SYNTHESIS AND HYBRIDIZATION PROPERTIES OF OLIGONUCLEOTIDES MODIFIED WITH 5-(1-ARYL-1,2,3-TRIAZOL-4-YL)-2'-DEOXYURIDINES

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SUPPORTING INFORMATION

Contents

General experimental section. Unless otherwise noted, 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. Dicholoromethane, 1,2-dichloroethane and *N,N'*-diisopropylethylamine were dried over activated molecular sieves (4Å). Reactions were monitored by TLC using silica gel coated plates with a fluorescence indicator (SiO_2-60, F-254) which were visualized under UV light and 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). Columns were built in the listed starting eluent containing 0.5% v/v pyridine. Evaporation of solvents was carried out under reduced pressure at temperatures below 45 °C. Following 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 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 (HSQC, COSY) 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 Q-TOF mass spectrometer using 2,5-dihydroxybenzoic acid as a matrix and mixture of polyethylene glycol (PEG 600) and (PEG 1000) as internal calibration standards.

Experimental protocols for preparation of nucleosides.

5-(1-phenyl-1*H*-1,2,3-triazol-4-yl)-2'-deoxyuridine (2X). Aqueous sodium ascorbate (1.0 M, 1.0 mL, 1.00 mmol), aq. $CuSO_4$ (0.73 mL, 7.5% w/v, 0.21 mmol) and 1-azidobenzene[S1] (83 mg, 0.70 mmol) were added to nucleoside **1** (200 mg, 0.35 mmol) in THF:H_2O:*t*BuOH (10 mL, 3:1:1, v/v/v). The reaction mixture was stirred for 4h at rt, whereupon it was diluted with EtOAc (30 mL) and brine (30 mL). The phases were separated and the organic phase was washed with sat.

aq. $NaHCO_3$ (30 mL), dried (Na_2SO_4) and evaporated to dryness. The resulting crude was purified by column chromatography (0-100% EtOAc in petroleum ether, v/v) to afford **2X** (180 mg, 74%) as a pale yellow solid material. R_f = 0.5 (80% EtOAc in petroleum ether, v/v); MALDI-HRMS: *m/z* 696.2456 ($[M+Na]^+$, $C_{38}H_{35}N_5O_7Na^+$, calc. 696.2434); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.79 (s, 1H, ex, NH), 8.81 (s, 1H, H6/Tz), 8.40 (s, 1H, H6/Tz), 7.91 (d, 2H, *J* = 7.8Hz, Ph), 7.60 (t, 2H, *J* = 7.8 Hz, Ph), 7.51-7.48 (t, 1H, *J* = 7.8 Hz, Ph), 7.38-7.37 (d, 2H, *J* = 7.5 Hz, DMTr), 7.29-7.23 (m, 6H, DMTr), 7.16-7.13 (m, 1H, DMTr), 6.84-6.81 (m, 4H, DMTr), 6.20 (ap t, 1H, *J* = 6.5 Hz, H1'), 5.35 (d, 1H, ex, *J* = 4.7 Hz, 3'-OH), 4.23-4.21 (m, 1H, H3'), 3.97-3.96 (m, 1H, H4'), 3.68-3.67 (2s, 6H, CH_3O), 3.25-3.23 (m, 2H, H5'), 2.30-2.28 (m, 2H, H2'); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.1, 158.0, 157.9, 149.5, 144.7, 139.8, 136.5, 136.3 (Ar), 135.5, 135.4, 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 128.6 (Ar), 127.7 (Ar), 127.6 (Ar), 126.5 (Ar), 120.1 (Ar), 120.0 (C6), 113.1 (Ar), 104.7, 85.72, 85.67 (C4'), 85.3 (C1'), 70.3 (C3'), 63.6 (C5'), 54.85 (CH_3O), 39.9 (C2'; overlap with DMSO, visible in DEPT).

5-[1-(Napth-1-yl)-1*H*-1,2,3-triazol-4-yl]-2'-deoxyuridine (2Y). Aqueous sodium ascorbate (1.0 M, 1.2 mL, 1.20 mmol), aq. $CuSO_4$ (1.1 mL, 7.5% w/v, 0.32 mmol) and 1-azidonapthalene[S2] (200 mg, 0.80 mmol) were added to nucleoside **1**(0.30 g, 0.53 mmol) in THF:H_2O:*t*BuOH (10 mL, 3:1:1, v/v/v). The reaction mixture was stirred for 4h at rt, whereupon it was diluted with EtOAc (30 mL) and brine (30 mL). The phases were separated and the organic phase was washed with sat. aq. $NaHCO_3$ (30 mL). The combined aqueous phase was back-extracted with EtOAc (30 mL). The combined organic phase was dried (Na_2SO_4), evaporated to dryness and the resulting crude purified by column chromatography (0-100% EtOAc in petroleum ether, v/v) to afford **2Y** (0.30 g, 78%) as a yellow solid material. R_f = 0.3 (70% EtOAc in petroleum ether, v/v); MALDI-HRMS: *m/z* 746.2625 ($[M+Na]^+$, $C_{42}H_{37}N_5O_7Na^+$ calc.746.2591); ^{1}H NMR (500

MHz, DMSO-d_6) δ 11.81 (s, 1H, ex, NH), 8.65 (s, 1H, H6/Tz), 8.49 (s, 1H, H6/Tz), 8.22-8.18 (m, 1H, Ar), 8.13 (d, 1H, J = 8.2 Hz, Ar), 7.72-7.70 (m, 2H, Ar), 7.69-7.65 (m, 1H, Ar), 7.61-7.57 (m, 1H, Ar), 7.48-7.46 (d, 1H, J = 8.5 Hz, Ar), 7.41-7.39 (m, 2H, Ar), 7.31-7.24 (m, 6H, Ar), 7.18-7.14 (m, 1H, Ar), 6.87-6.84 (m, 4H, Ar), 6.24 (ap t, 1H, J = 6.3 Hz, H1'), 5.37 (d, 1H, ex, J = 4.7 Hz, 3'-OH), 4.26-4.22 (m, 1H, H3'), 4.00-3.97 (m, 1H, H4'), 3.68-3.67 (2s, 6H, CH_3O), 3.27-3.26 (m, 2H, H5'), 2.34-2.31 (m, 2H, H2'); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.1, 157.97, 157.96, 149.6, 144.8, 139.0, 136.1 (Ar), 135.5, 135.4, 133.6, 133.1, 130.2 (Ar), 129.7 (Ar), 129.6 (Ar), 128.3 (Ar), 127.9, 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.0 (Ar), 126.5 (Ar), 125.4 (Ar), 124.4 (C6), 123.8 (Ar), 121.9 (Ar), 113.1 (Ar), 104.8, 85.74, 85.71(C4'), 85.2 (C1'), 70.4 (C3'), 63.7 (C5'), 54.9 (CH_3O), 39.9 (C2'; overlap with DMSO, visible in DEPT).

3'-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-5-(1-phenyl-1*H*-1,2,3-triazol-4-yl)-2'-deoxyuridine (3X). Nucleoside **2X** (0.30 g, 0.44 mmol) was co-evaporated with anhydrous 1,2-dichloroethane (3×10 mL) and redissolved in anhydrous CH_2Cl_2 (6 mL). To this was dropwise added *N*,*N*′-diisopropylethylamine (DIPEA; 300 µL, 1.75 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosporamidite (PCl-reagent; 200 µL, 0.60 mmol). The reaction mixture was stirred under an argon atmosphere for 3.5h at rt, evaporated to dryness and the resulting crude purified by column chromatography (0-3% MeOH in CH_2Cl_2, v/v) to afford phosphoramidite **3X** (230 mg, 60%) as a slightly yellow solid material. R_f = 0.7 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z:* 896.3505 ([M+Na]$^+$, $C_{47}H_{52}N_7O_8PNa^+$, calc.896.3512); ^{31}P NMR (121MHz, $CDCl_3$) δ 149.4, 149.0. The NMR data are in close agreement with previously published data.[S3]

3'-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-5-[1-(napth-1-yl)-1*H*-1,2,3-triazol-4-yl]-2'-deoxyuridine (3Y). Nucleoside **2Y** (200 mg, 0.24mmol) was co-evaporated with anhydrous 1,2-dichloroethane (3×10 mL) and redissolved in anhydrous CH_2Cl_2 (4 mL). DIPEA (170 µL, 0.98 mmol) and PCl-reagent (76 µL, 0.34 mmol) were added dropwise and the reaction mixture was stirred under an argon atmosphere for 3.5h at rt, evaporated to dryness and the resulting crude purified by column chromatography (0-3% MeOH in CH_2Cl_2, v/v) to afford phosphoramidite **3Y** (180 mg, 73%) as a slightly yellow solid material. R_f = 0.5 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS: *m/z* 946.3685 ($[M+Na]^+$, $C_{51}H_{54}N_7O_8PNa^+$, calc.946.3669; ^{31}P NMR (121 MHz, $CDCl_3$) δ 149.4, 149.0.

Synthesis and purification of ONs. ONs were made on a DNA synthesizer (0.2 µmol scale) using succinyl linked LCAA-CPG (long chain alkyl amine controlled pore glass) columns with 500Å pore size. Standard protocols for incorporation of DNA phosphoramidites were used. A ~50-fold molar excess of modified phosphoramidites in anhydrous acetonitrile (0.05M) was used along with extended oxidation (45s) and hand-coupling (20 min, 4,5-dicyanoimidazole as activator), which resulted in coupling yields of >95%, >95% and ~92% for monomers **X**, **Y** and **Z**, respectively. Cleavage from solid support and removal of nucleobase protecting groups was realized using 32% aq. ammonia (55 °C, 16 h). Crude 5'-DMTr-ONs were purified on HPLC (XTerra MS C18 10 µm 7.8 × 10 mm pre-column; XTerra MS C18 10 µm, 7.8 × 150 mm column) using a 0.05 mM TEAA (triethylammonium acetate) buffer - 25% water/acetonitrile (v/v) gradient. Purified ONs were detritylated using 80% aq. AcOH (20 min) and precipitated from acetone (1 mL) at -18 °C for 12-16h. The identity of the synthesized ONs was established through MS analysis recorded in positive ion mode on a quadrupole time-of-flight tandem mass

spectrometer equipped with a MALDI source using anthranilic acid as a matrix (Table S1), while purity (>80%) was verified by analytical RP-HPLC running in analytical mode.

