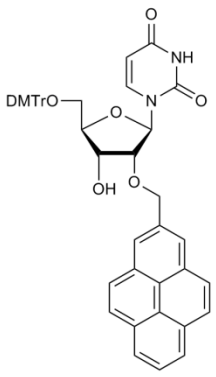
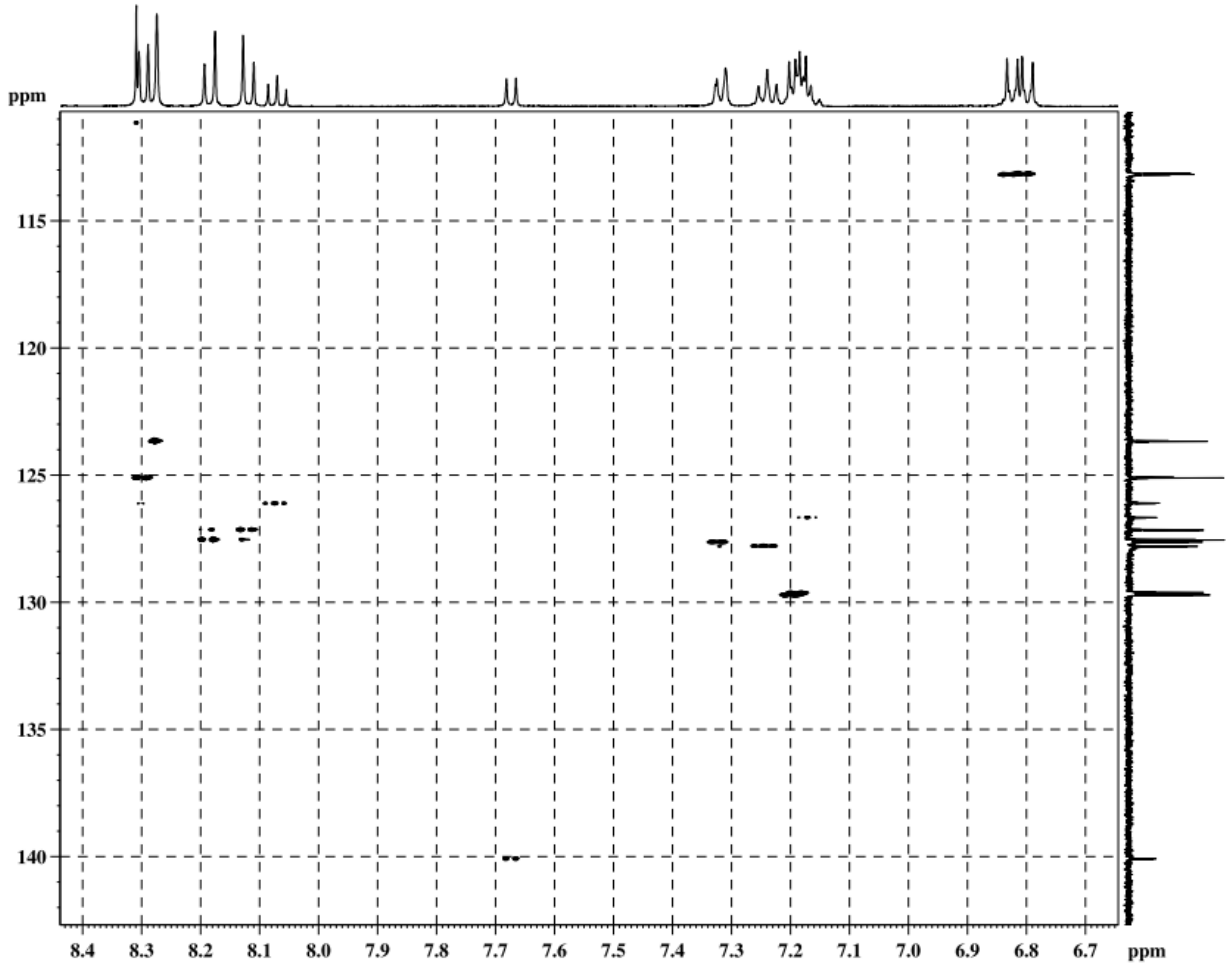
129.620
127.797
127.625
127.540
127.151
126.665
126.097
125.099
123.661
113.170
113.143
101.466
87.162
82.949
80.562
71.338
68.695
62.716
54.934
54.922
DMTrO
O
NH
N
O
O
OH
O
3Y
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
ppm

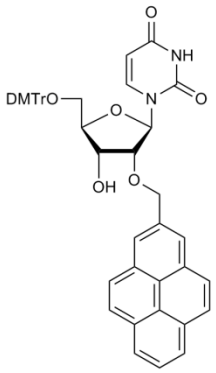

O
NH
DMTrO
N
O
O
OH
O
ppm
55
60
65
70
75
80
85
90
95
100
6.0
5.5
5.0
4.5
4.0
3.5
ppm