Table S1. MALDI-ToF MS and ε_{260} of synthesized ONs.[a]

ON	Sequence	Calc. $(M+H)^+$	Exp. $(M+H)^+$	ε_{260} (OD/μmol)
X1	5'-GTGT **X** TTGC	2864	2864	84.5
X2	5'-GTG**X**T**X** TGC	2993	2993	84.0
X3	5'-GTGT**XX** TGC	2993	2993	84.0
X4	5'-GTG **XXXX**GC	3251	3251	83.0
Y1	5'-GTGT **Y** TTGC	2914	2914	89.0
Y2	5'-GTG**Y**T**Y** TGC	3094	3094	92.5
Y3	5'-GTGT**YY** TGC	3094	3093	92.5
Y4	5'-GTG **YYYY**GC	3452	3452	100.0
Z1	5'-GTGT **Z** TTGC	2988	2988	107.5
Z2	5'-GTG**Z**T**Z** TGC	3242	3242	130.0
Z3	5'-GTGT**ZZ** TGC	3242	3242	130.0
Z4	5'-GTG **ZZZZ**GC	3748	3748	175.0

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

Experimental protocol for thermal denaturation studies. ON concentrations were estimated using the following extinction coefficients for DNA (OD/μmol): G (12.01), A (15.20), T (8.40), C (7.05); for RNA (OD/μmol): G (13.70), A (15.40), U (10.00), C (9.00). The contributions from the chromophores were estimated at (OD/μmol): phenyl-1*H*-1,2,3-triazol-4-yl (7.8),[S4] naphthalene (3.8)[S5] and pyrene (22.4).[S6] The strands comprising a given duplex were mixed and annealed. Thermal denaturation temperatures of duplexes (1.0 μM final concentration of each strand) were determined on a temperature-controlled UV/VIS spectrophotometer using quartz optical cells with 1.0 cm pathlengths. T_m's were determined as the first derivative maximum of thermal denaturation curves (A_{260} vs. T) recorded in either medium or high salt buffer (100 mM or 710 mM NaCl, 0.1 mM EDTA, pH 7.0 adjusted with 10 mM Na_2HPO_4 and 5 mM Na_2HPO_4). The temperature of the denaturation experiments ranged from at least 15 °C below T_m to 20 °C above T_m (although not below 5 °C). A temperature ramp of 0.5 °C/min was used in all experiments. Reported T_m-values are reported as averages of two experiments within ± 1.0 °C.

Experimental protocol for steady-state fluorescence emission studies. Steady-state fluorescence emission spectra of **Z**-modified ONs and their duplexes with matched/mismatched DNA/RNA targets, were recorded in non-deoxygenated thermal denaturation buffer (each strand 1.0 μM) using an excitation wavelength of λ_{ex} = 344 nm, excitation slit 5.0 nm, emission slit 5.0 nm and a scan speed of 600 nm/min. Experiments were performed at 10 °C. Emission spectra (360–600 nm range) were obtained as an average of five scans.

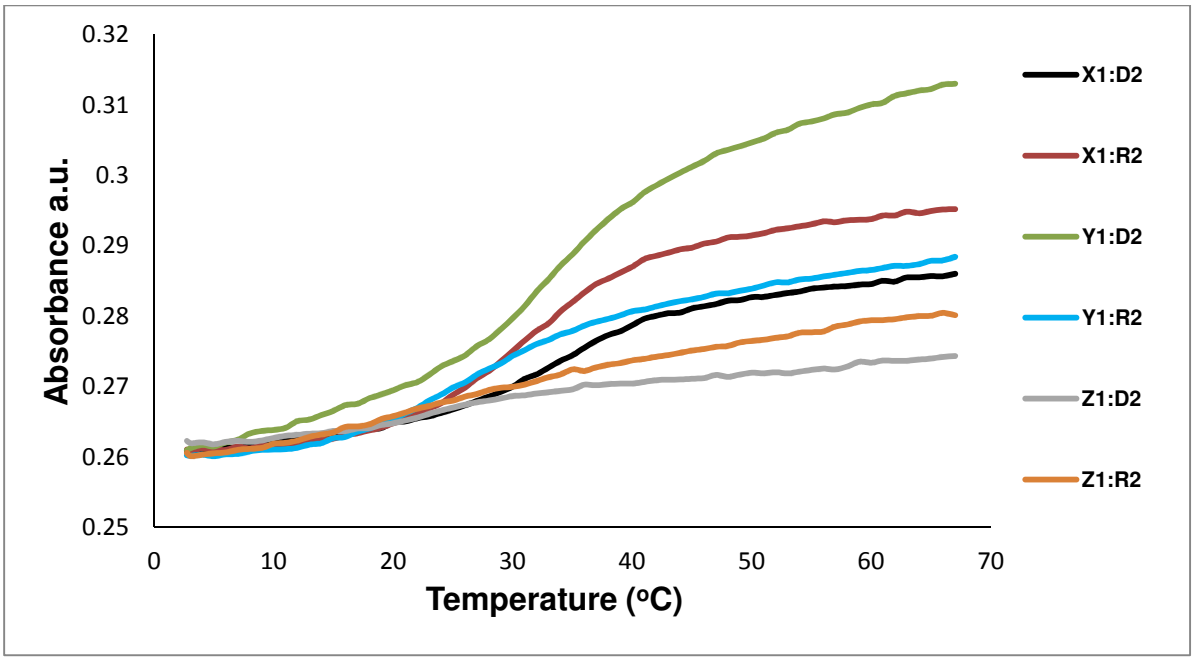

Figure S1. Thermal denaturation curves of duplexes involving **B1**-series ONs.

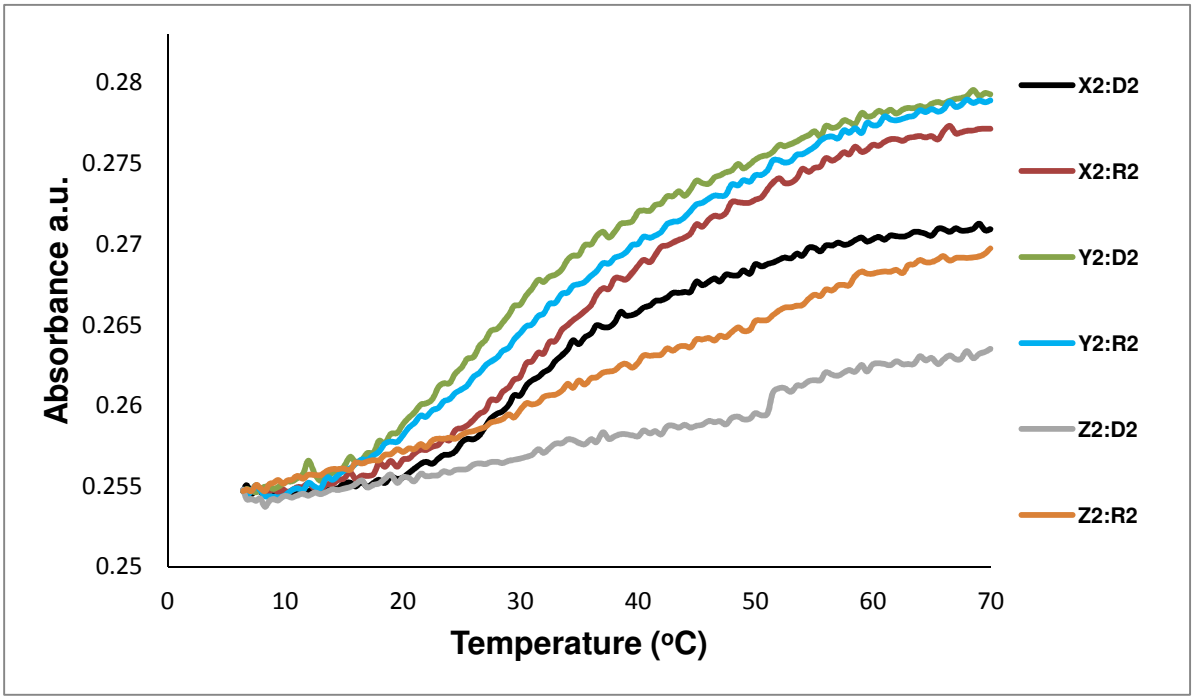

Figure S2. Thermal denaturation curves of duplexes involving **B2**-series ONs.

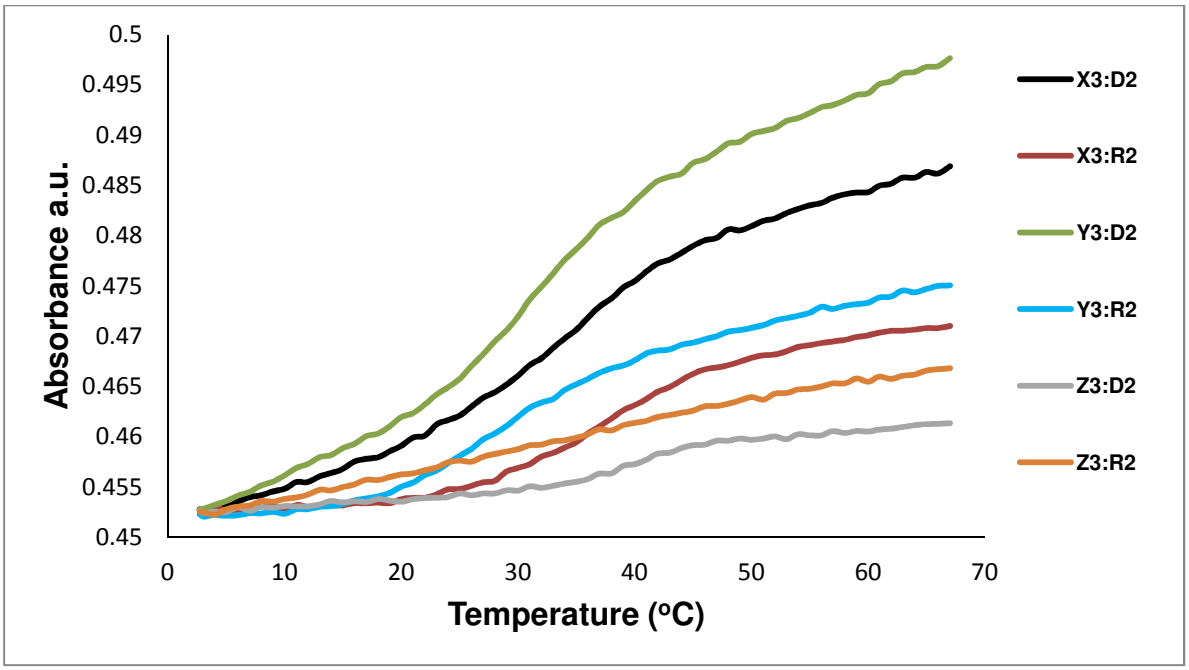

Figure S3. Thermal denaturation curves of duplexes involving **B3**-series ONs.