3Y

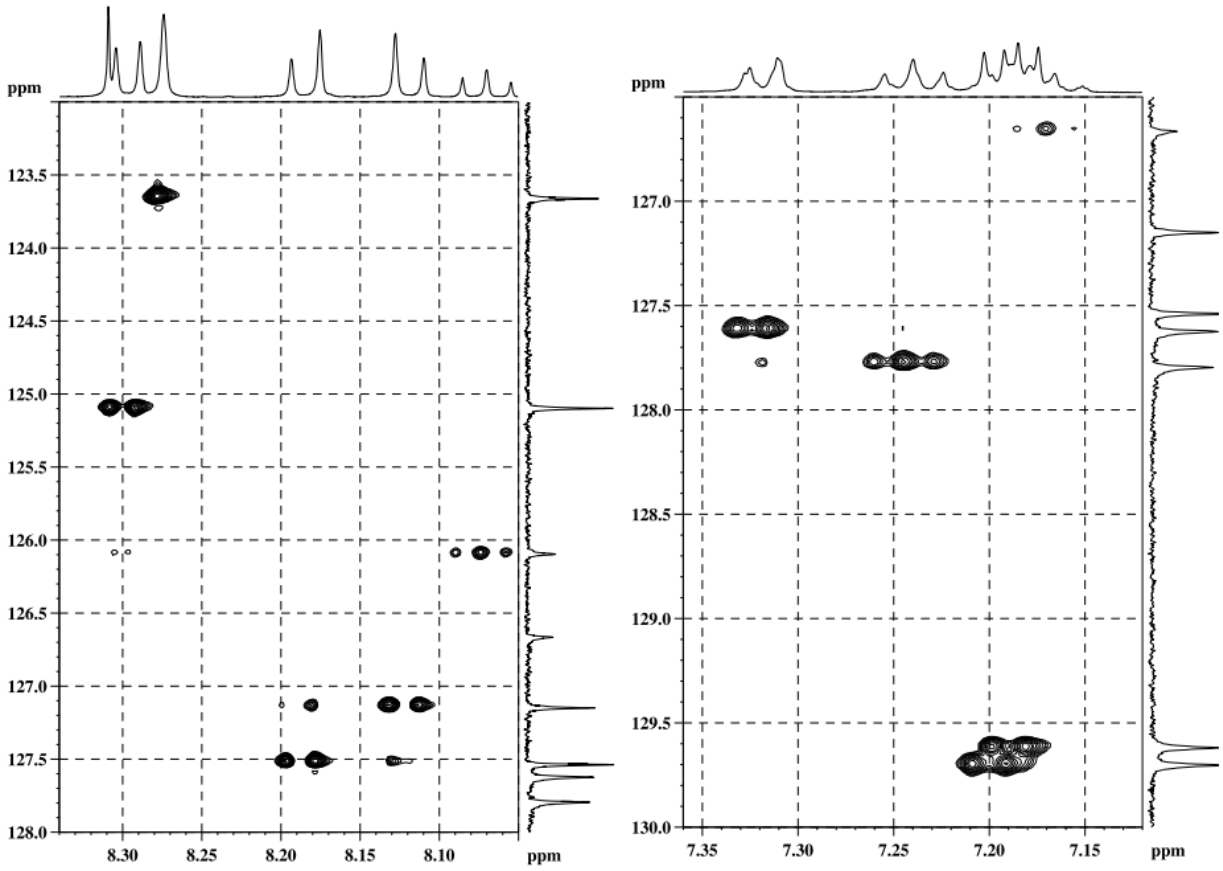

O
NH
DMTrO
N
O
O
OH
O

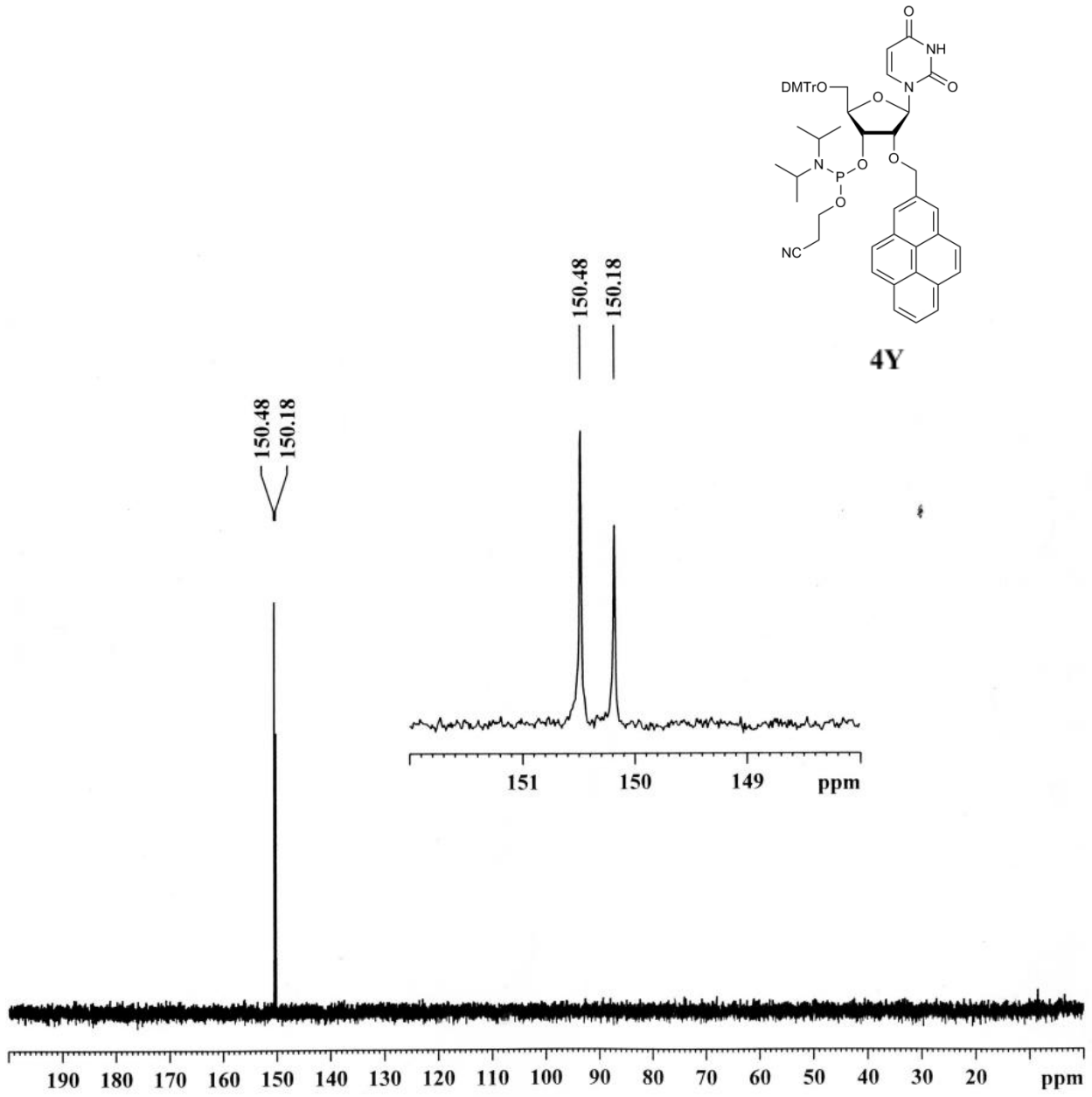
3Y

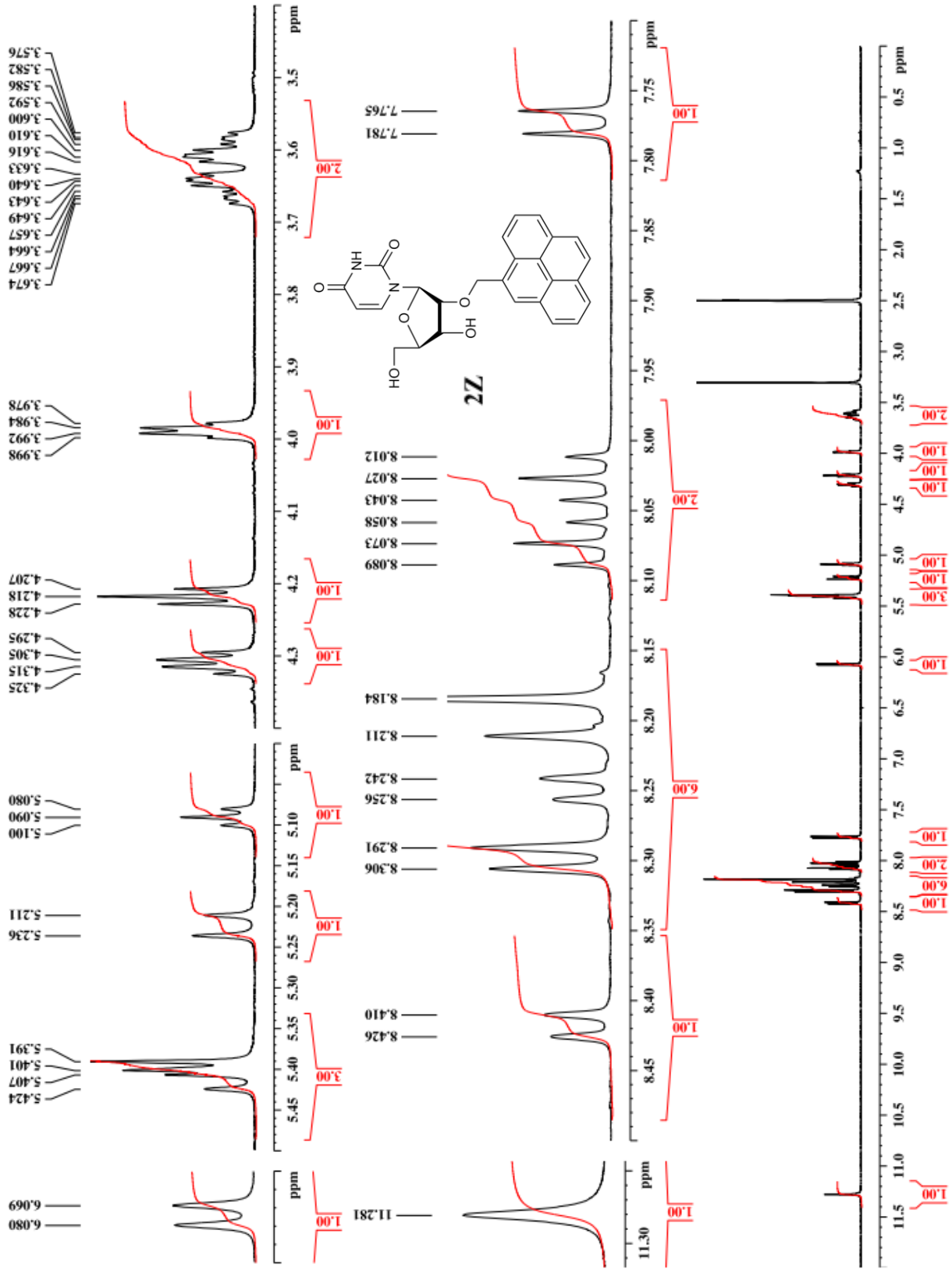

ppm
115
120
125
130
135
140
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
ppm

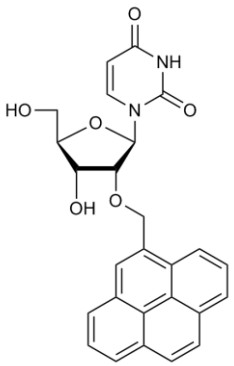

O
NH
DMTrO
N
O
O
OH
O

3Y

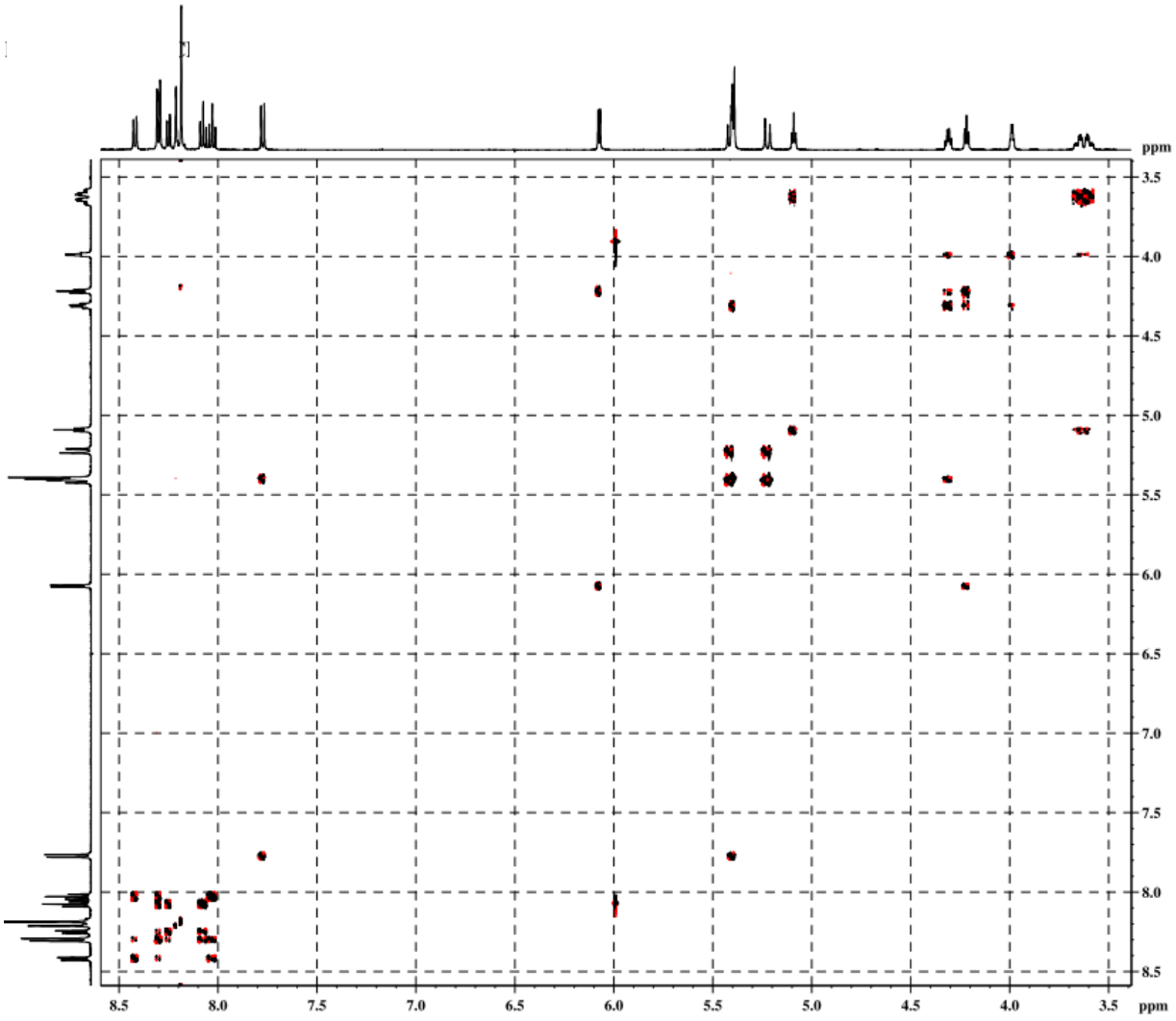

ppm
123.5
124.0
124.5
125.0
125.5
126.0
126.5
127.0
127.5
128.0
8.30
8.25
8.20
8.15
8.10
ppm
ppm
127.0
127.5
128.0
128.5
129.0
129.5
130.0
7.35
7.30
7.25
7.20
7.15
ppm

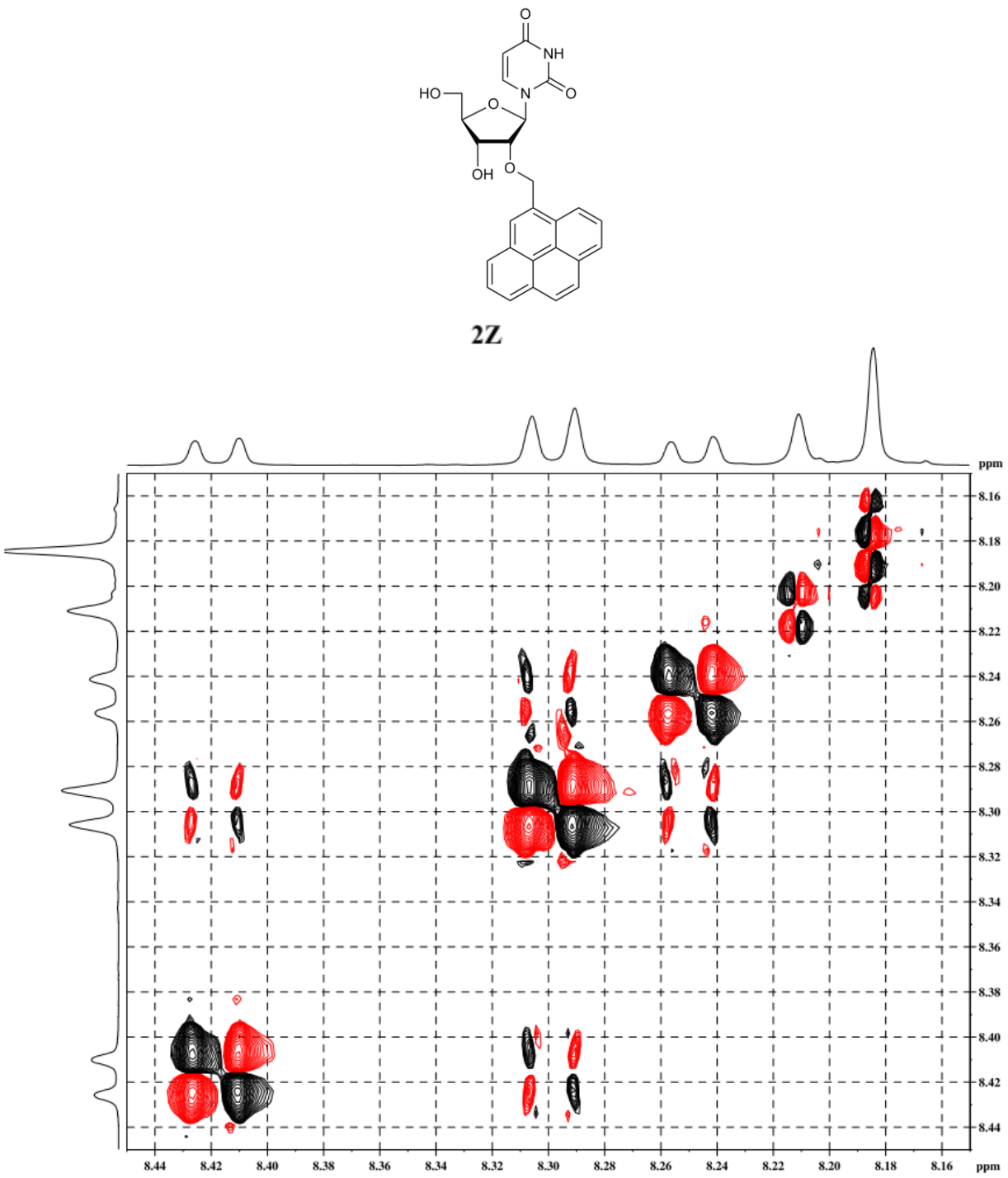

DMTrO
NH
O
N
O
O
O
N
P
O
O
NC
4Y
150.48
150.18
150.48
150.18
151
150
149
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