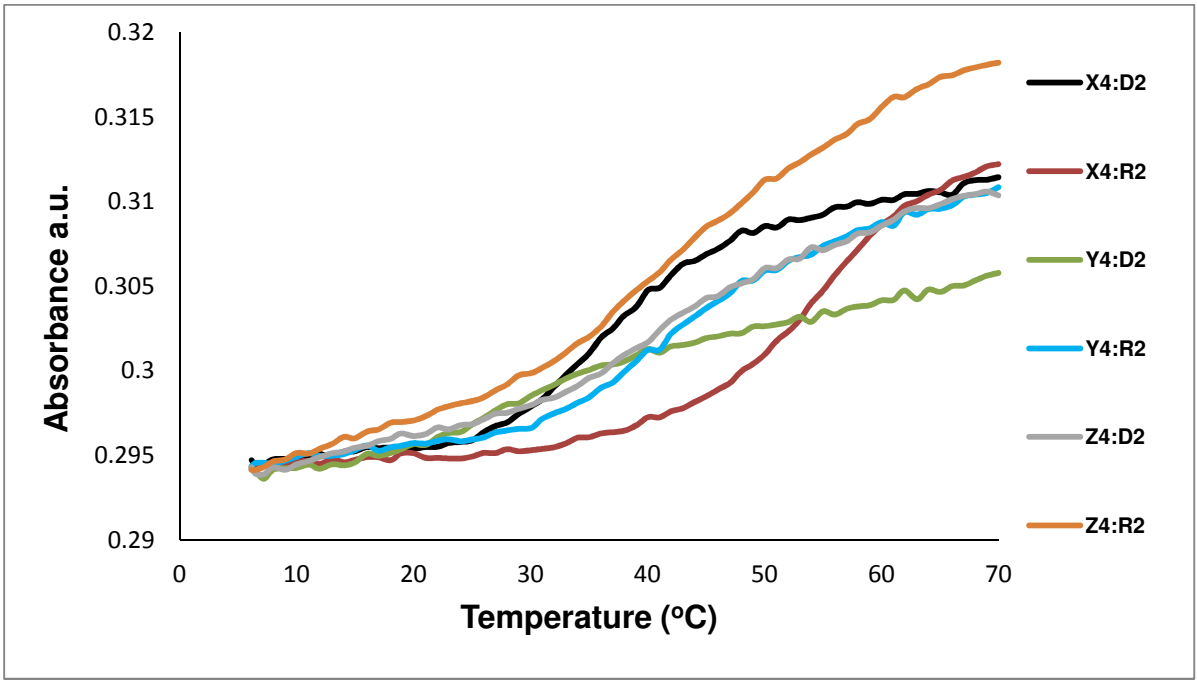

Figure S4. Thermal denaturation curves of duplexes involving **B4**-series ONs.

Table S2. Thermal denaturation temperatures (T_m values)for duplexes between **B1/B2/B4** and complementary DNA or RNA in medium salt buffer.[a]

			T_m (ΔT_m/mod) [°C]					
			DNA			RNA		
			3'-CAC AAA ACG			3'-CAC AAA ACG		
ON	Sequence	B =	**X**	**Y**	**Z**	**X**	**Y**	**Z**
B1	5'-GTGT **B** TTGC		27.0 [-6.0]	22.5 [-10.5]	14.5 [-18.5]	28.0 [-3.0]	-	-
B2	5'-GTGT **BB** TGC		26.0 [-3.5]	22.0 [-5.5]	-	34.5 [+1.8]	27.5 [-1.8]	-
B4	5'-GTG **BBBB** GC		28.0 [-1.3]	17.5 [-3.9]	-	45.5 [+3.6]	37.5 [+1.6]	-

[a] T_m's determined as the first derivative maximum of thermal denaturation curves (A_{260} vs T) recorded in medium salt buffer ($[Na^+]$ = 110 mM, $[Cl^-]$ = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), using 1.0 µM of each strand. T_m's are averages of at least two measurements within 1.0 °C. ΔT_m/mod = change in T_m's per modification relative to unmodified reference duplexes (+cDNA: T_m= 33.0 °C; +cRNA: T_m = 31.0 °C). "-" denotes weak or no transition.

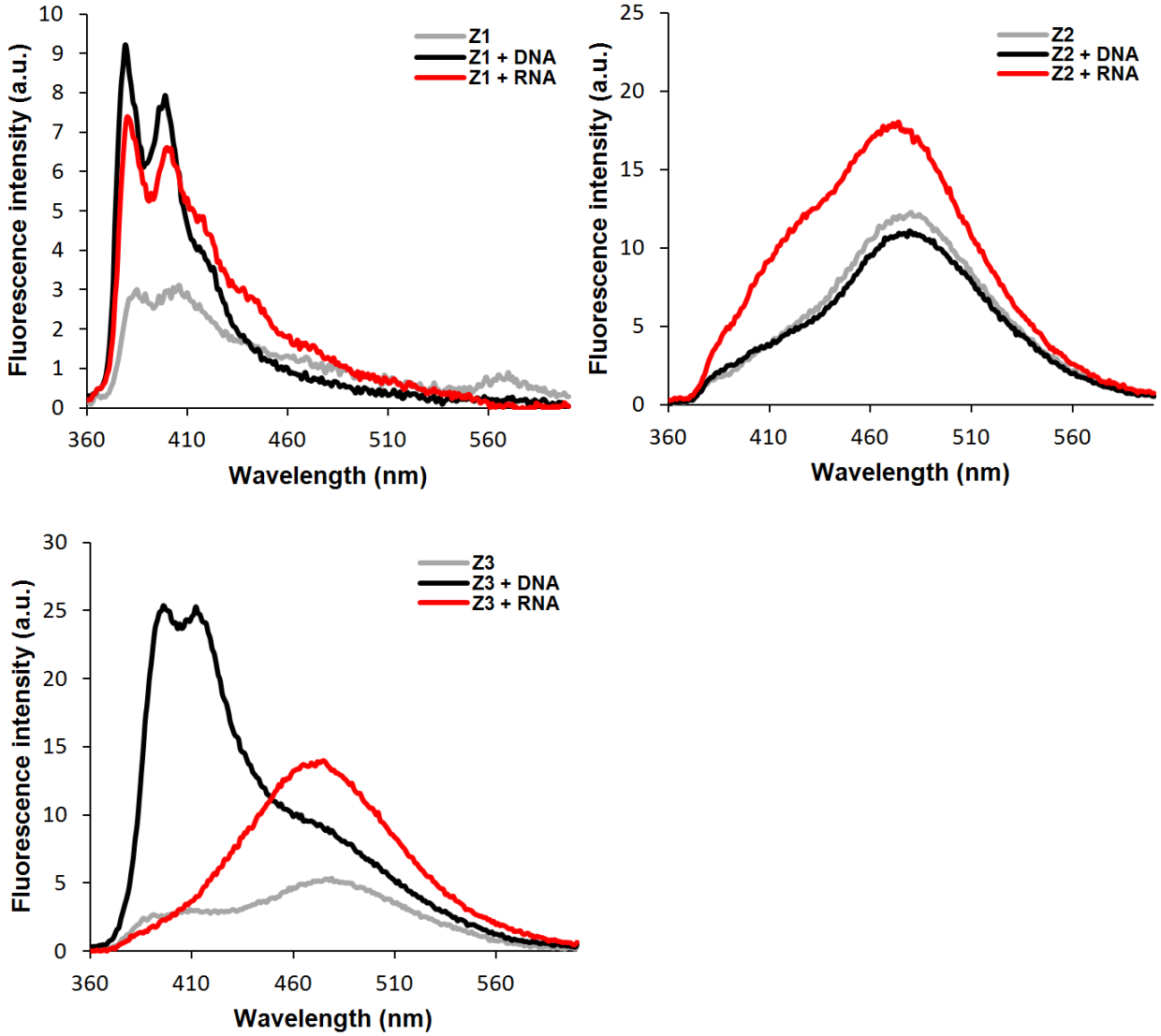

Figure S5. Steady-state fluorescence emission spectra of **Z1**-**Z3** in the presence or absence of complementary DNA/RNA (λ_{em} = 344 nm; T = 10 °C).

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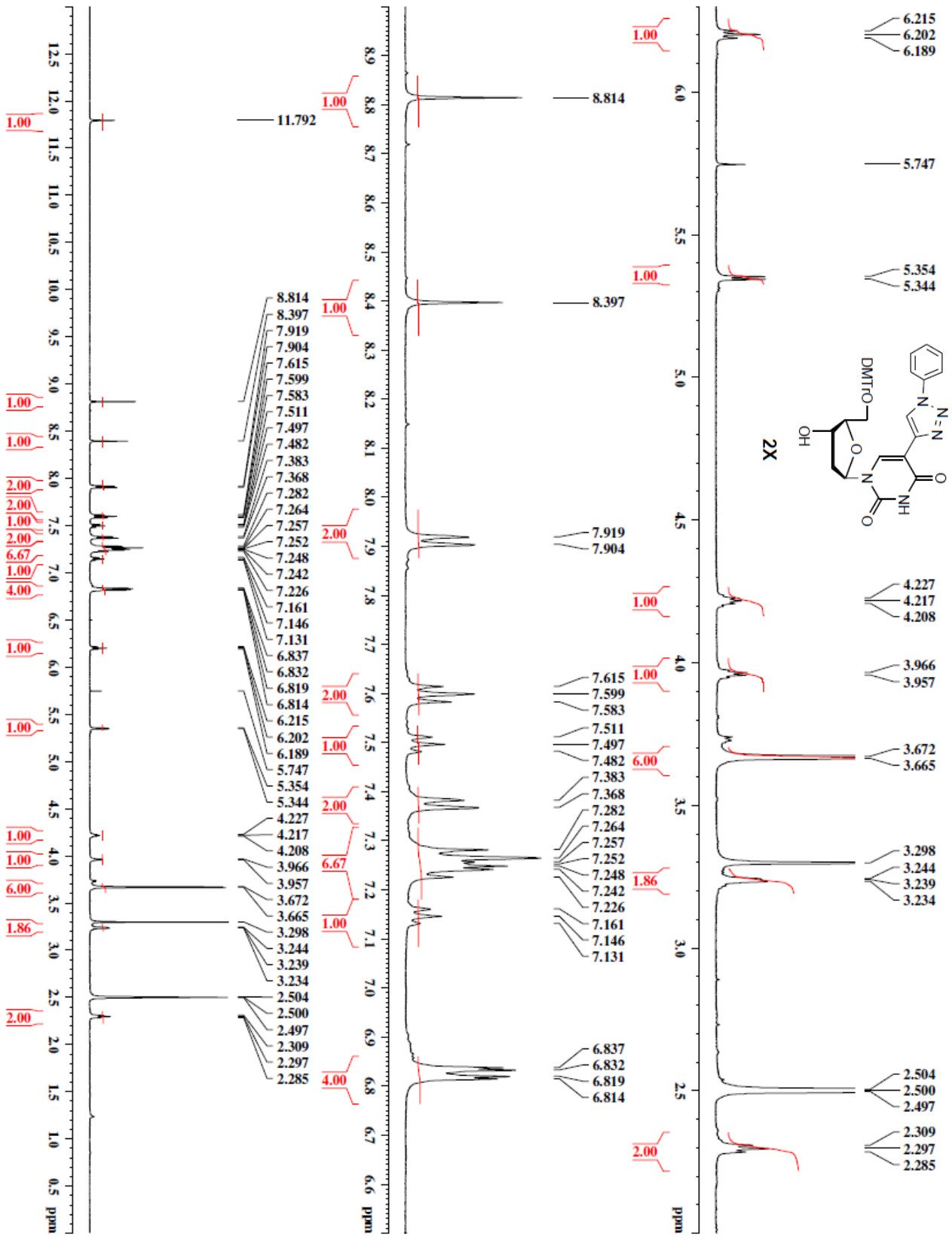

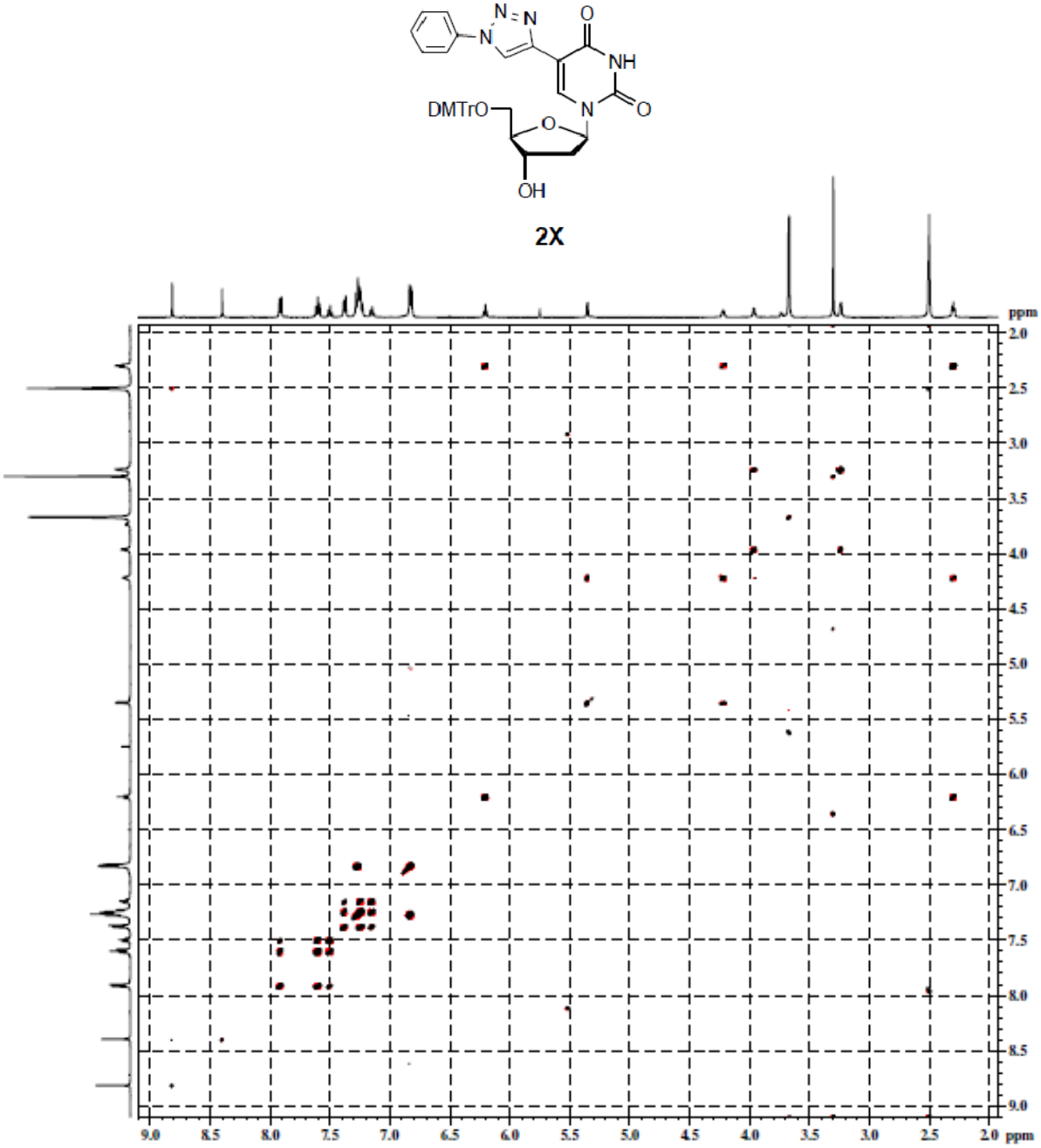

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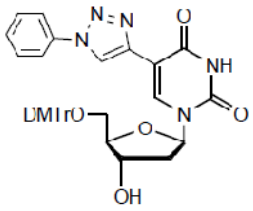

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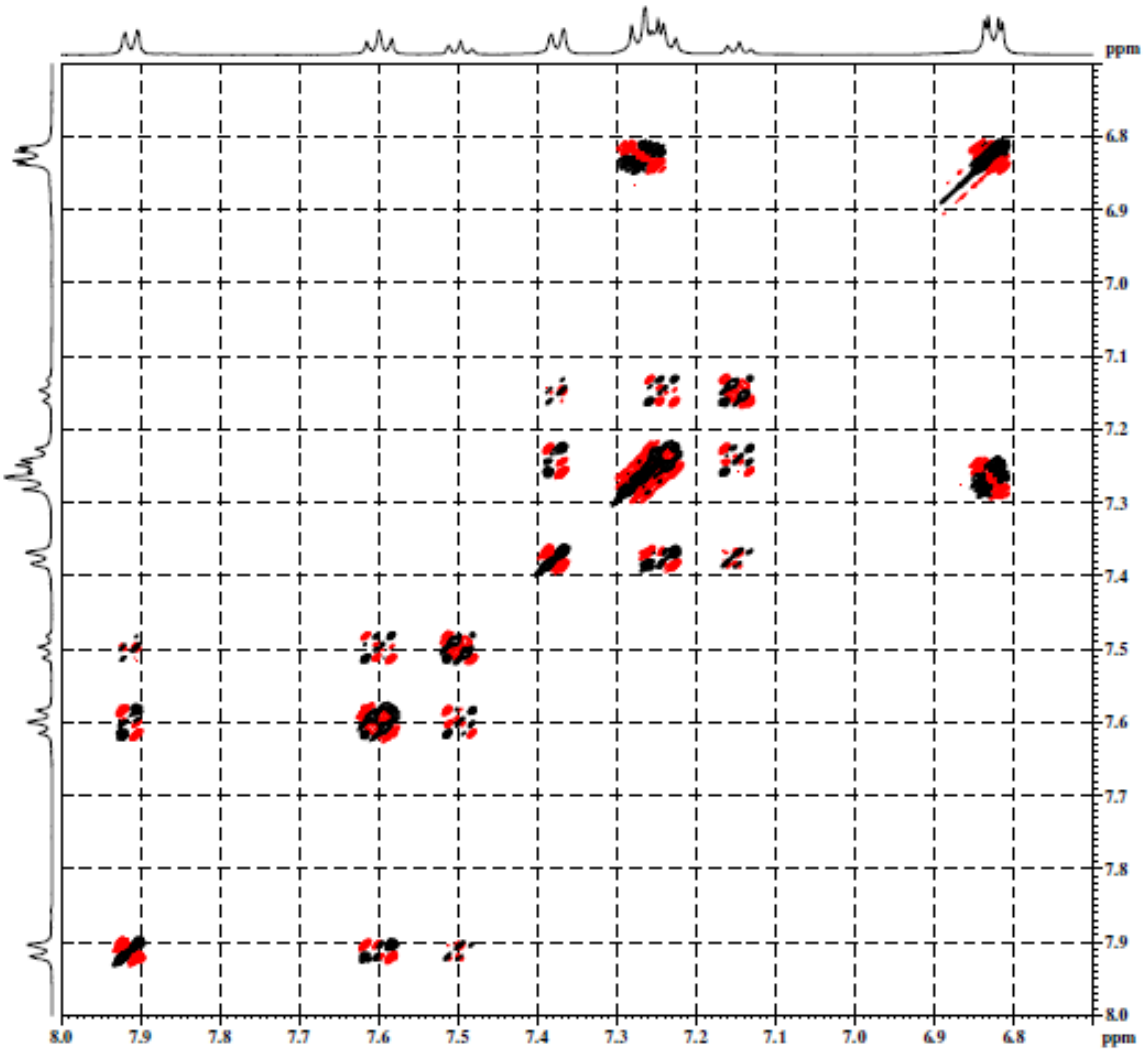