2Z

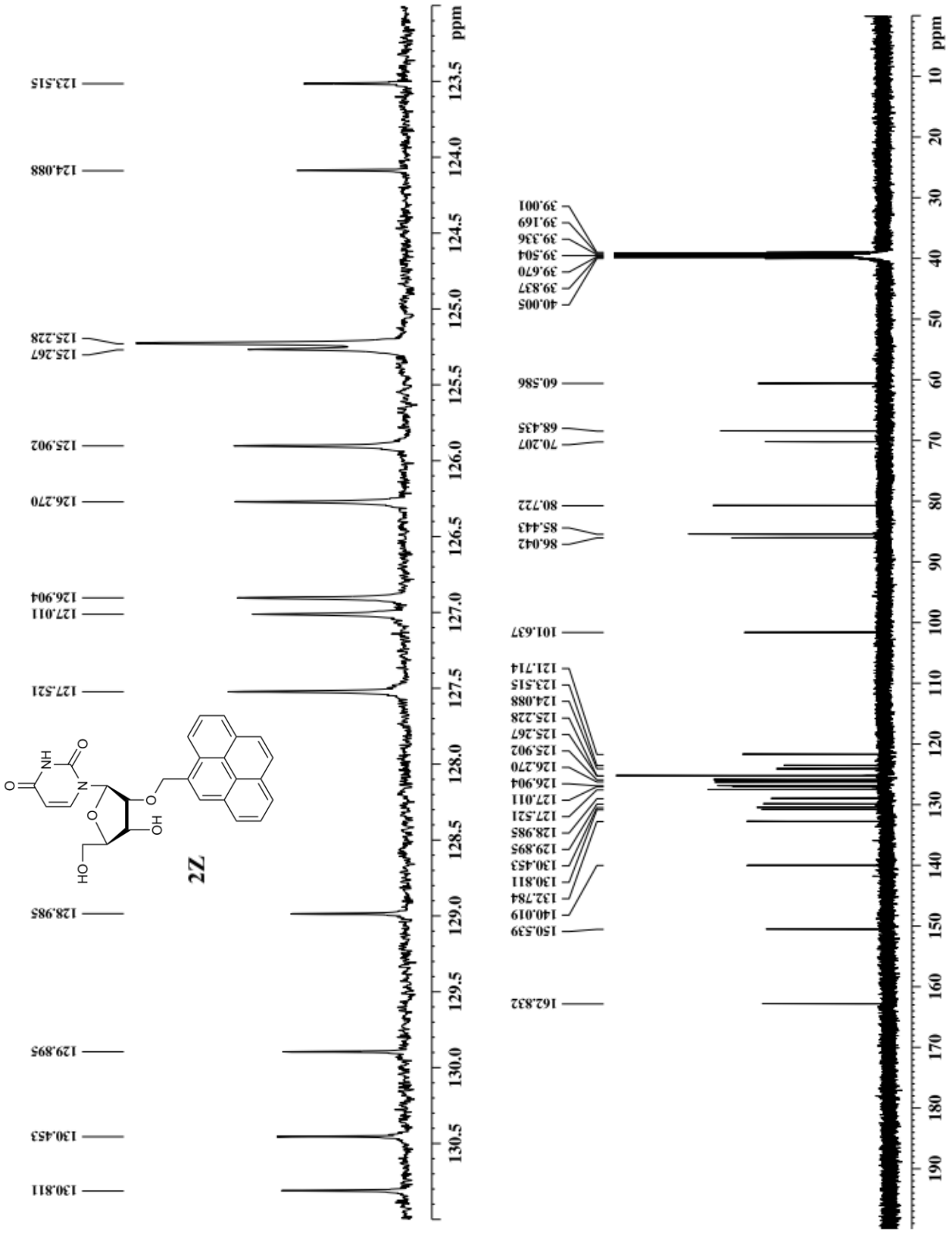

O
NH
N
O
HO
O
OH
O

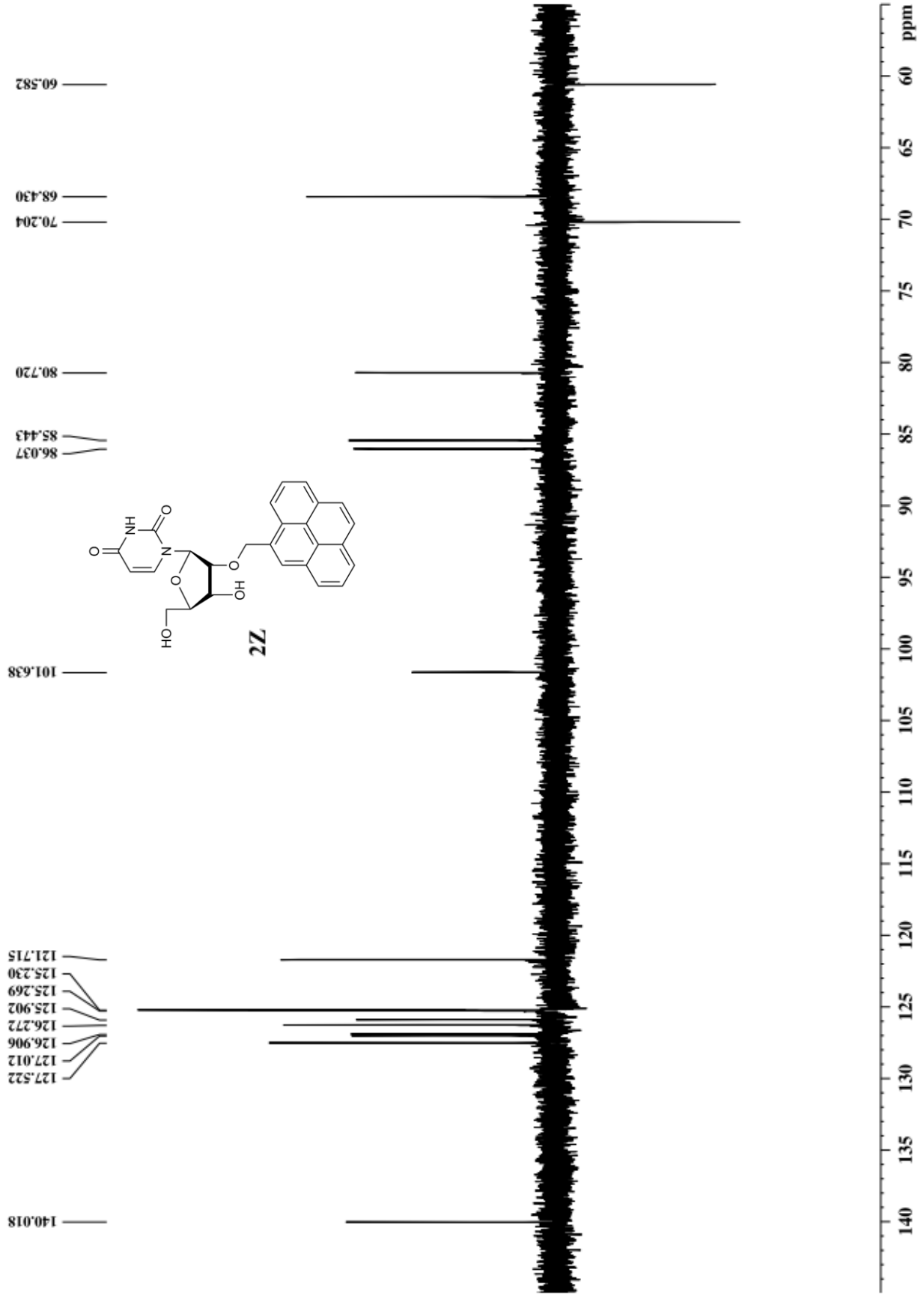
2Z

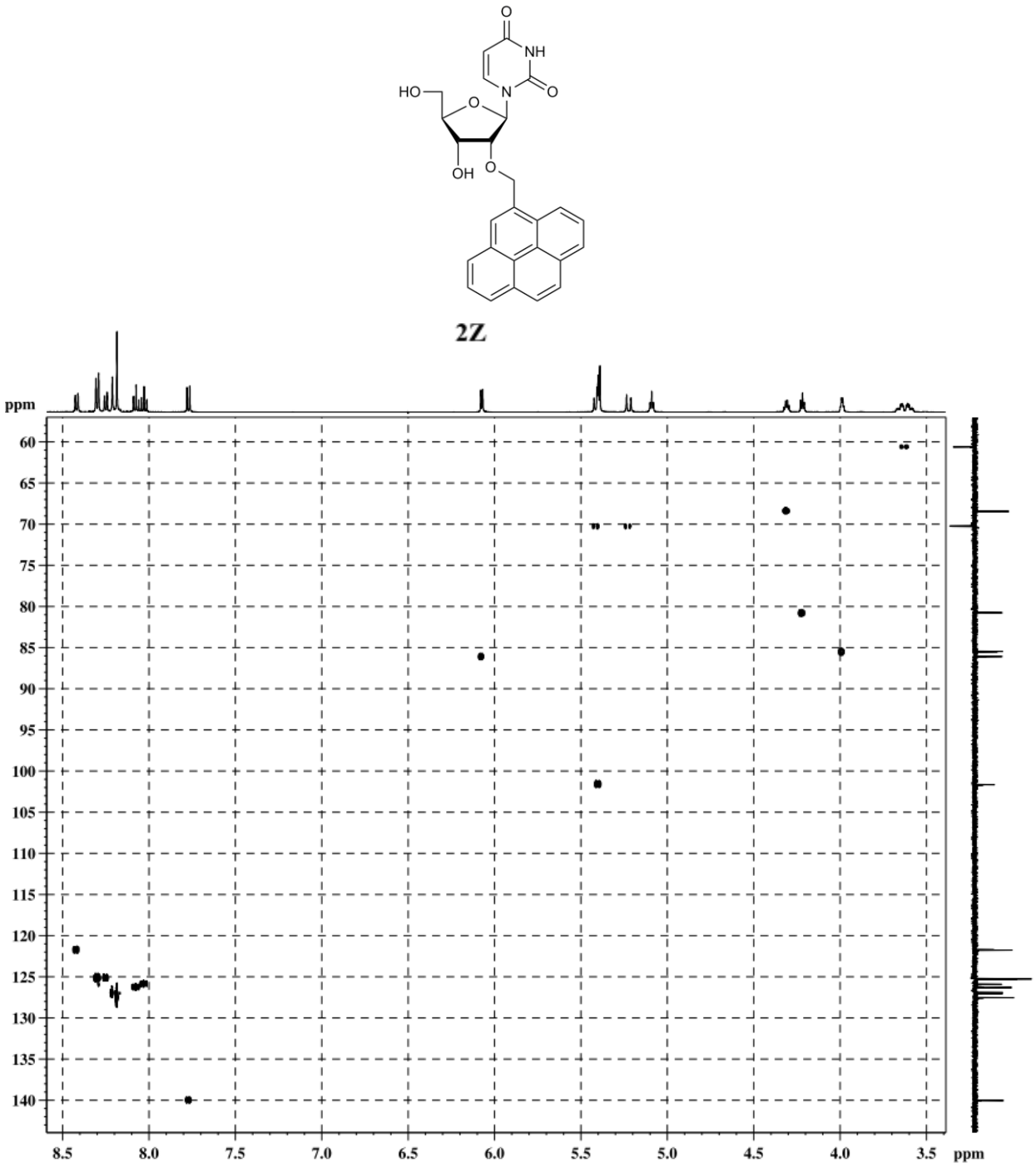

ppm
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm

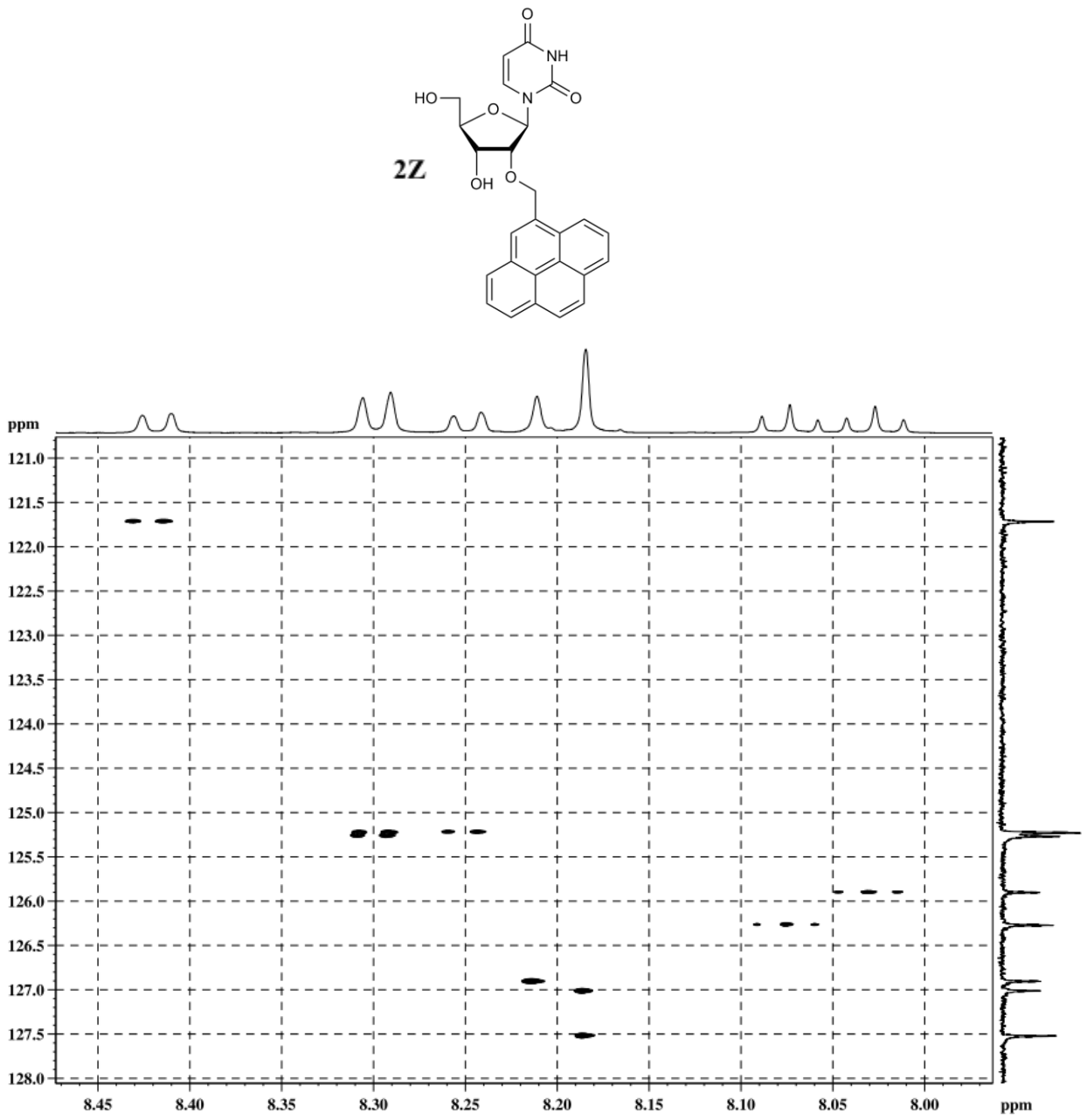

O
NH
N
O
HO
O
OH
O
ppm
8.16
8.18
8.20
8.22
8.24
8.26
8.28
8.30
8.32
8.34
8.36
8.38
8.40
8.42
8.44
8.44
8.42
8.40
8.38
8.36
8.34
8.32
8.30
8.28
8.26
8.24
8.22
8.20
8.18
8.16
ppm

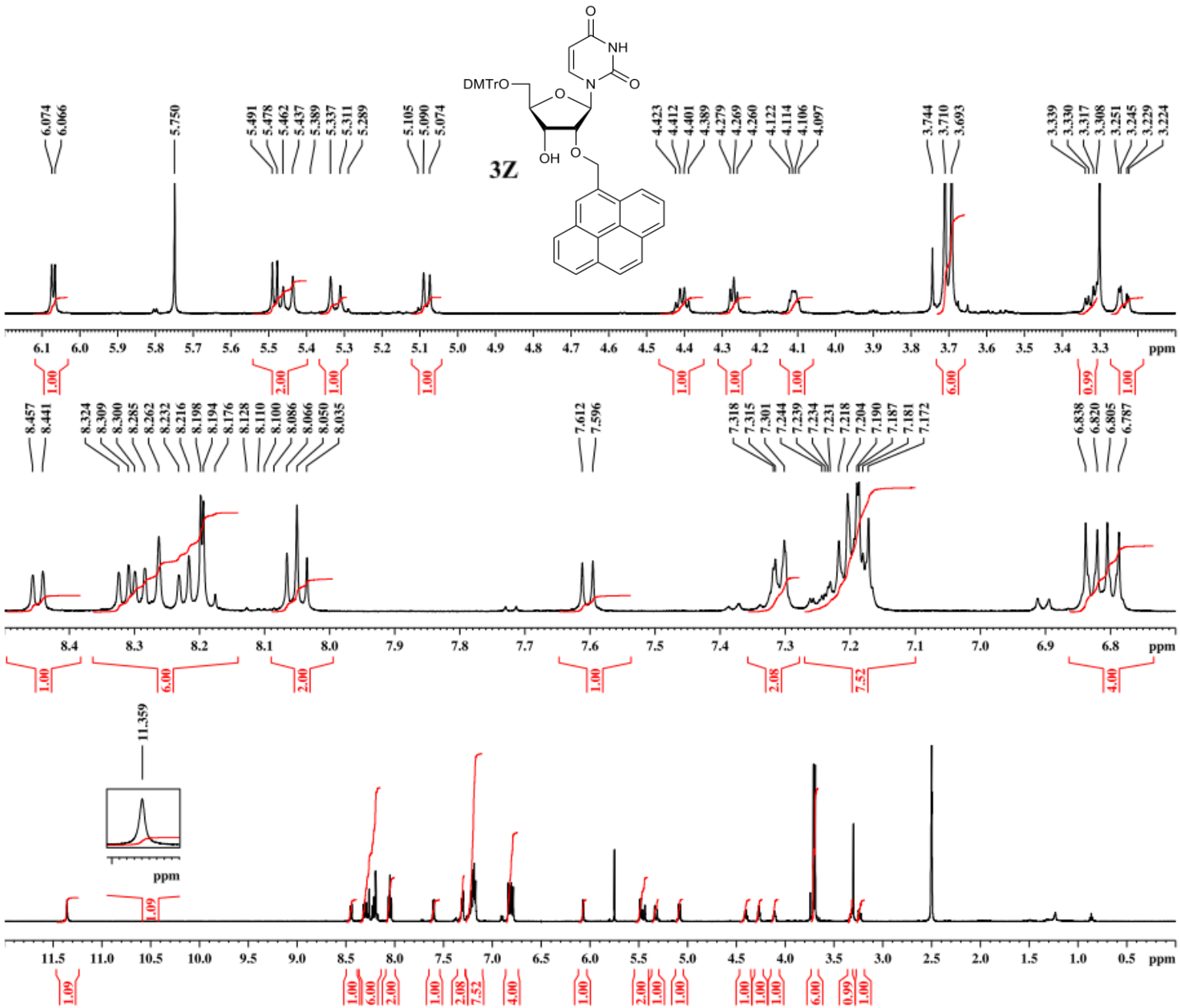
2Z

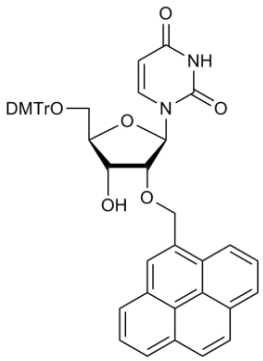

2Z
ppm
123.515
124.088
125.228
125.267
125.902
126.270
126.904
127.011
127.521
128.985
129.895
130.453
130.811
ppm
39.001
39.169
39.336
39.504
39.670
39.837
40.005
60.586
68.435
70.207
80.722
85.443
86.042
101.637
121.714
123.515
124.088
125.228
125.267
125.902
126.270
126.904
127.011
127.521
128.985
129.895
130.453
130.811
132.784
140.019
150.539
162.832


60.582
68.430
70.204
80.720
85.443
86.037
101.638
121.715
125.230
125.269
125.902
126.272
126.906
127.012
127.522
140.018
2Z
ppm

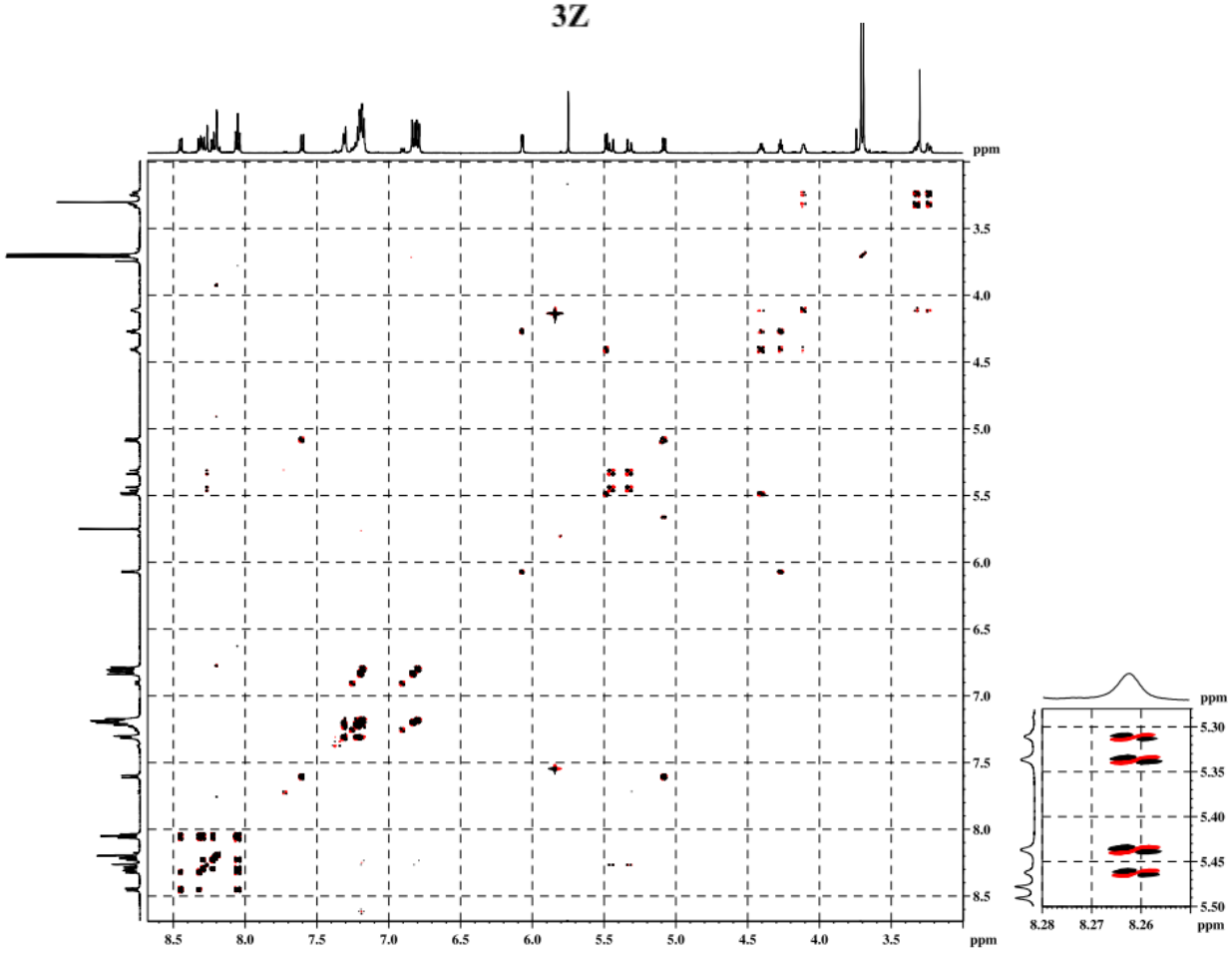

O
NH
HO
N
O
O
OH
O
2Z
ppm
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm

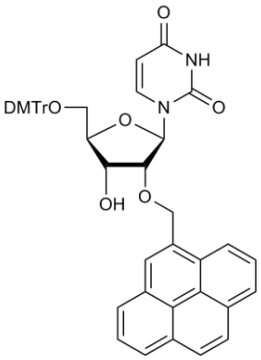

2Z
HO
O
NH
N
O
OH
O
ppm
121.0
121.5
122.0
122.5
123.0
123.5
124.0
124.5
125.0
125.5
126.0
126.5
127.0
127.5
128.0
8.45
8.40
8.35
8.30
8.25
8.20
8.15
8.10
8.05
8.00
ppm


3Z
DMTrO
NH
O
N
OH
6.074
6.066
5.750
5.491
5.478
5.462
5.437
5.389
5.337
5.311
5.289
5.105
5.090
5.074
4.423
4.412
4.401
4.389
4.279
4.269
4.260
4.122
4.114
4.106
4.097
3.744
3.710
3.693
3.339
3.330
3.317
3.308
3.251
3.245
3.229
3.224
8.457
8.441
8.324
8.309
8.300
8.285
8.262
8.232
8.216
8.198
8.194
8.176
8.128
8.110
8.100
8.086
8.066
8.050
8.035
7.612
7.596
7.318
7.315
7.301
7.244
7.239
7.234
7.231
7.218
7.204
7.190
7.187
7.181
7.172
6.838
6.820
6.805
6.787
11.359
ppm