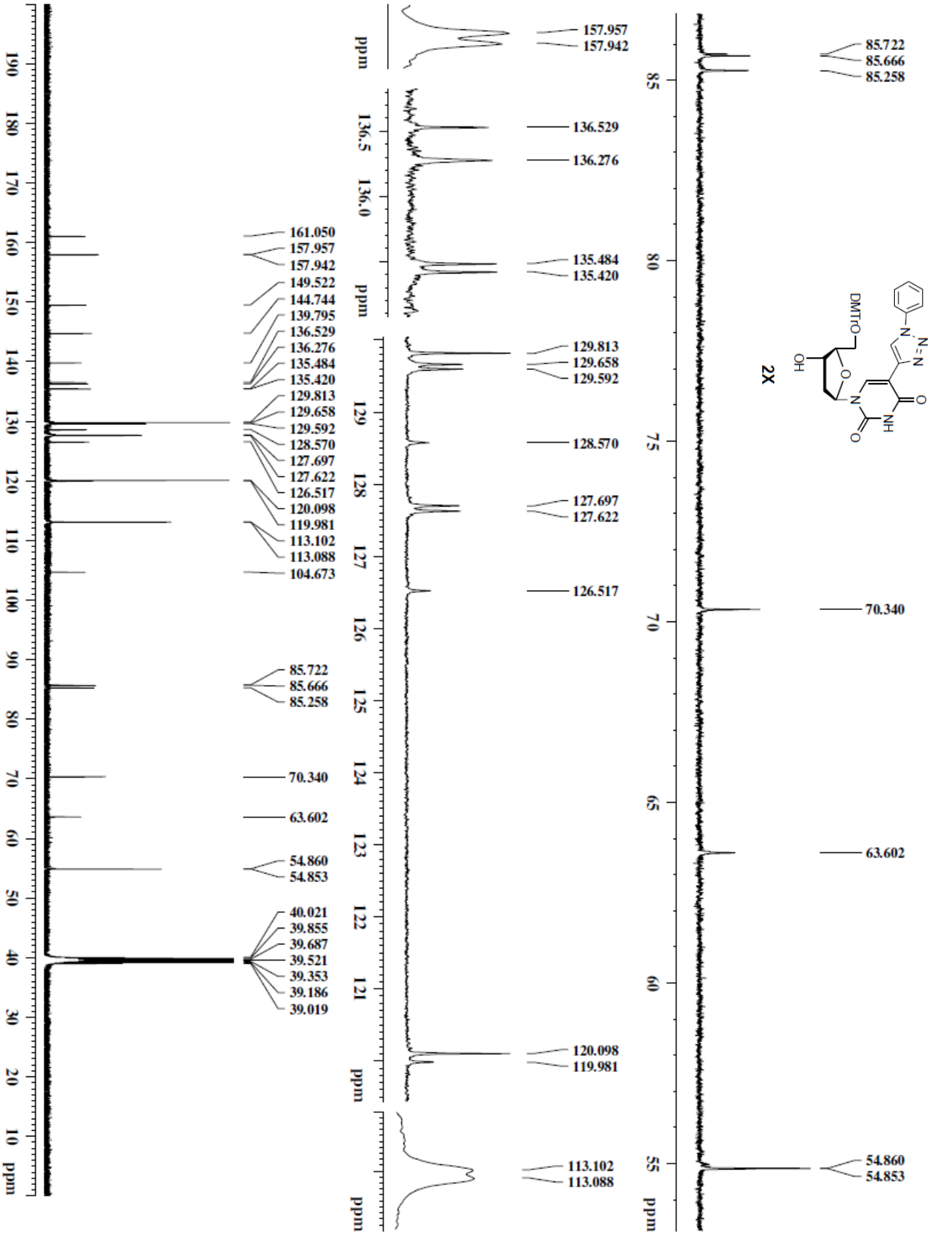

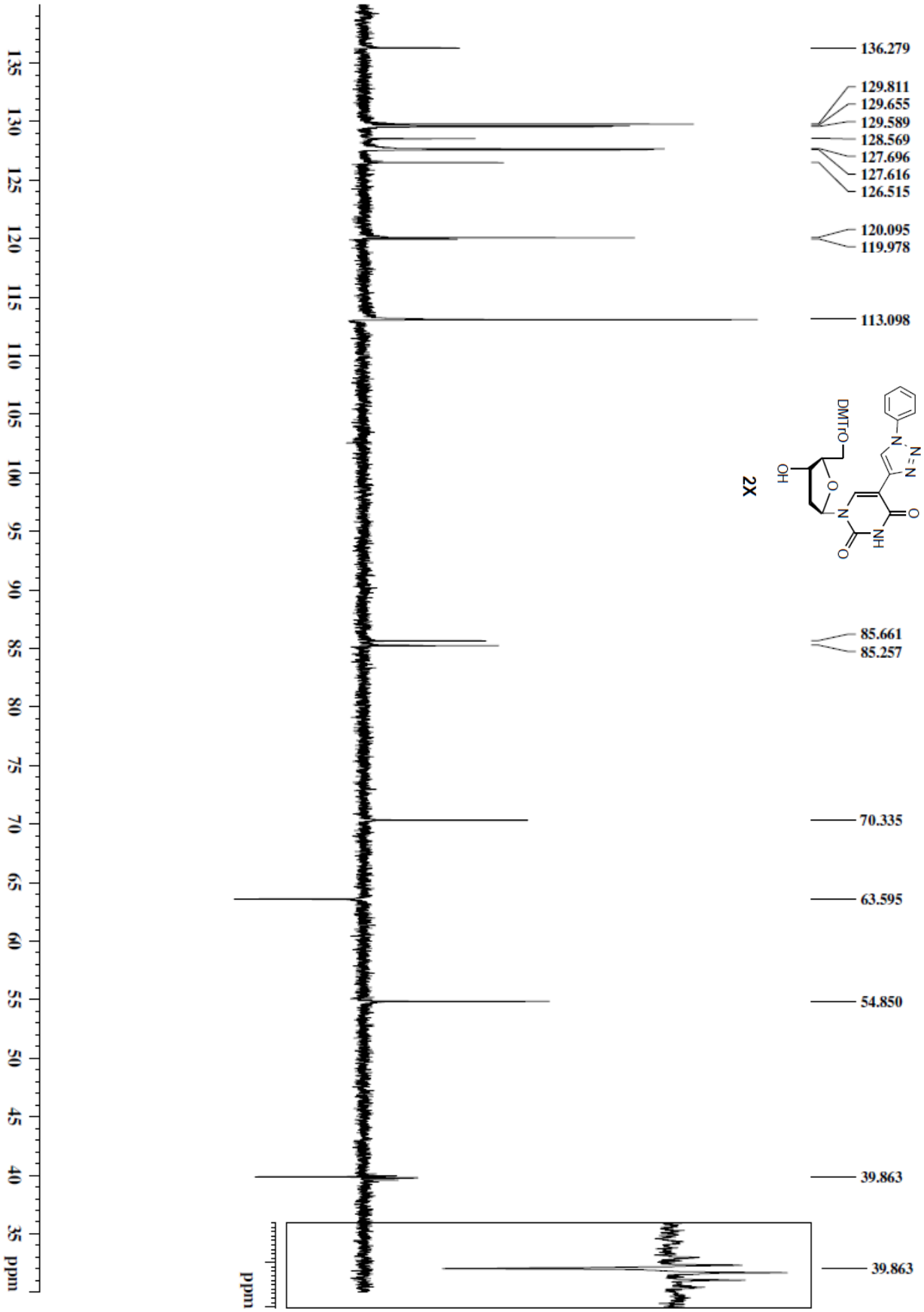

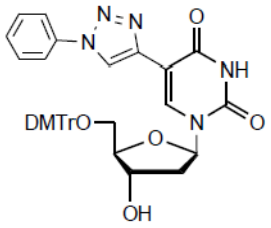

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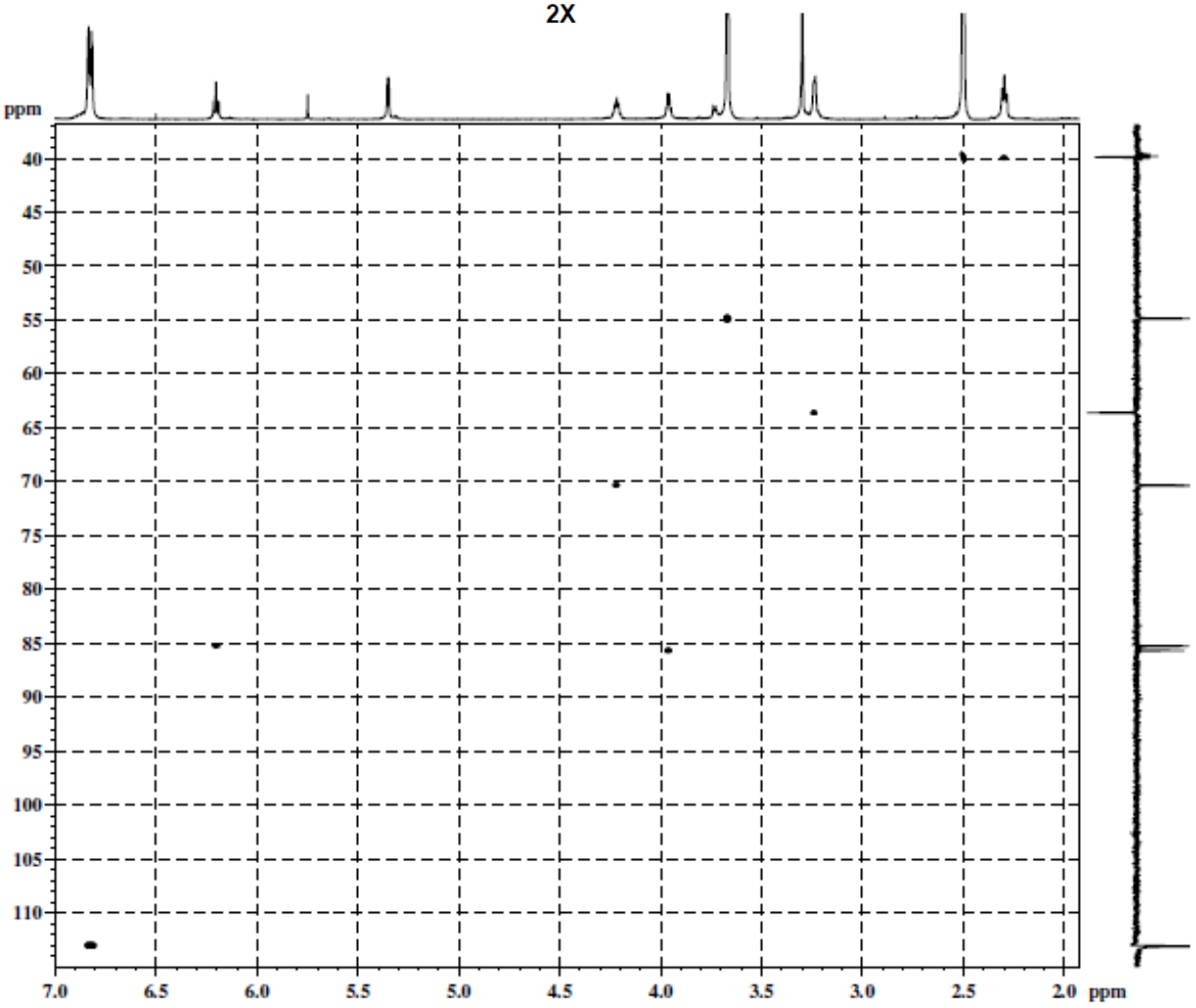

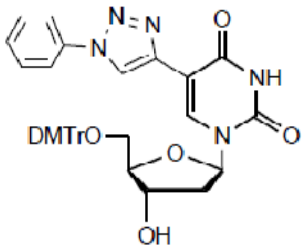

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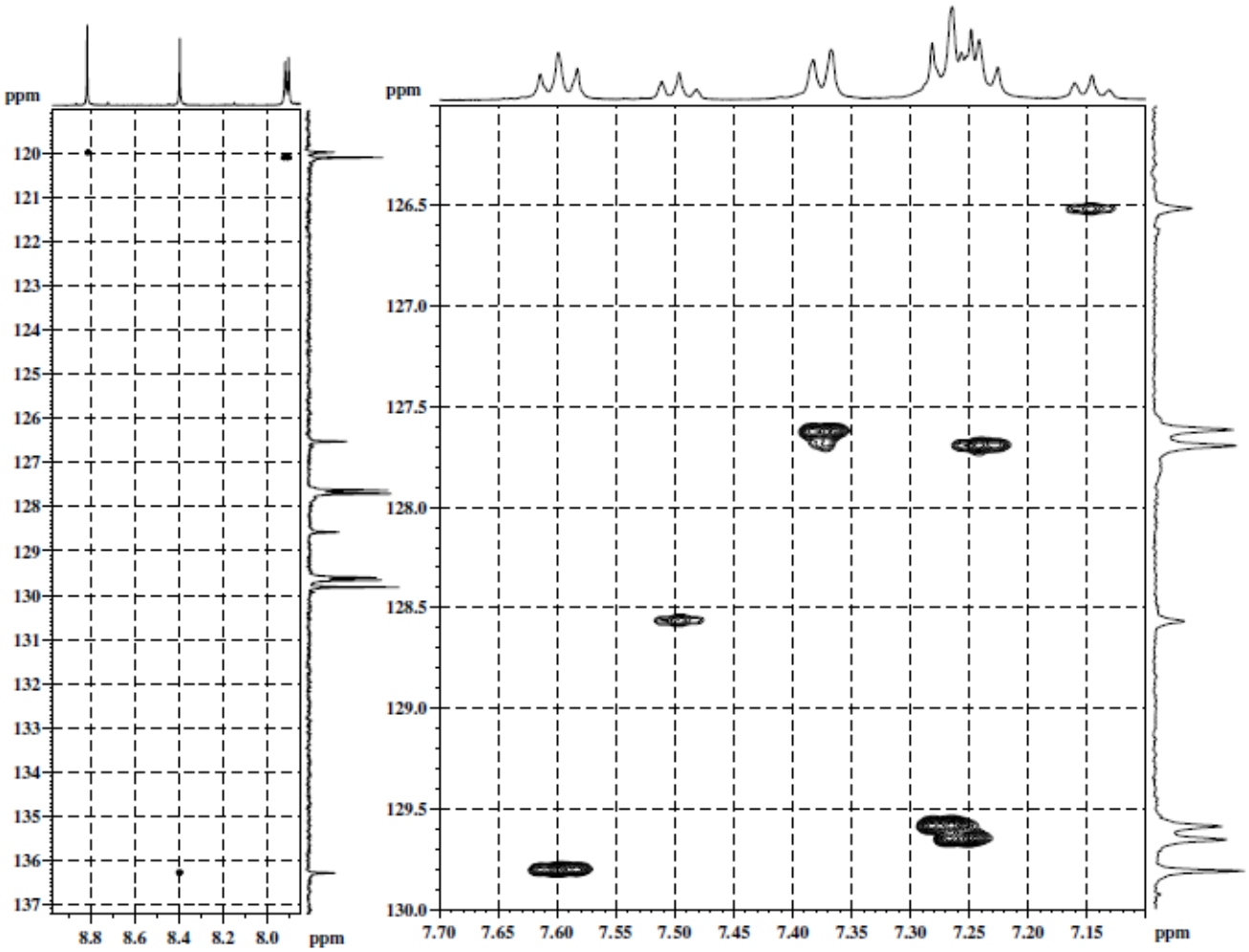