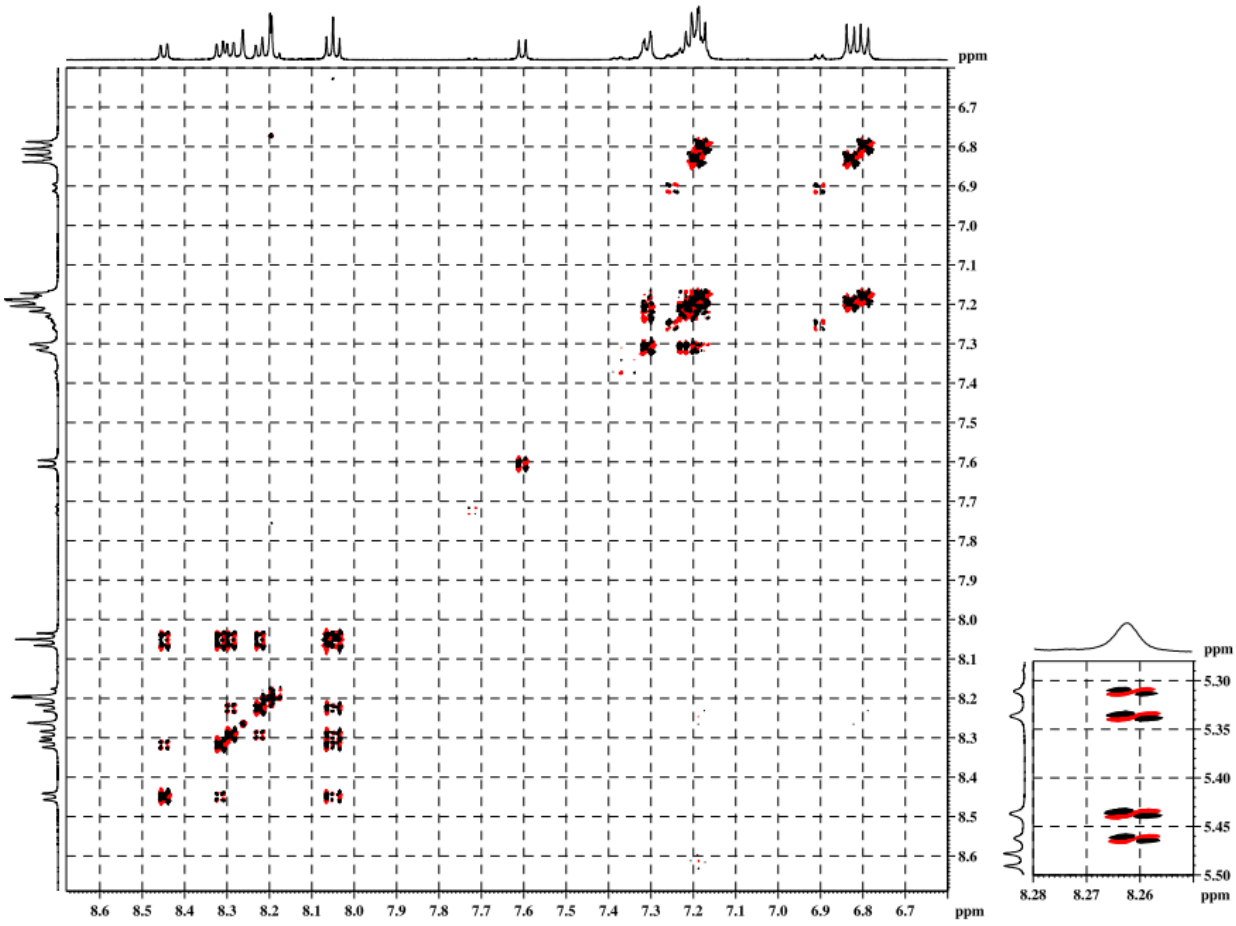

O
NH
DMTrO
N
O
O
OH
O

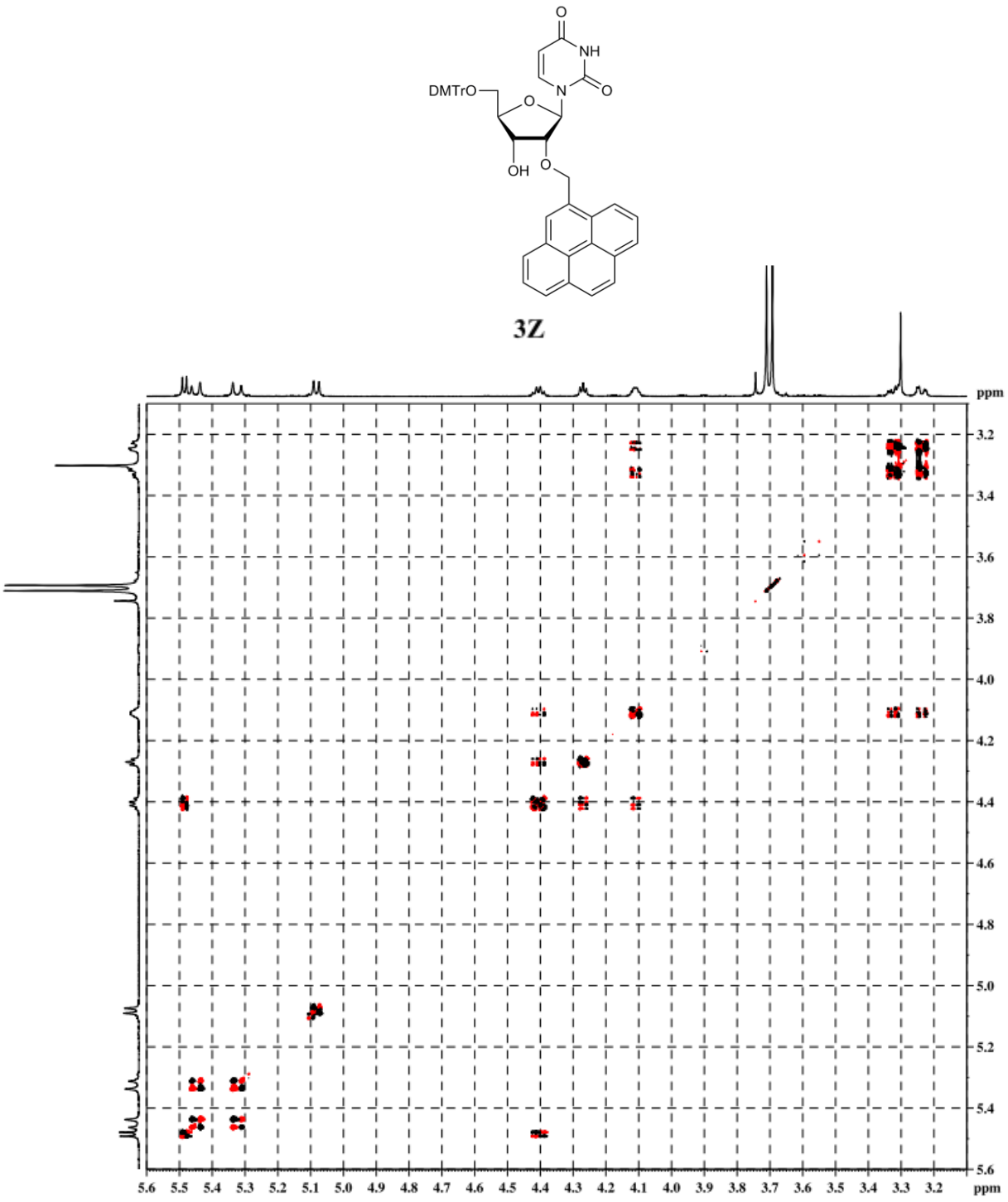
3Z

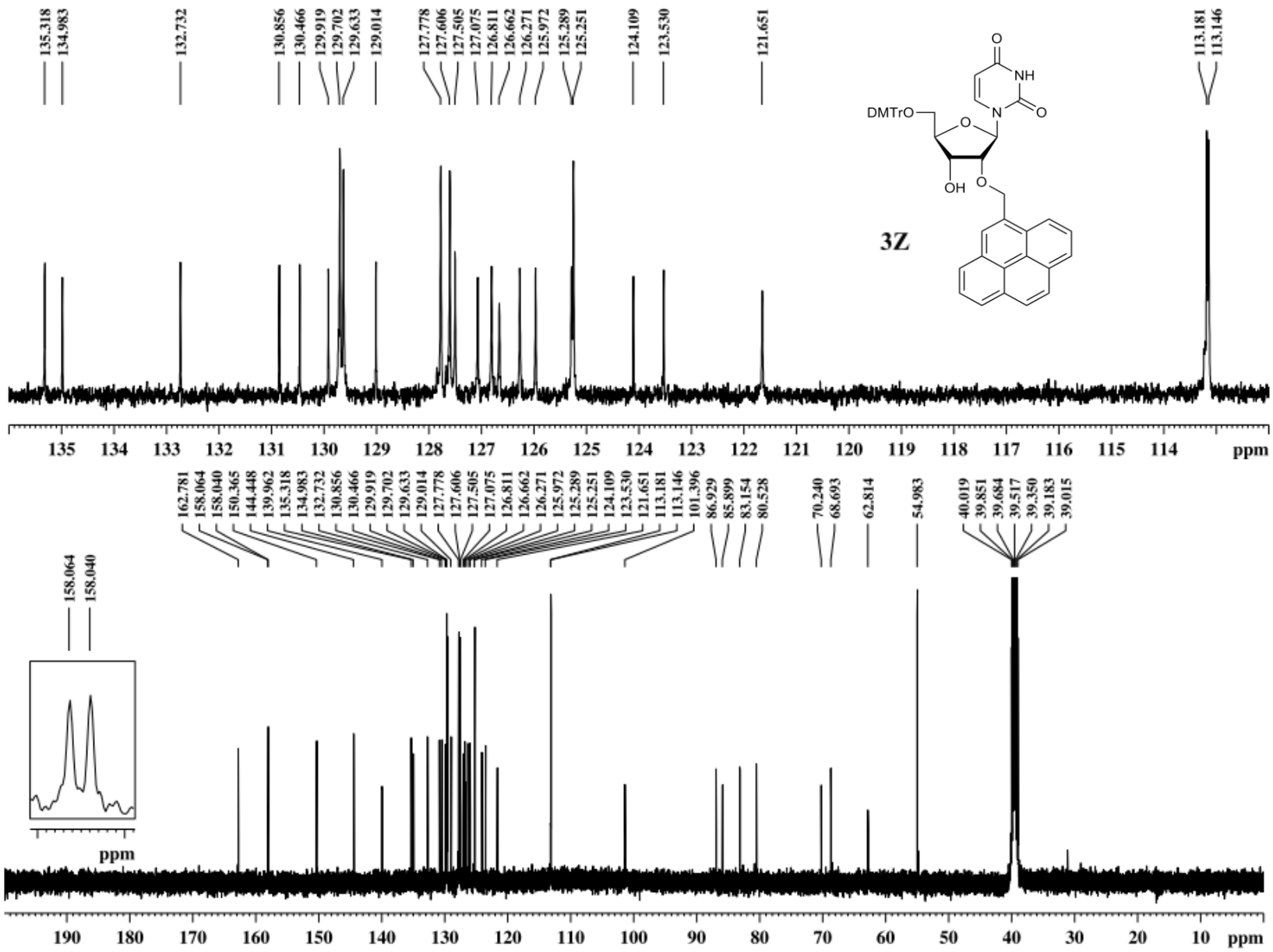

ppm
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm
ppm
5.30
5.35
5.40
5.45
5.50
8.28
8.27
8.26
ppm

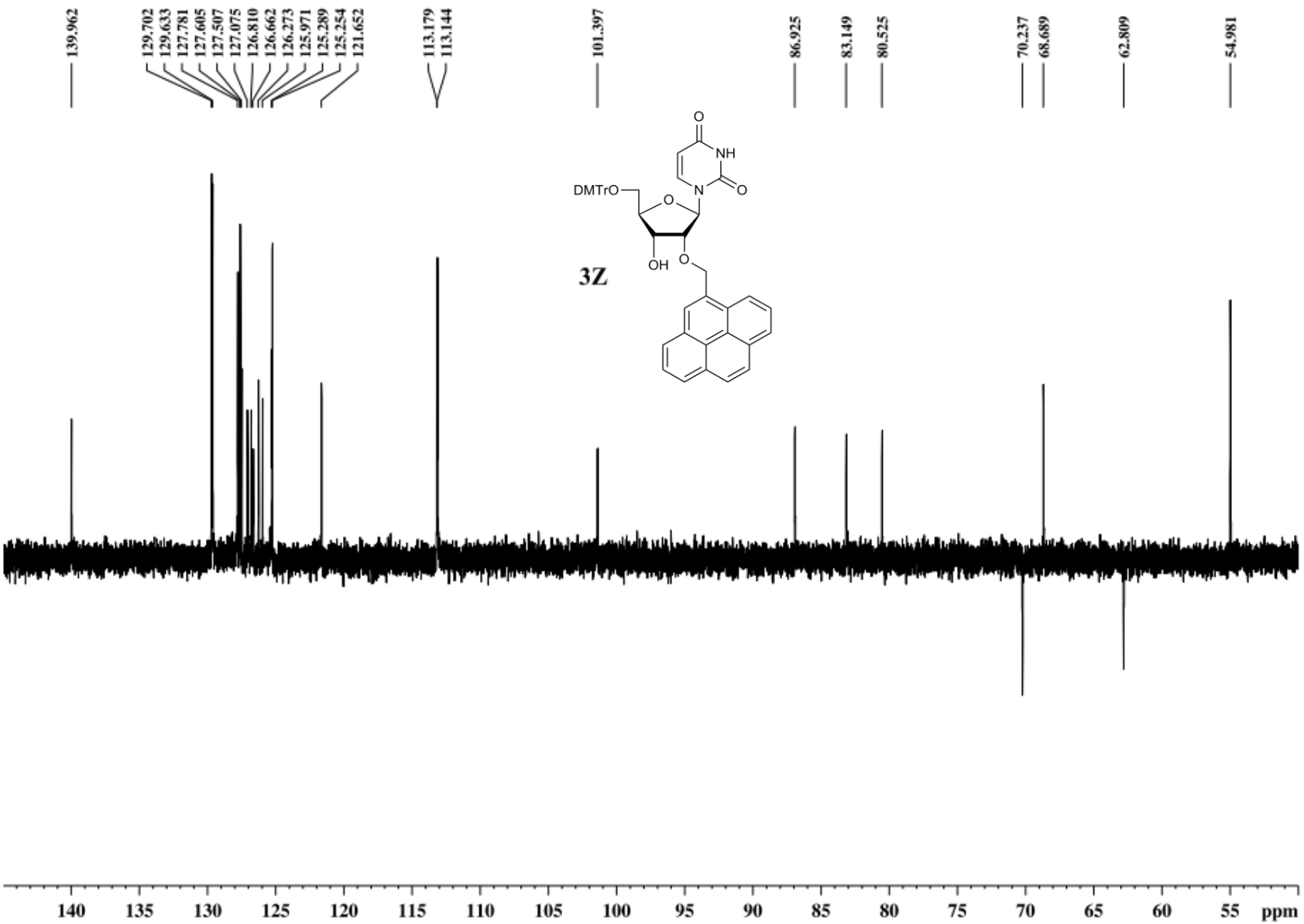

O
NH
N
O
DMTrO
O
OH
O

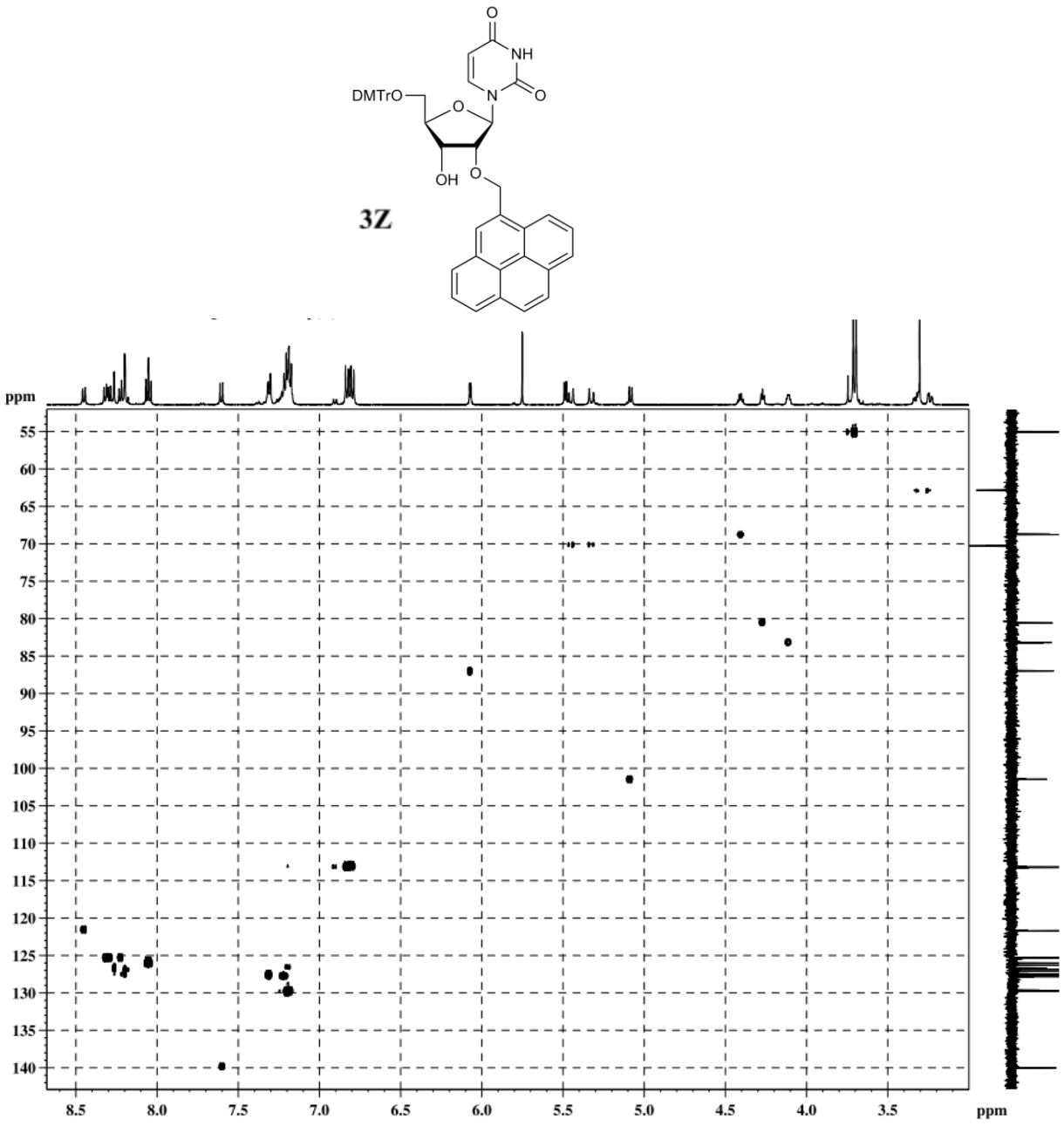
3Z

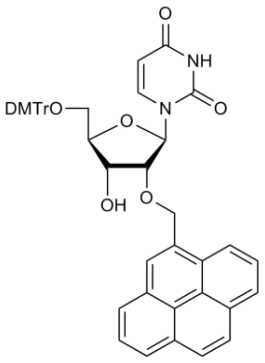

ppm
6.7
6.8
6.9
7.0
7.1
7.2
7.3
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
8.3
8.4
8.5
8.6
8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7
ppm
ppm
5.30
5.35
5.40
5.45
5.50
8.28 8.27 8.26
ppm


O
NH
N
O
DMTrO
O
OH
O
3Z
ppm
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2
ppm

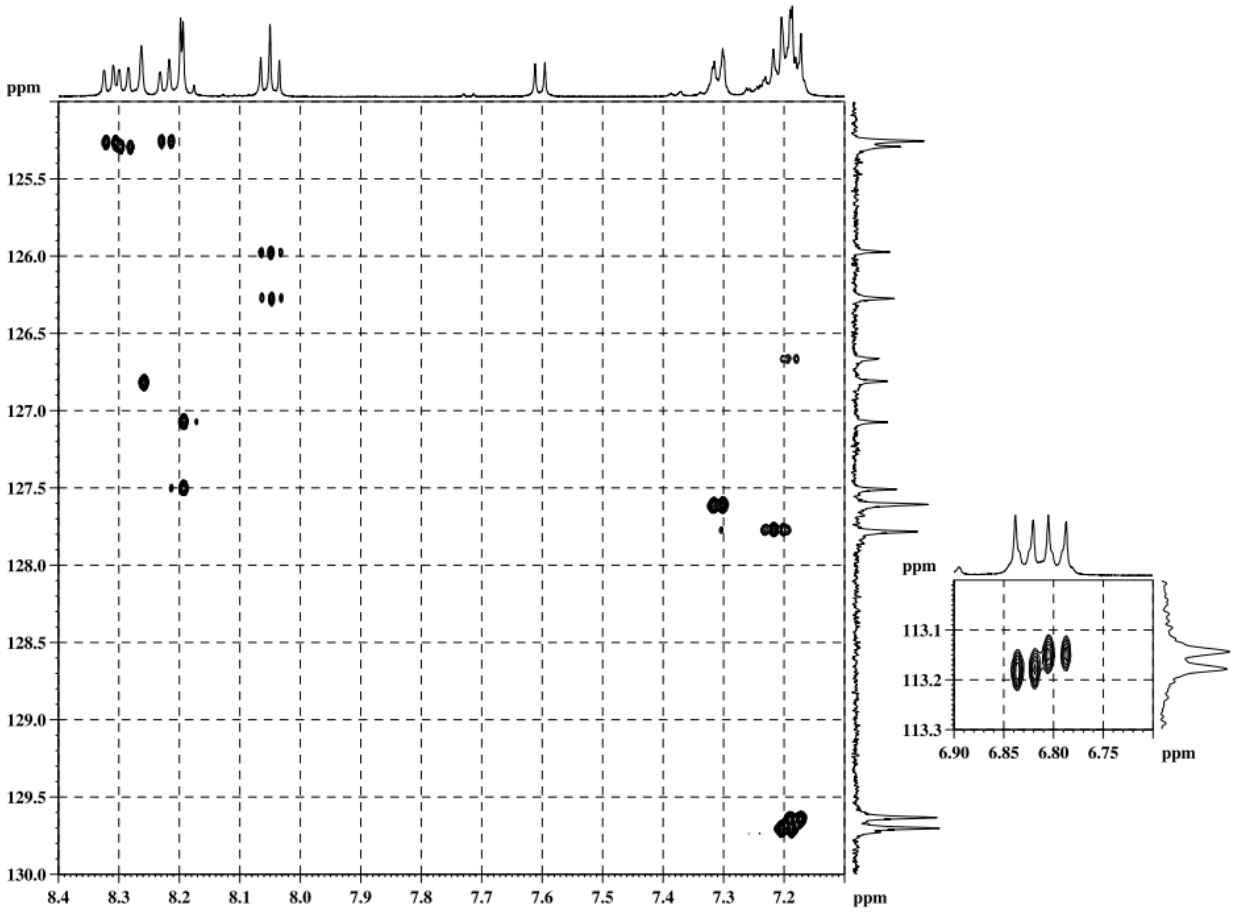

135.318
134.983
132.732
130.856
130.466
129.919
129.702
129.633
129.014
127.778
127.606
127.505
127.075
126.811
126.662
126.271
125.972
125.289
125.251
124.109
123.530
121.651
113.181
113.146
DMTrO
NH
O
N
OH
3Z
ppm
162.781
158.064
158.040
150.365
144.448
139.962
135.318
134.983
132.732
130.856
130.466
129.919
129.702
129.633
129.014
127.778
127.606
127.505
127.075
126.811
126.662
126.271
125.972
125.289
125.251
124.109
123.530
121.651
113.181
113.146
101.396
86.929
85.899
83.154
80.528
70.240
68.693
62.814
54.983
40.019
39.851
39.684
39.517
39.350
39.183
39.015
158.064
158.040
ppm