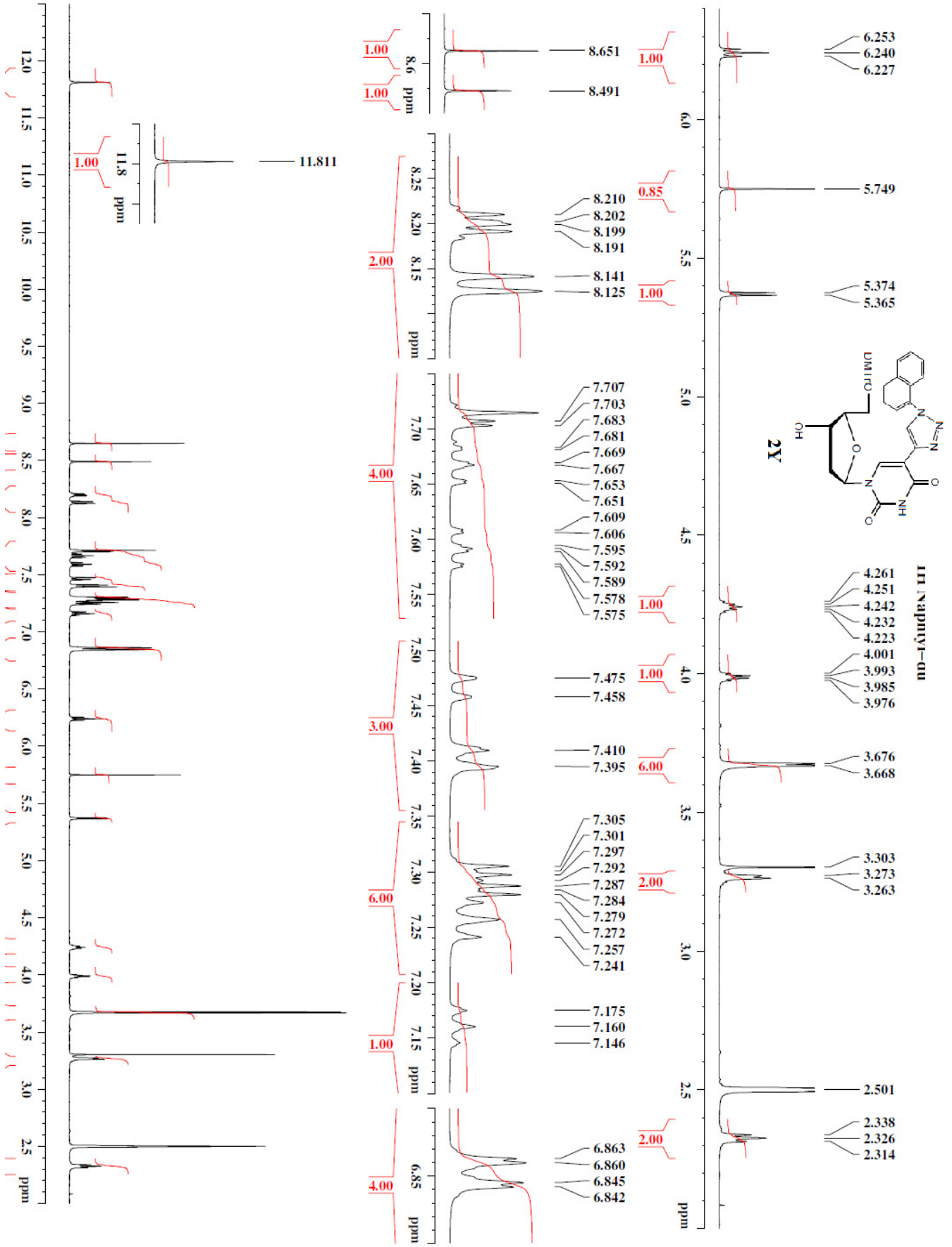

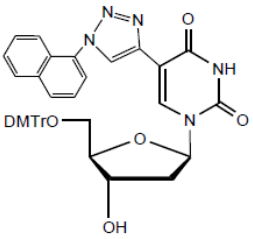

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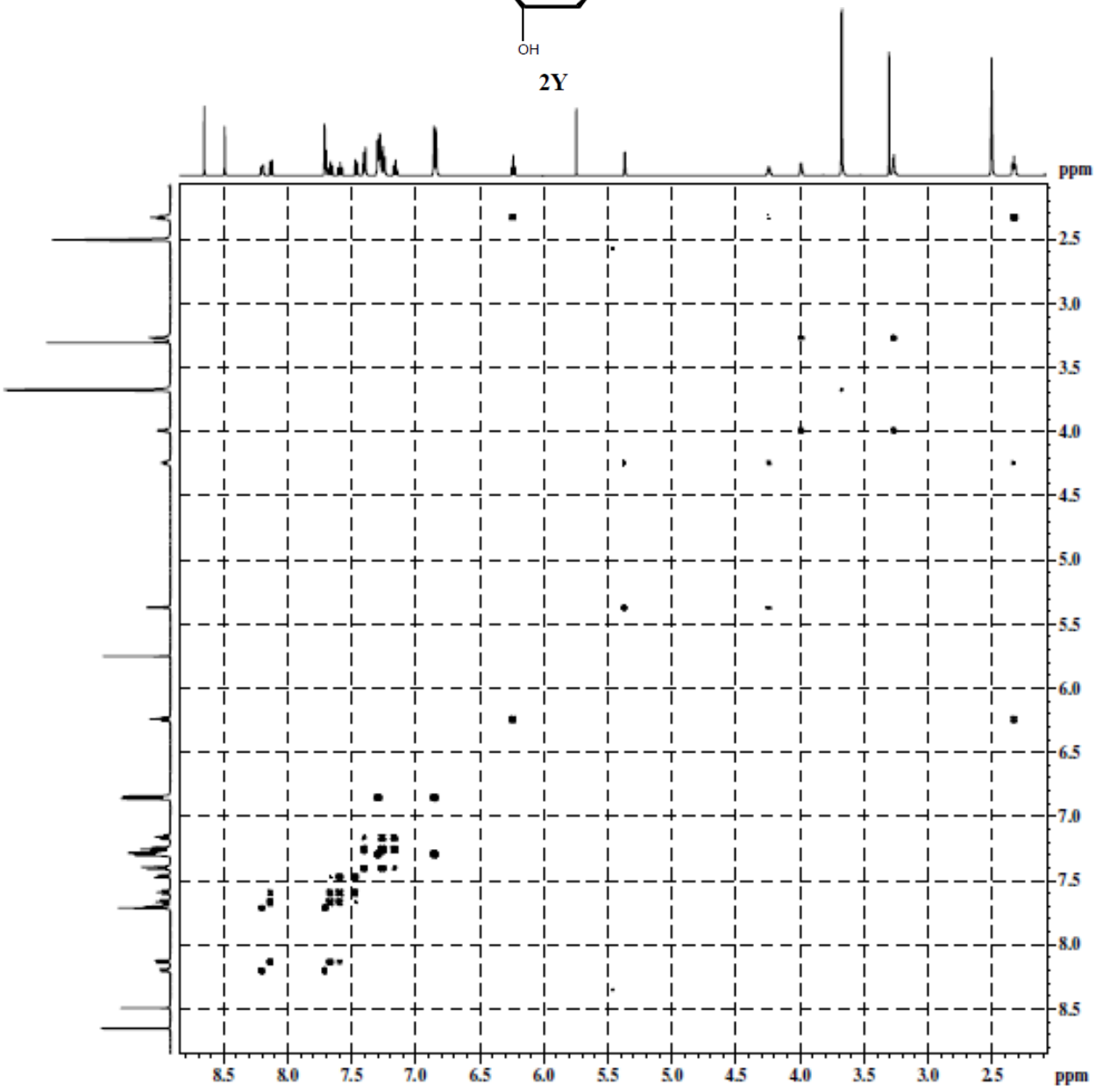

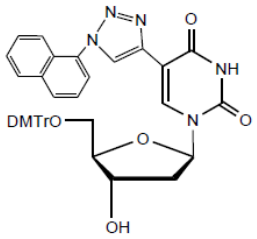

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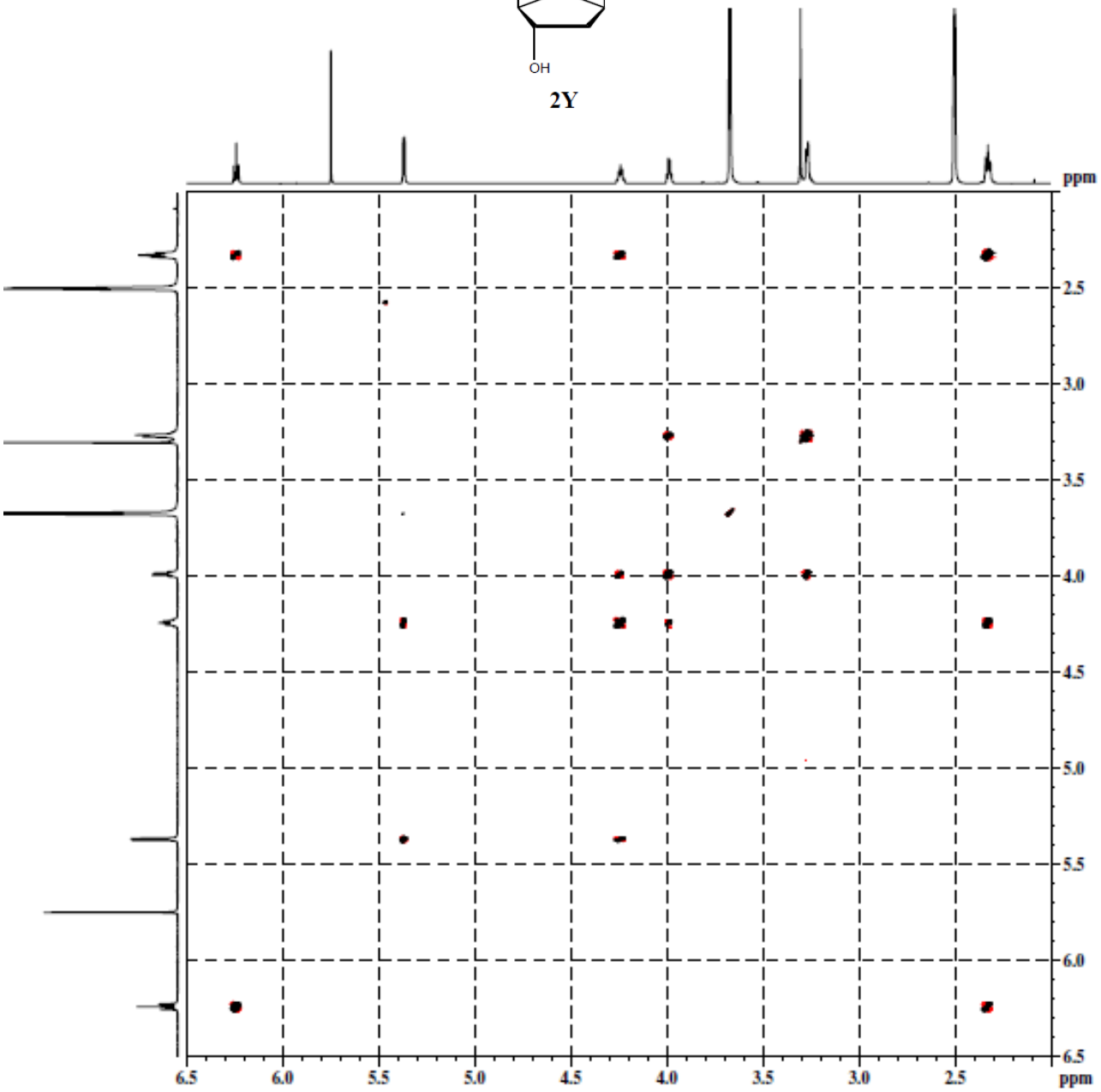

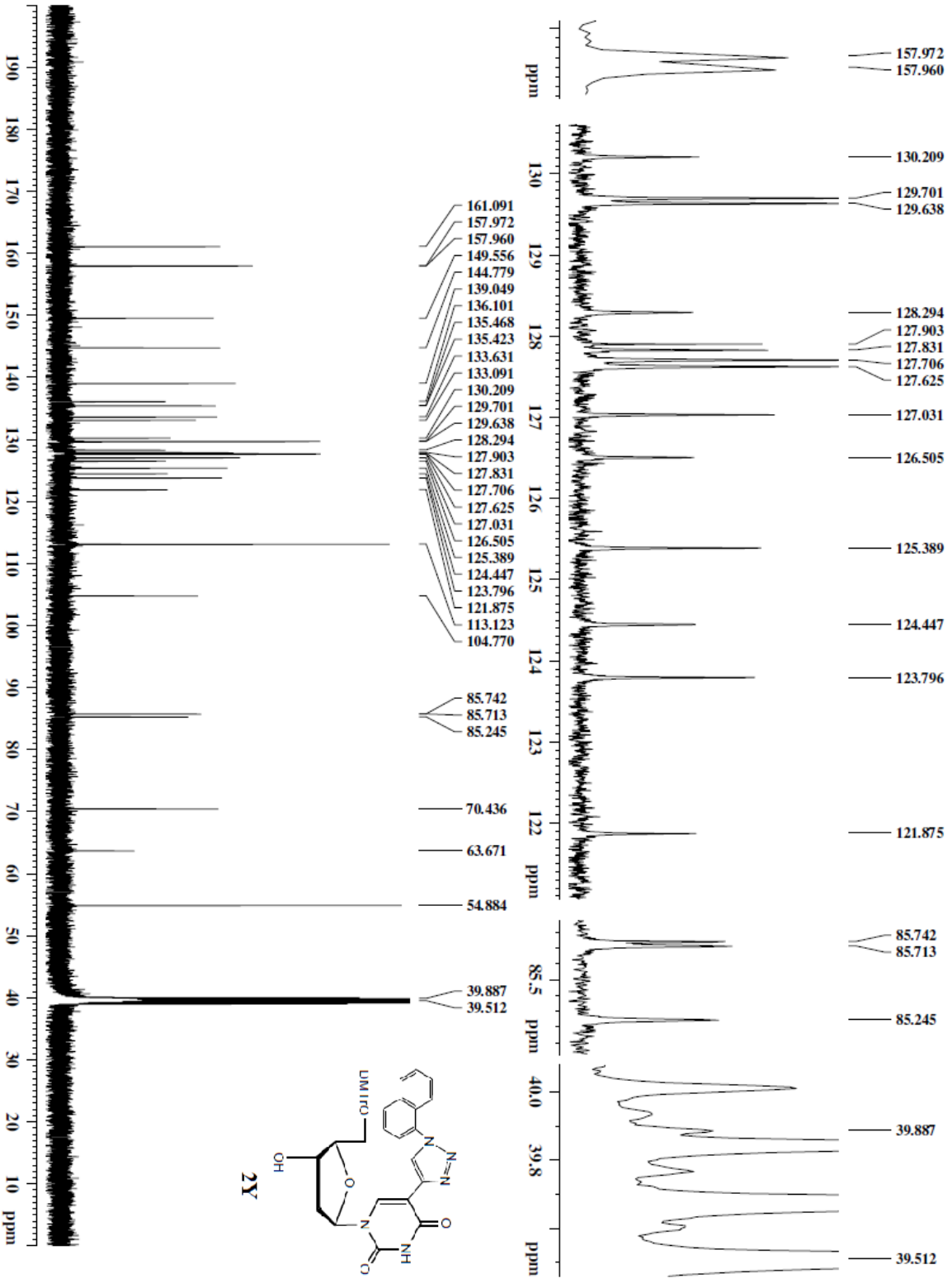

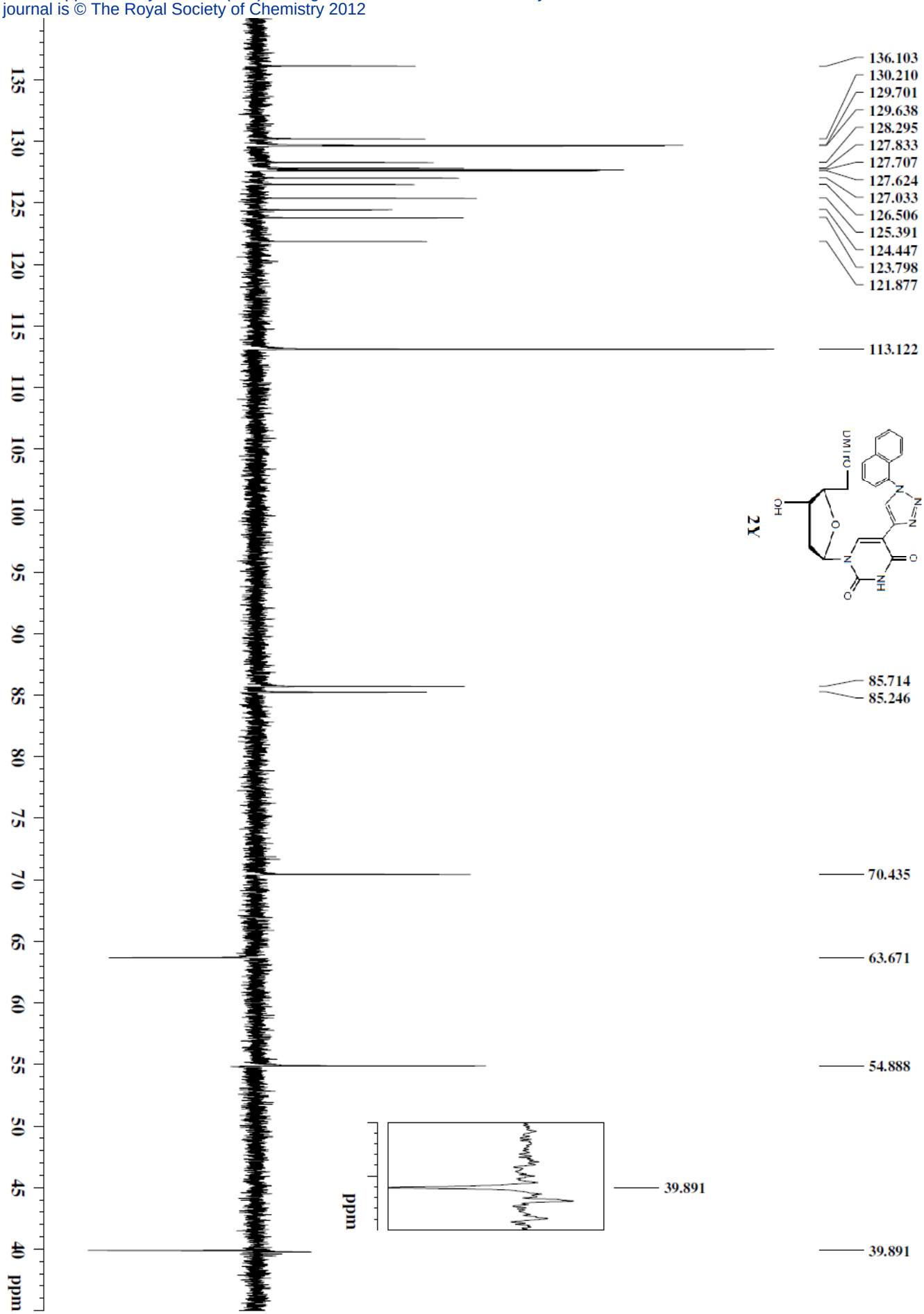

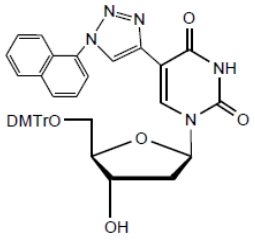
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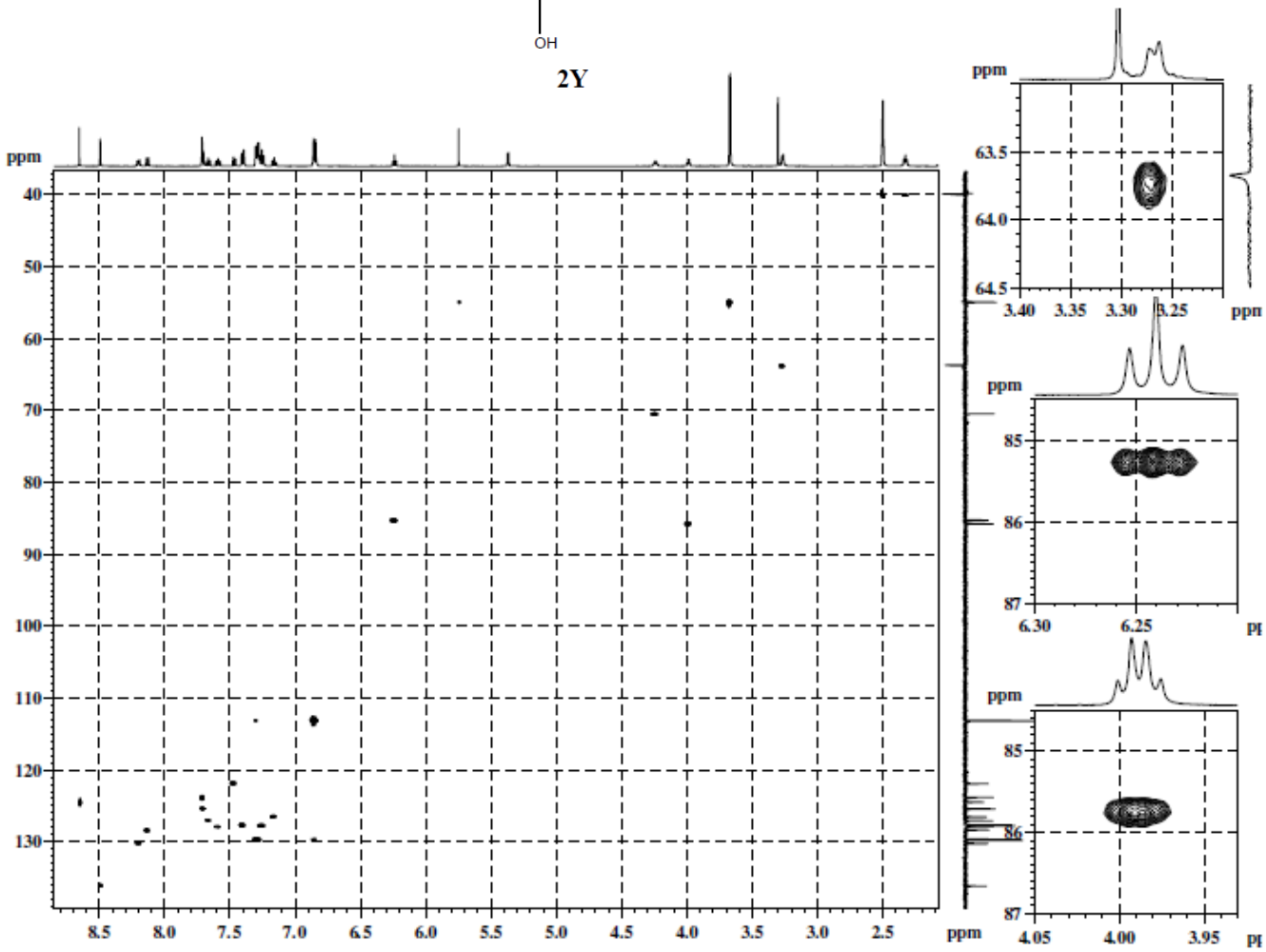
NH
DMTrO
N
O
O
OH

2Y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70
80
90
100
110
120
130
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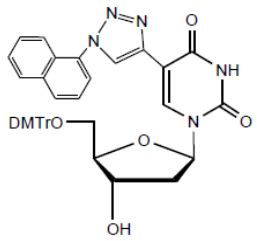
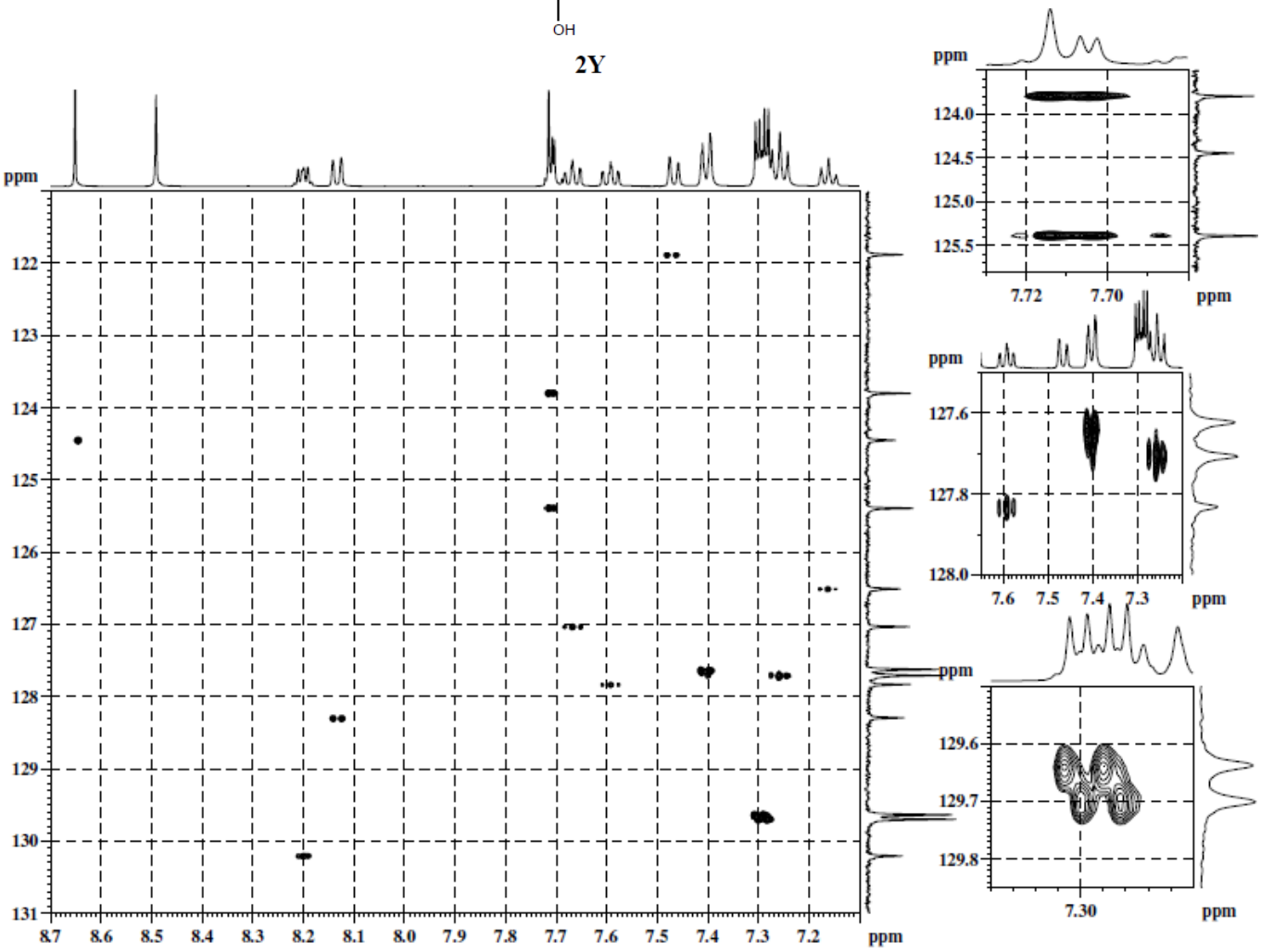
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
63.5
64.0
64.5
3.40
3.35
3.30
3.25
ppm
85
86
87
6.30
6.25
ppm
85
86
87
4.05
4.00
3.95


N
N
N
O
NH
N
O
DMTrO
O
OH

2Y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124
125
126
127
128
129
130
131
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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
ppm
ppm
124.0
124.5
125.0
125.5
7.72
7.70
ppm
ppm
127.6
127.8
128.0
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7.4
7.3
ppm
ppm
129.6
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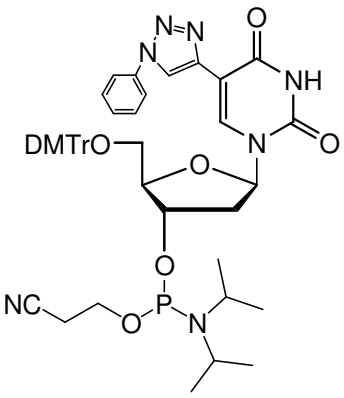
129.8
7.30
ppm

148.968
149.411



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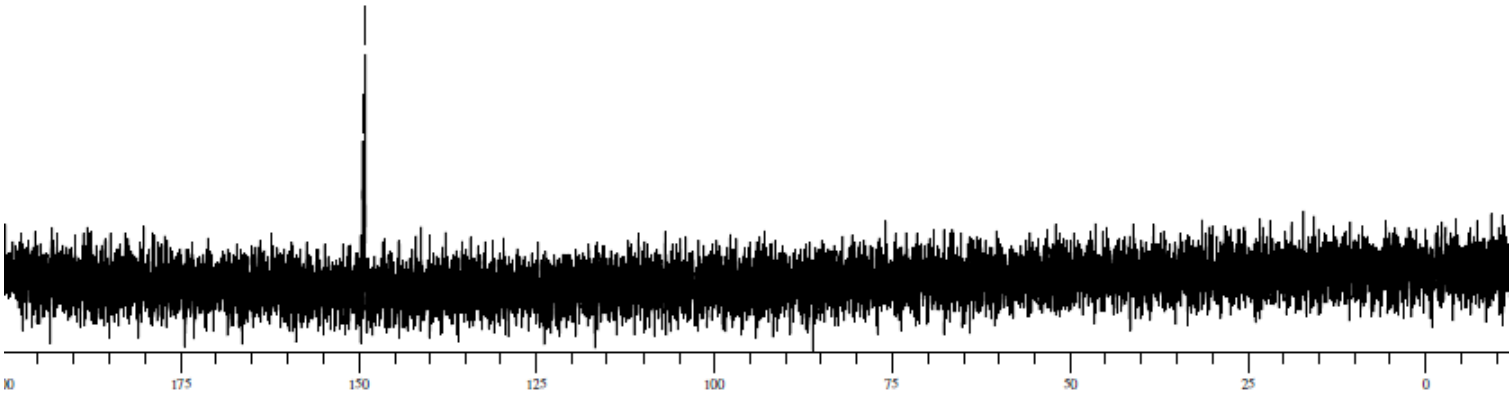

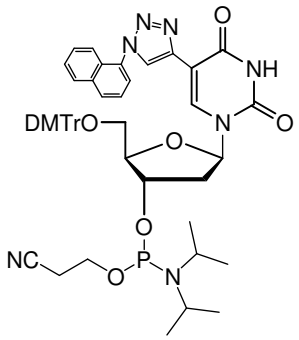

3Y