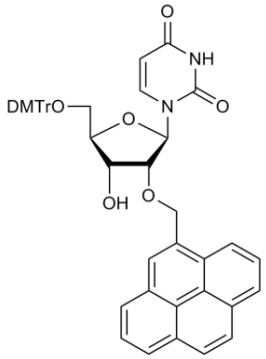

139.962
129.702
129.633
127.781
127.605
127.507
127.075
126.810
126.662
126.273
125.971
125.289
125.254
121.652
113.179
113.144
101.397
86.925
83.149
80.525
70.237
68.689
62.809
54.981
DMTrO
O
NH
N
O
O
OH
O
3Z
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
ppm


O
NH
N
O
DMTrO
O
OH
O
3Z
ppm
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm

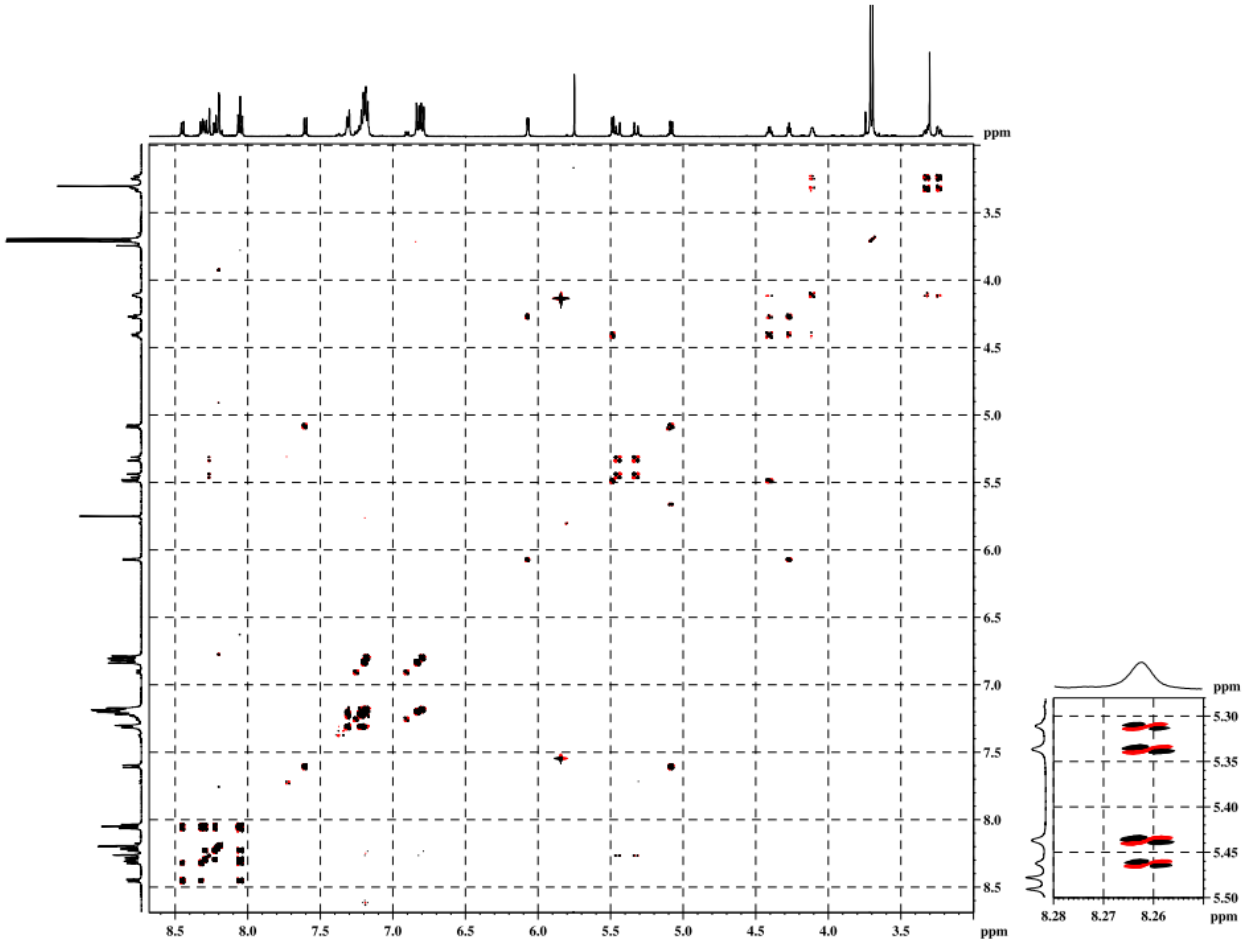

O
NH
DMTrO
N
O
O
OH
O

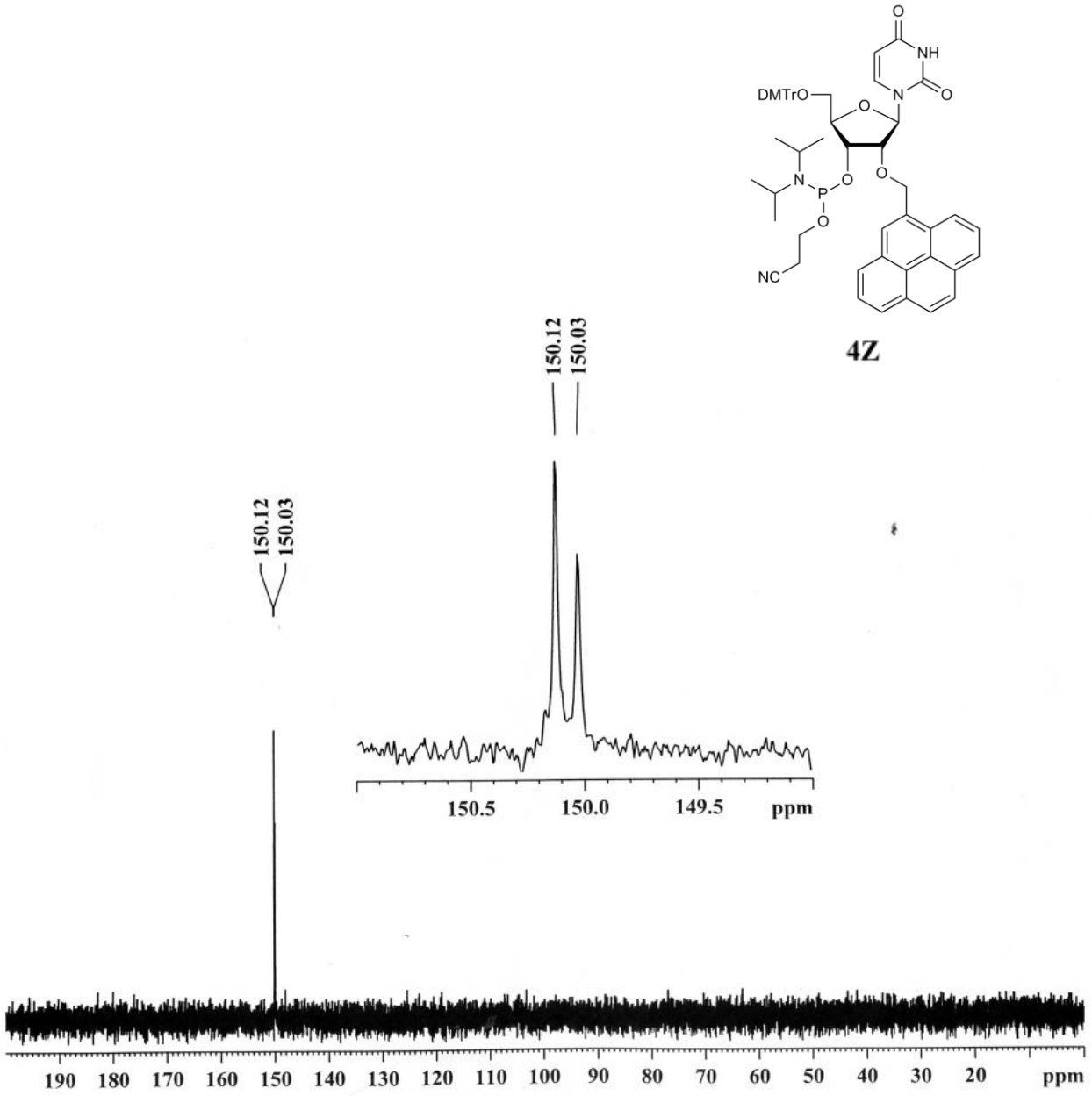
3Z


ppm
125.5
126.0
126.5
127.0
127.5
128.0
128.5
129.0
129.5
130.0
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
ppm
ppm
113.1
113.2
113.3
6.90
6.85
6.80
6.75
ppm


O
NH
DMTrO
N
O
O
OH
O

3Z


ppm
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm
ppm
5.30
5.35
5.40
5.45
5.50
8.28
8.27
8.26
ppm


DMTrO
NH
O
N
O
O
N
P
O
O
O
NC
4Z
150.12
150.03
150.12
150.03
150.5
150.0
149.5
ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm