

Supporting Information

Three pyrene modified nucleotides; synthesis and effects in secondary nucleic acid structures

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Table S1. MALDI-TOF MS data of synthesized ON's

ON	Sequence	MW (calcd./exp.)
K1	5'-d(GCG AAT AKG CG)-3'	3662.0/3660
K2	5'-d(GCG AAK ATG CG)-3'	3662.0/3660
K3	5'-d(CGC ATA TKC GC)-3'	3573.5/3574
K4	5'-d(CGC ATA KTC GC)-3'	3573.5/3574
K5	5'-d(CGC AKA TTC GC)-3'	3573.5/3572.9
K6	5'-d(GCG AAK AKG CG)-3'	3943.0/3940
K7	5'-d(CGC AKA KTC GC)-3'	3854.0/3852
L1	5'-d(GCG AAT ALG CG)-3'	3634.5/3632.9
L2	5'-d(GCG AAL ATG CG)-3'	3634.5/3636.0
L3	5'-d(CGC ATA TLC GC)-3'	3545.5/3542.7
L4	5'-d(CGC ATA LTC GC)-3'	3545.5/3541.7
L5	5'-d(CGC ALA TTC GC)-3'	3545.5/3545.6
L6	5'-d(GCG AAL ALG CG)-3'	3887.8/3886.3
L7	5'-d(CGC ALA LTC GC)-3'	3798.7/3798.4
M1	5'-d(GCG AAT AMG CG)-3'	3649.0/3647.2
M2	5'-d(GCG AAM ATG CG)-3'	3649.0/3646.4
M3	5'-d(CGC ATA TMC GC)-3'	3559.4/3559.6
M4	5'-d(CGC ATA MTC GC)-3'	3559.4/3558.5
M5	5'-d(CGC AMA TTC GC)-3'	3559.4/3552.7
M6	5'-d(GCG AAM AMG CG)-3'	3915.0/3915.2
M7	5'-d(CGC AMA MTC GC)-3'	3825.8/3827.2
K8	5'-d(GCT CAC KCT CCC A)-3'	4111.0/4109.0
L8	5'-d(GCT CAC LCT CCC A)-3'	4083.6/4082.6
M8	5'-d(GCT CAC MCT CCC A)-3'	4097.4/4097.4
KK	5'-d(GCT CAC KKC TCC CA)-3'	4697.3/4697.8
LL	5'-d(GCT CAC LLC TCC CA)-3'	4641.3/4639.7
MM	5'-d(GCT CAC MMC TCC CA)-3'	4668.6/4667.6
KL	5'-d(GCT CAC KLC TCC CA)-3'	4669.3/4670.9
LK	5'-d(GCT CAC LKC TCC CA)-3'	4669.3/4668.8
MK	5'-d(GCT CAC MKC TCC CA)-3'	4682.6/4680.5

Additional thermal denaturation data

Table S2. T_m data for multizippers

Entry	ON	Duplex	Zipper	X =	$T_m/^{\circ}\text{C}^a$ $\Delta T_m/^{\circ}\text{C}^b$ [$\Delta\Delta T_m/^{\circ}\text{C}^c$]		
					K	L	M
1	X3	5'-d(CGC ATA TXC GC)-3'	(-2)(-4)	X =	48.5	14.5	51.0
	X6	3'-d(GCG XAX AAG CG)-5'			+3.0[+1.0]	-31.0[-5.5]	+5.5[+2.5]
2	X4	5'-d(CGC ATA XTC GC)-3'	(-1)(-3)	X =	36.0	31.0	51.5
	X6	3'-d(GCG XAX AAG CG)-5'			-9.5[-10.0]	-14.5[+8.0]	+6.0[0.0]
3	X5	5'-d(CGC AXA TTC GC)-3'	(+)(-1)	X =	41.0	33.5	38.5
	X6	3'-d(GCG XAX AAG CG)-5'			-4.5[-5.5]	-12.0[+11.5]	-7.0[-17.0]
4	X7	5'-d(CGC AXA XTC GC)-3'	(-3)(-1)	X =	35.5	25.0	49.5
	X1	3'-d(GCG XAT AAG CG)-5'			-6.0[-9.0]	-20.5[+7.5]	+4.0[-1.5]
5	X7	5'-d(CGC AXA XTC GC)-3'	(-1)(+1)	X =	43.0	41.0	40.5
	X2	3'-d(GCG TAX AAG CG)-5'			-2.5[-1.5]	-4.5[+15.0]	-5.0[-15.5]
6	X7	5'-d(CGC AXA XTC GC)-3'	(-1)(-3)	X =	35.0	29.0	41.0
	X6	3'-d(GCG XAX AAG CG)-5'			-10.5[-10.0]	-16.0[+21.5]	-4.0[-15.0]

^a Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs. temperature) recorded in a buffer containing 2.5 mM Na_2HPO_4 , 5.0 mM NaH_2PO_4 , 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.0 μM concentrations of each strand. **K**, **L** and **M** correspond to the incorporation of the amidites, **4**, **7** and **11**, respectively. ^bDifferences in melting temperatures as compared to the unmodified duplexes; $\Delta T_m = T_{m(\text{duplex})} - T_{m(\text{ref})}$, $T_{m(\text{ref})} = 45.5^{\circ}\text{C}$. ^cDifferences in melting temperatures as compared to singly modified duplexes; $\Delta\Delta T_m = \Delta T_{m(a:b)} - (\Delta T_{m(a:\text{ref})} + \Delta T_{m(\text{ref}:b)})$.

The zipper arrangements obtained when mixing ON's with one or two incorporations of either of the monomer **K**, **L** or **M** were studied (Table S2). The data revealed that in general the total of two or three incorporations of monomer **K** in an 11-mer DNA duplex led to the destabilization of the resulting modified duplex. Hence, the favorable zipper contacts found in (+1) zipper arrangement for monomer **K** disappeared in the presence of an additional pyrene in the duplex (entries 3 and 5). Three or four incorporations of monomer **L** in general led to significant destabilization of the resulting duplex, and the positive zipper contacts between two monomers found in (-1) zipper arrangement (Table 1) disappeared in these multi-zipper arrangements (Table S2, entries 2-6). For

monomer **M**, positive value of ΔT_m 's in three cases (Table S2, entries 1, 2, and 4) suggested that three incorporations of monomer **M** were well tolerated in the 11-mer dsDNA except when two monomers from opposite strands were present in a (+1) zipper arrangement (Table S2, entry 3, 5 and 6).

Table S3. T_m data for duplexes with two consecutive incorporations of same or different monomer

		$T_m/^\circ\text{C}^a(\Delta T_m/^\circ\text{C}^b)$	
		Complementary DNA	Complementary RNA
ON	Sequence	3'- d(CGA GTG AAG AGG GT)	3'- r(CGA GUG AAG AGG GU)
TT	5'-d(GCT CAC TT C TCC CA)-3'	53.5	60.5
KK	5'-d(GCT CAC KK C TCC CA)-3'	52.0 (-1.5)	55.5 (-5.0)
LL	5'-d(GCT CAC LL C TCC CA)-3'	45.0 (-8.5)	56.0 (-4.5)
MM	5'-d(GCT CAC MM C TCC CA)-3'	52.0 (-1.5)	55.5 (-5.0)
KL	5'-d(GCT CAC KL C TCC CA)-3'	49.0 (-4.5)	58.0 (-2.5)
LK	5'-d(GCT CAC LK C TCC CA)-3'	48.0 (-5.5)	55.0 (-5.5)
KM	5'-d(GCT CAC KM C TCC CA)-3'	48.0 (-5.5)	55.0 (-5.5)

^a Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs. temperature) recorded in a buffer containing 2.5 mM Na_2HPO_4 , 5.0 mM NaH_2PO_4 , 100 mM NaCl , 0.1 mM EDTA, pH 7.0 using 1.0 μM concentrations of each strand. **K**, **L** and **M** correspond to the incorporation of the amidites, **4**, **7** and **11**, respectively. ^bDifferences in melting temperatures as compared to the unmodified duplexes.

Steady-state fluorescence emission spectra for modified ON's

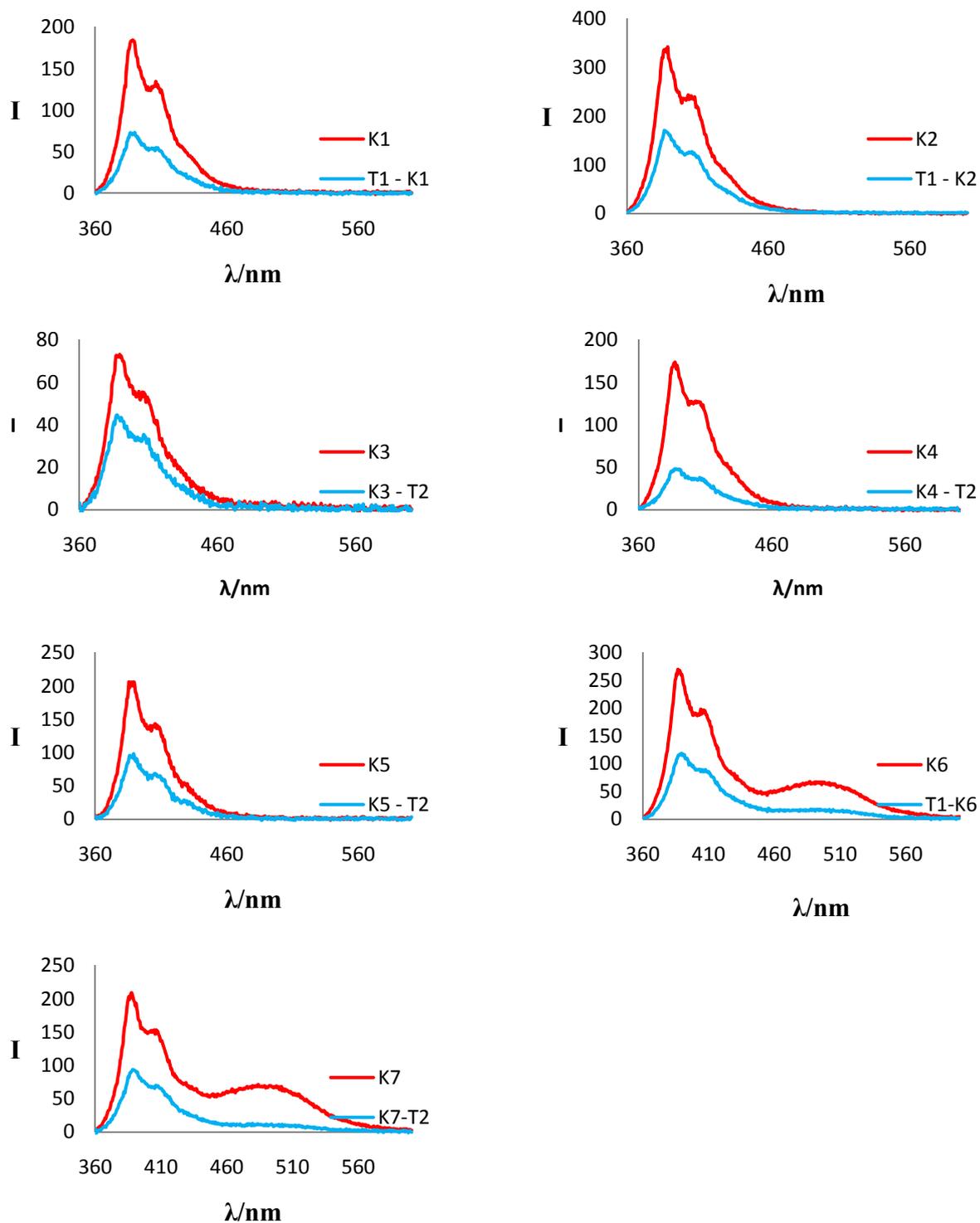


Figure S1. Steady-state fluorescence emission spectra for modified ON's **K1-K7** in the absence (red) and presence (blue) of complementary DNA ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 1.0 \text{ }\mu\text{M}$). Different scaling of Y-axes is used.

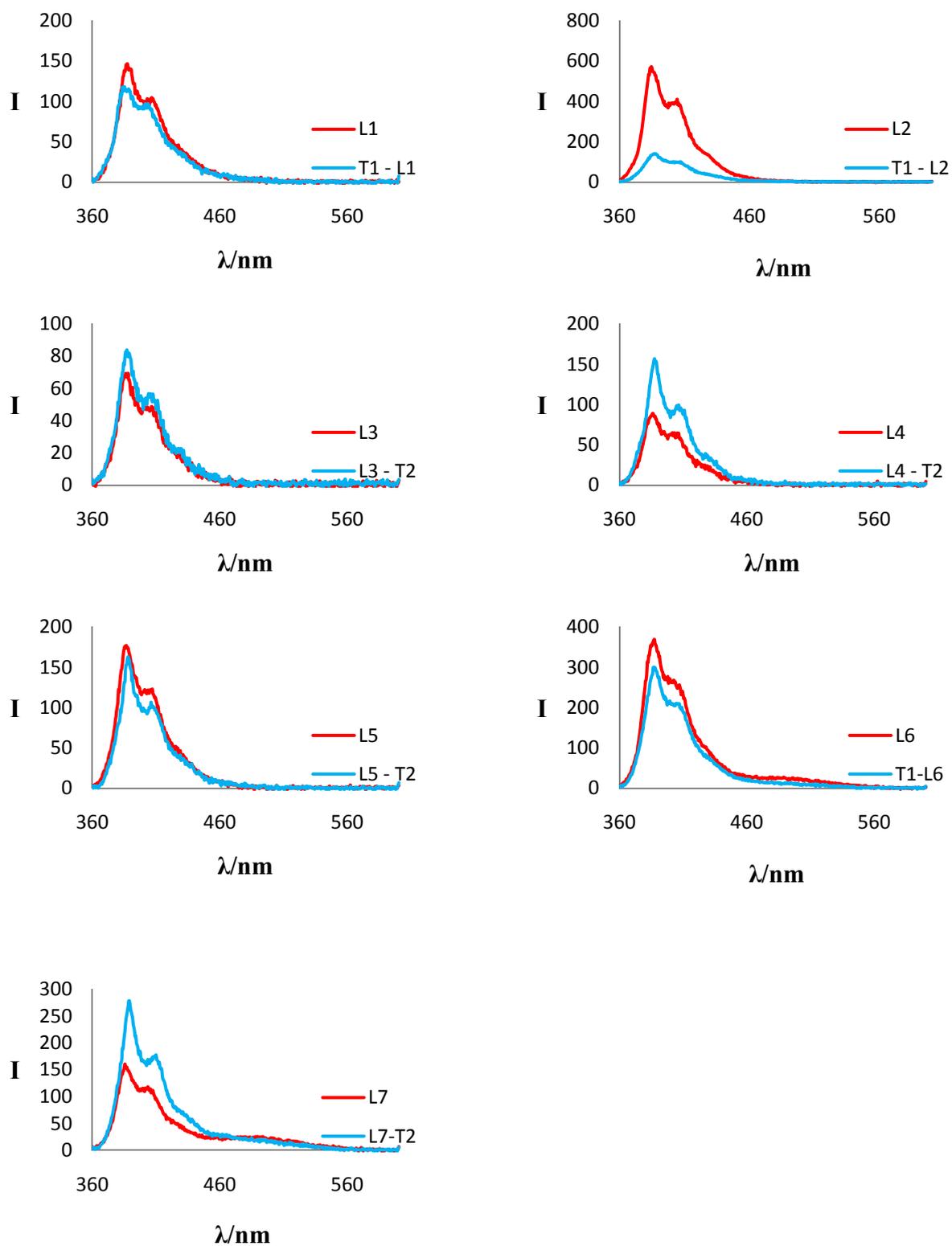


Figure S2. Steady-state fluorescence emission spectra for modified ON's **L1-L7** in the absence (red) and presence (blue) of complementary DNA ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 1.0 \text{ }\mu\text{M}$). Different scaling of Y-axes is used.

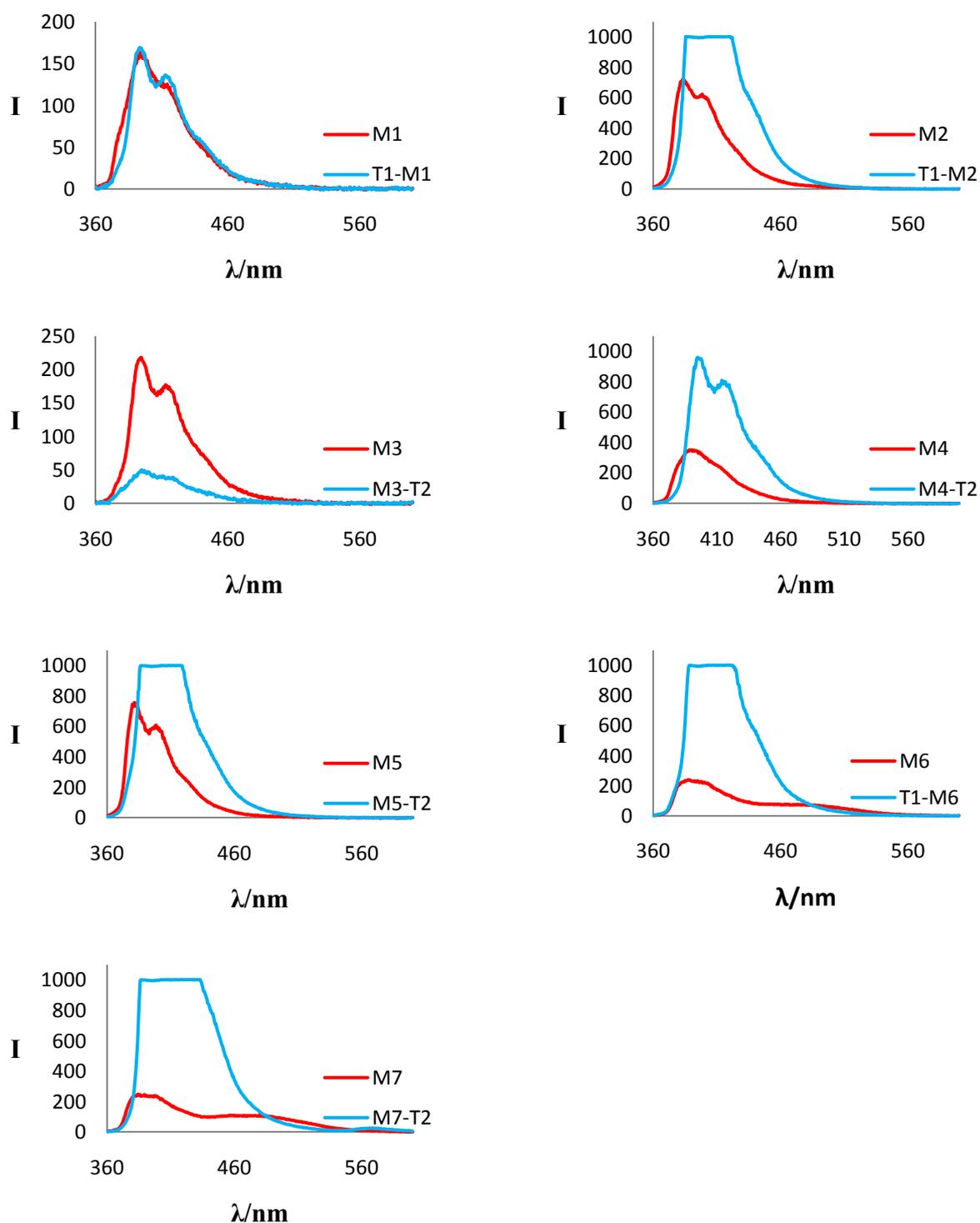


Figure S3. Steady-state fluorescence emission spectra for modified ON' s **M1-M7** in the absence (red) and presence (blue) of complementary DNA ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 1.0 \text{ }\mu\text{M}$). Different scaling of Y-axes is used. For full spectra of duplexes **T1-M2**, **M5-T2**, **T1-M6** and **M7-T2** ($[\text{ON}] = 0.25 \text{ }\mu\text{M}$) see Figure S4.

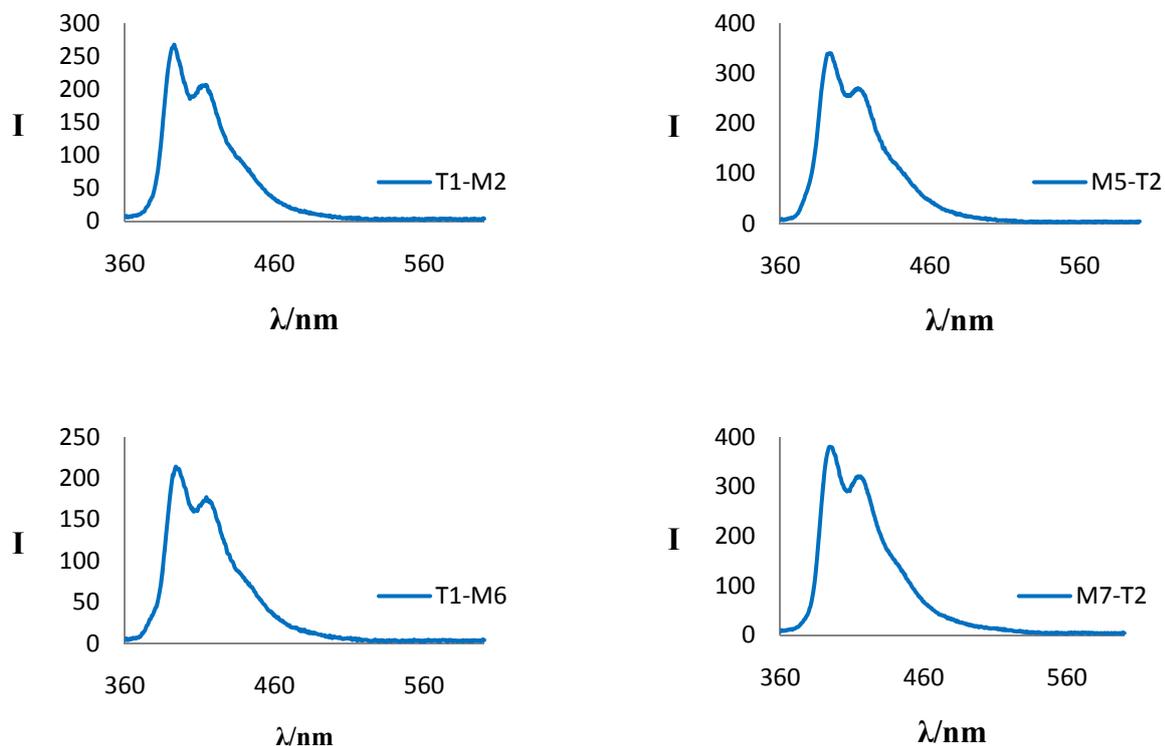


Figure S4. Steady-state fluorescence emission spectra for modified ON's **M2** and **M5-M7** in the presence of complementary DNA ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 0.25 \text{ }\mu\text{M}$). Different scaling of Y-axes is used.

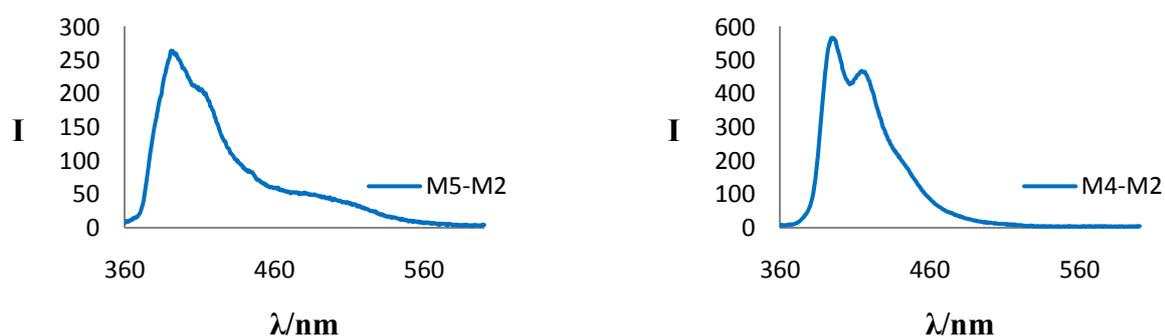


Figure S5. Steady-state fluorescence emission spectra for modified duplexes **M5-M2** and **M4-M2** ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 0.25 \text{ }\mu\text{M}$). Different scaling of Y-axes is used.

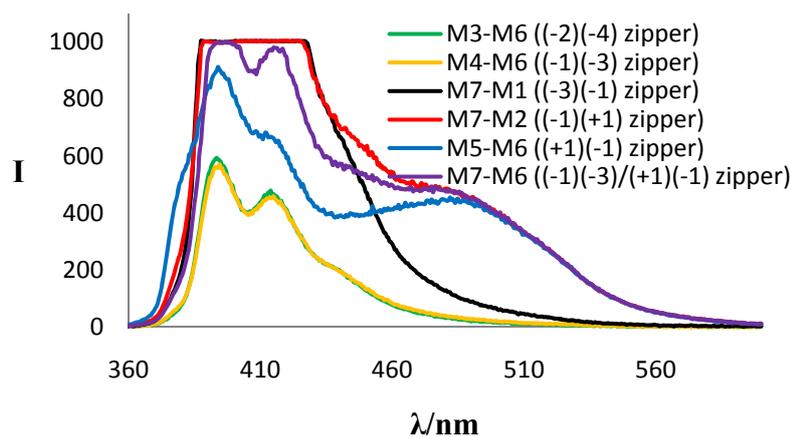
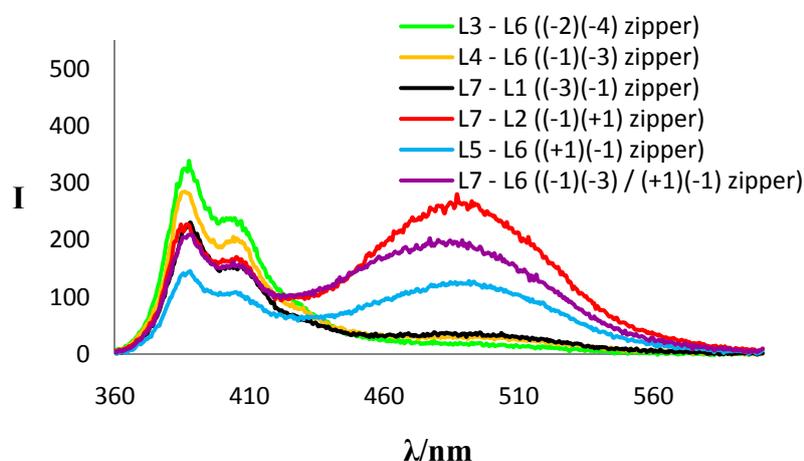
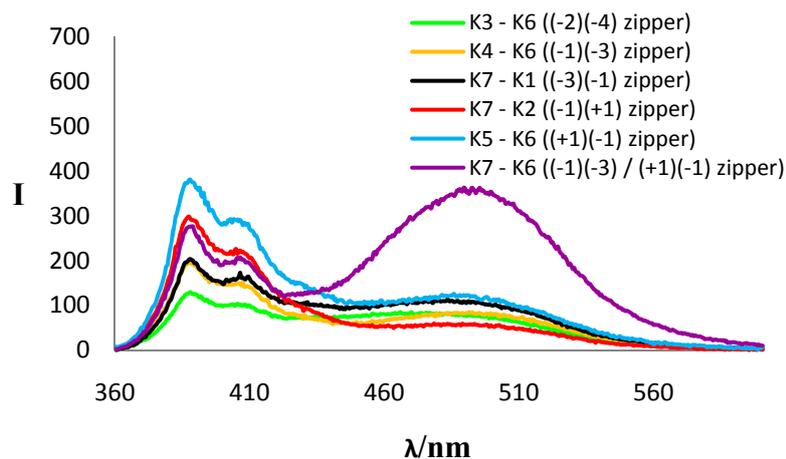


Figure S6. Steady-state fluorescence emission spectra of interstrand multizippers (Table 1 and Table S2) ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10^\circ\text{C}$; $[\text{ON}] = 1.0 \mu\text{M}$). Different scaling of Y-axes is used. For full spectra of duplexes **M7-M1** and **M7-M2** ($[\text{ON}] = 0.25 \mu\text{M}$) see Figure S7.

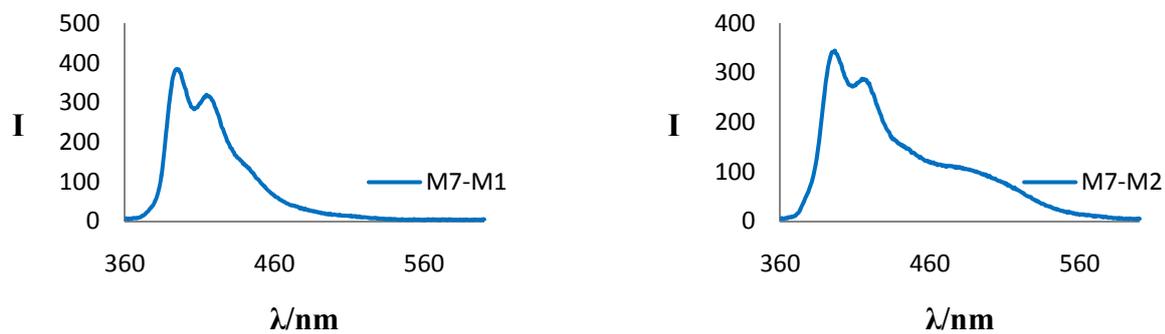


Figure S7. Steady-state fluorescence emission spectra for modified duplexes **M7-M1** and **M7-M2** ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 0.25 \text{ } \mu\text{M}$). Different scaling of Y-axes is used.

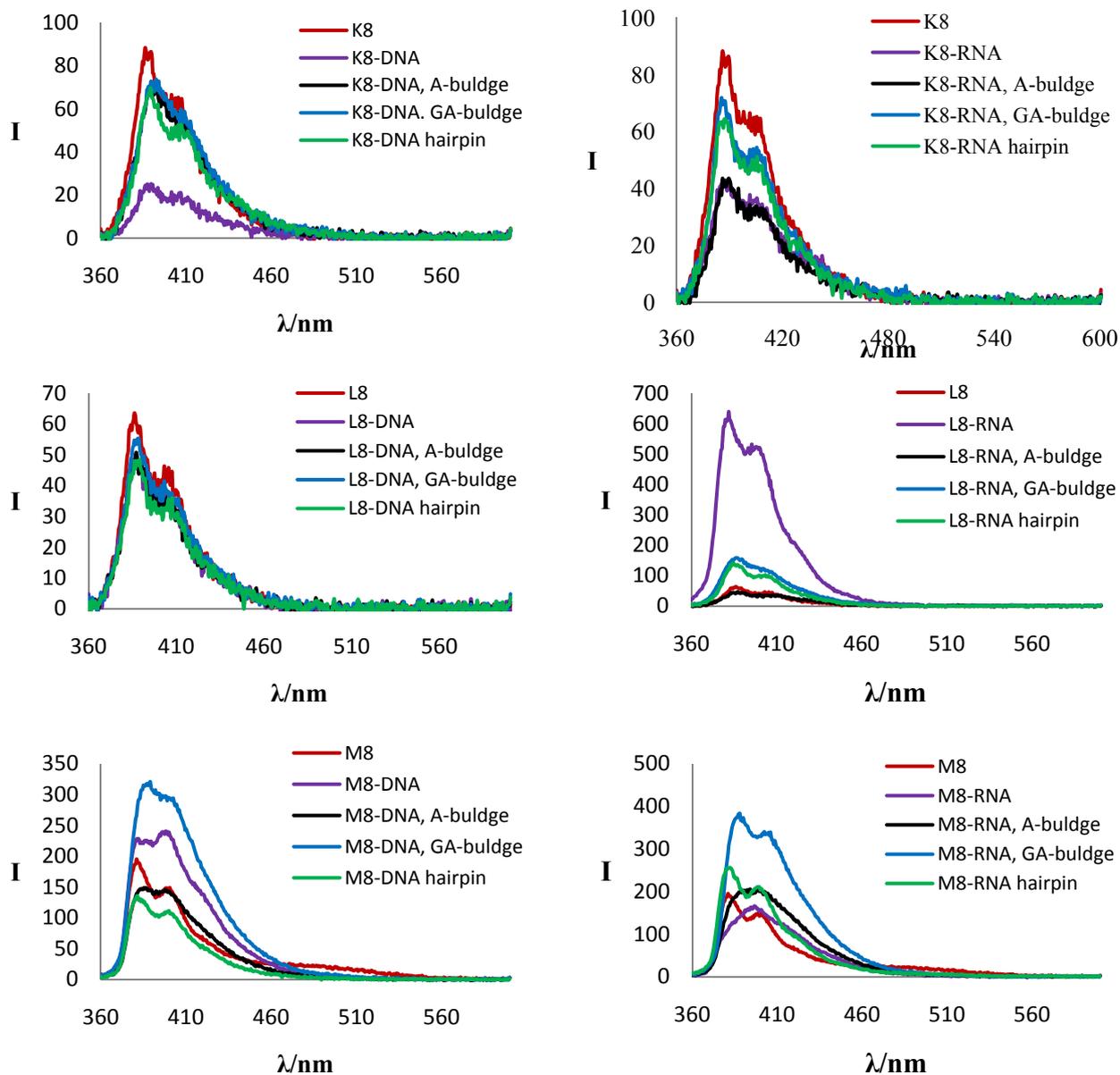
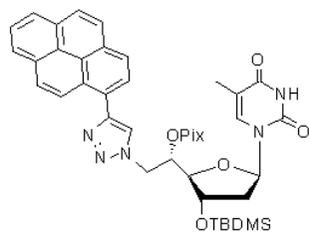
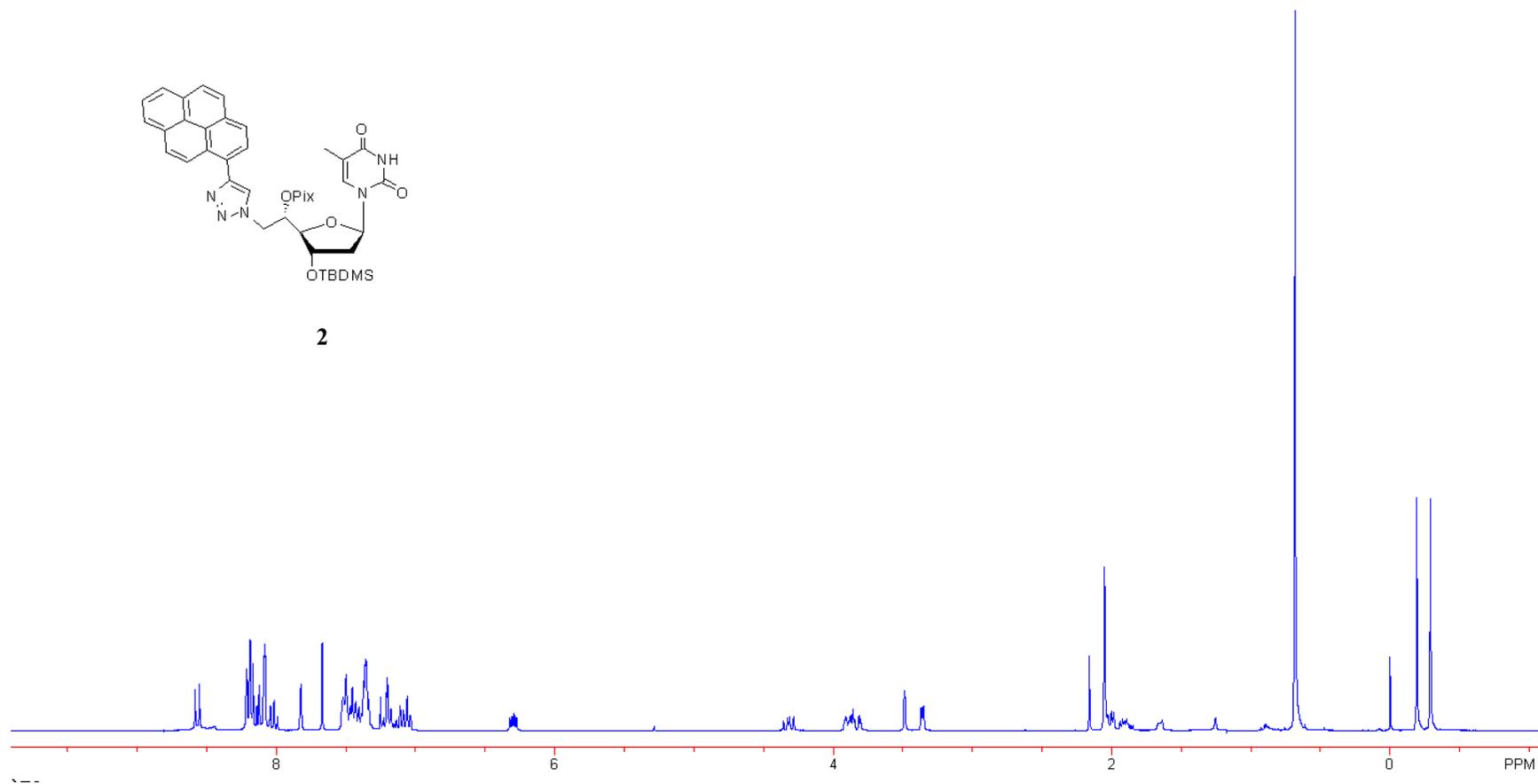
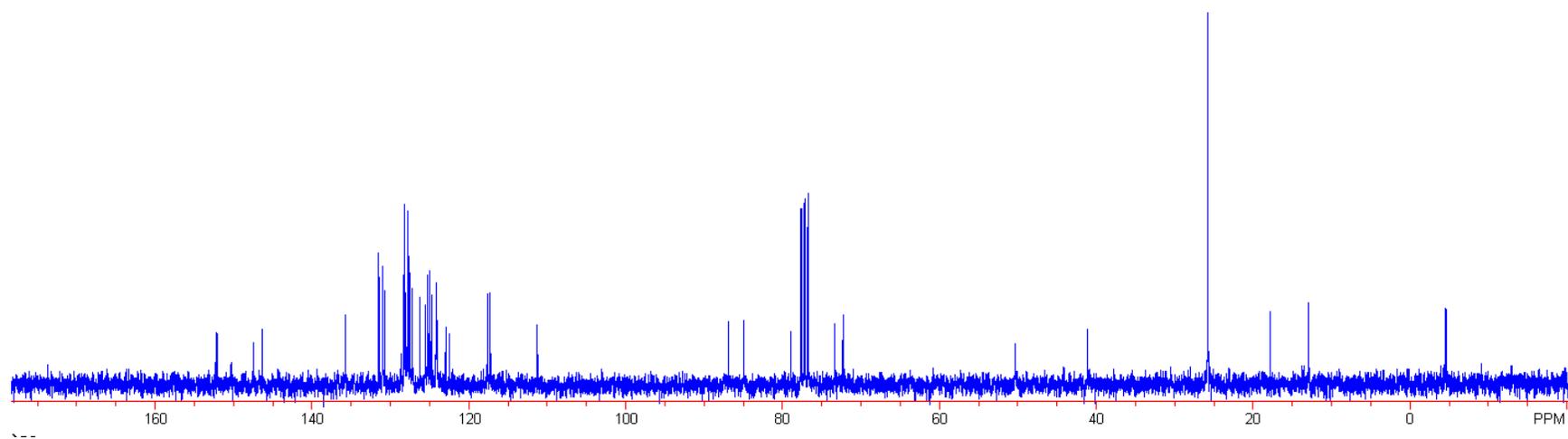
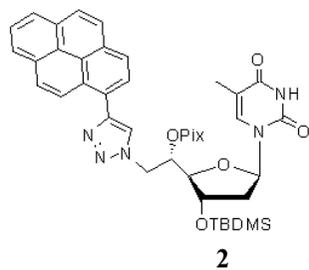


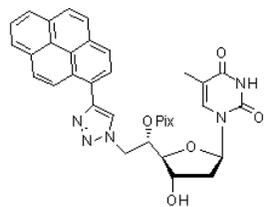
Figure S8. Steady-state fluorescence emission spectra for modified ON's **K8**, **L8** and **M8** in the absence and presence of complementary DNA or RNA ($\lambda_{\text{ex}} = 350 \text{ nm}$; $T = 10 \text{ }^\circ\text{C}$; $[\text{ON}] = 1.0 \text{ } \mu\text{M}$). Different scaling of Y-axes is used.



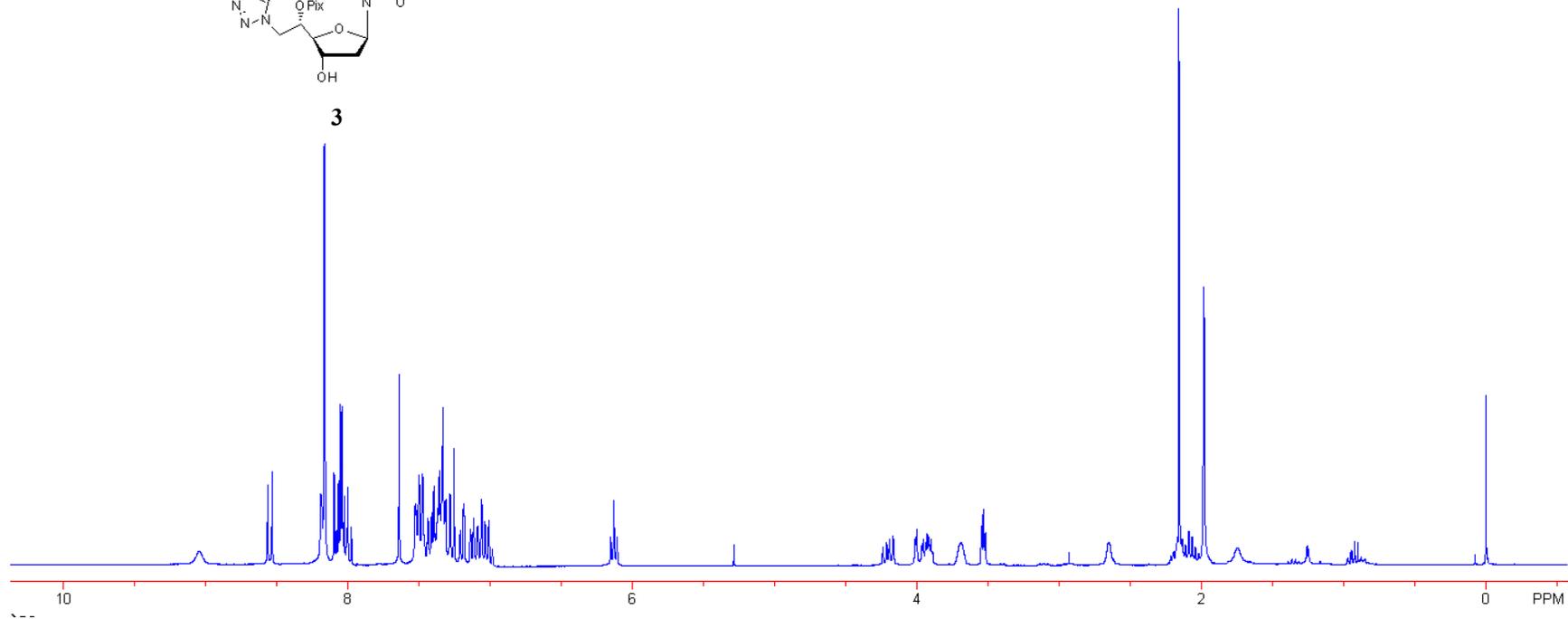
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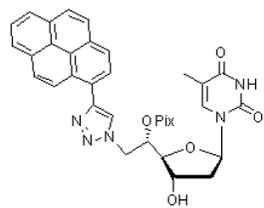




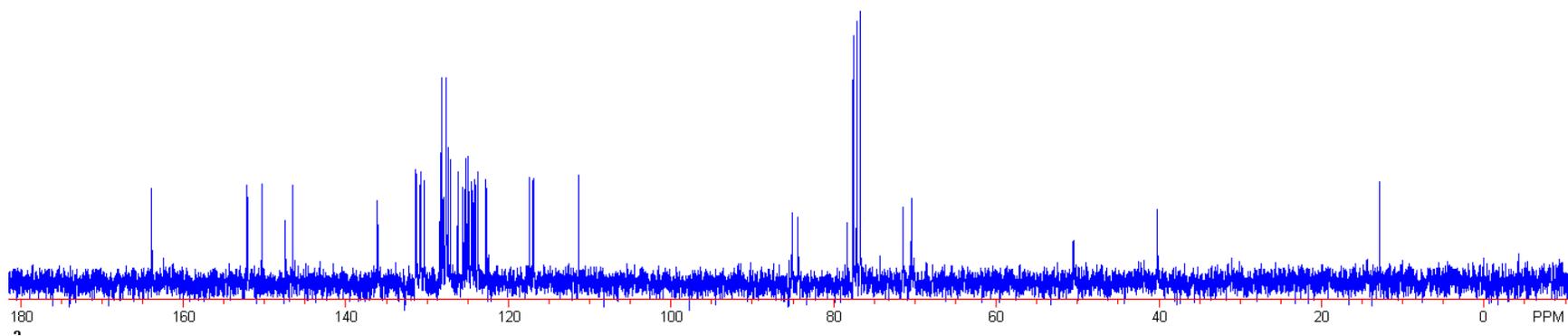


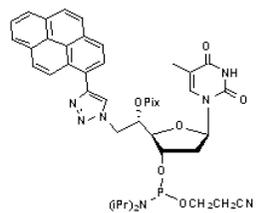
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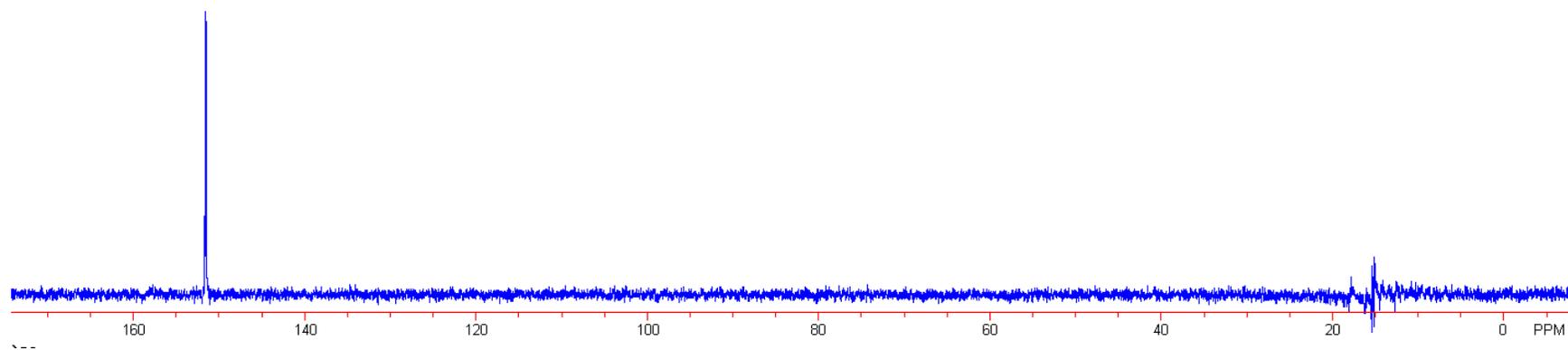


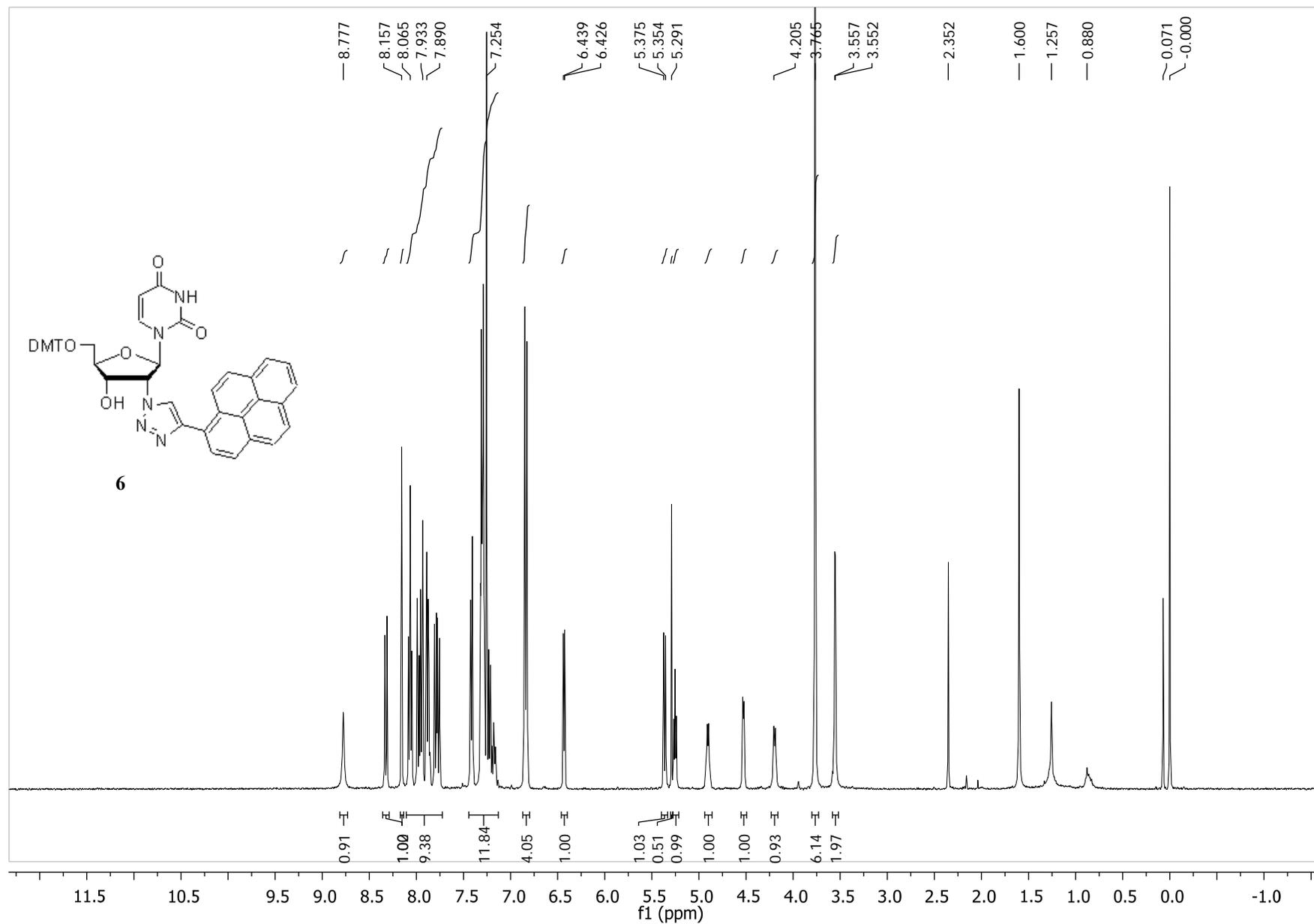
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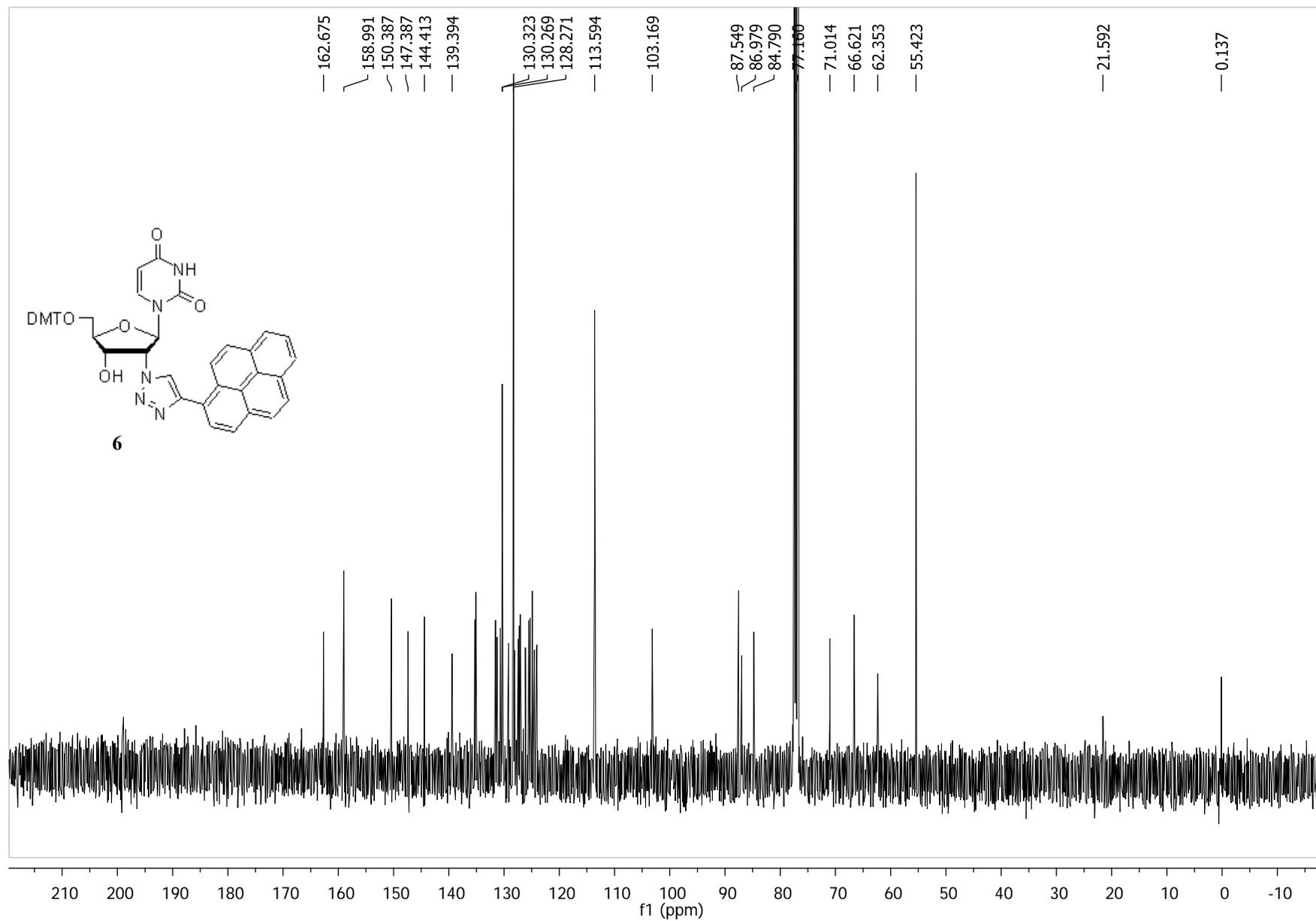


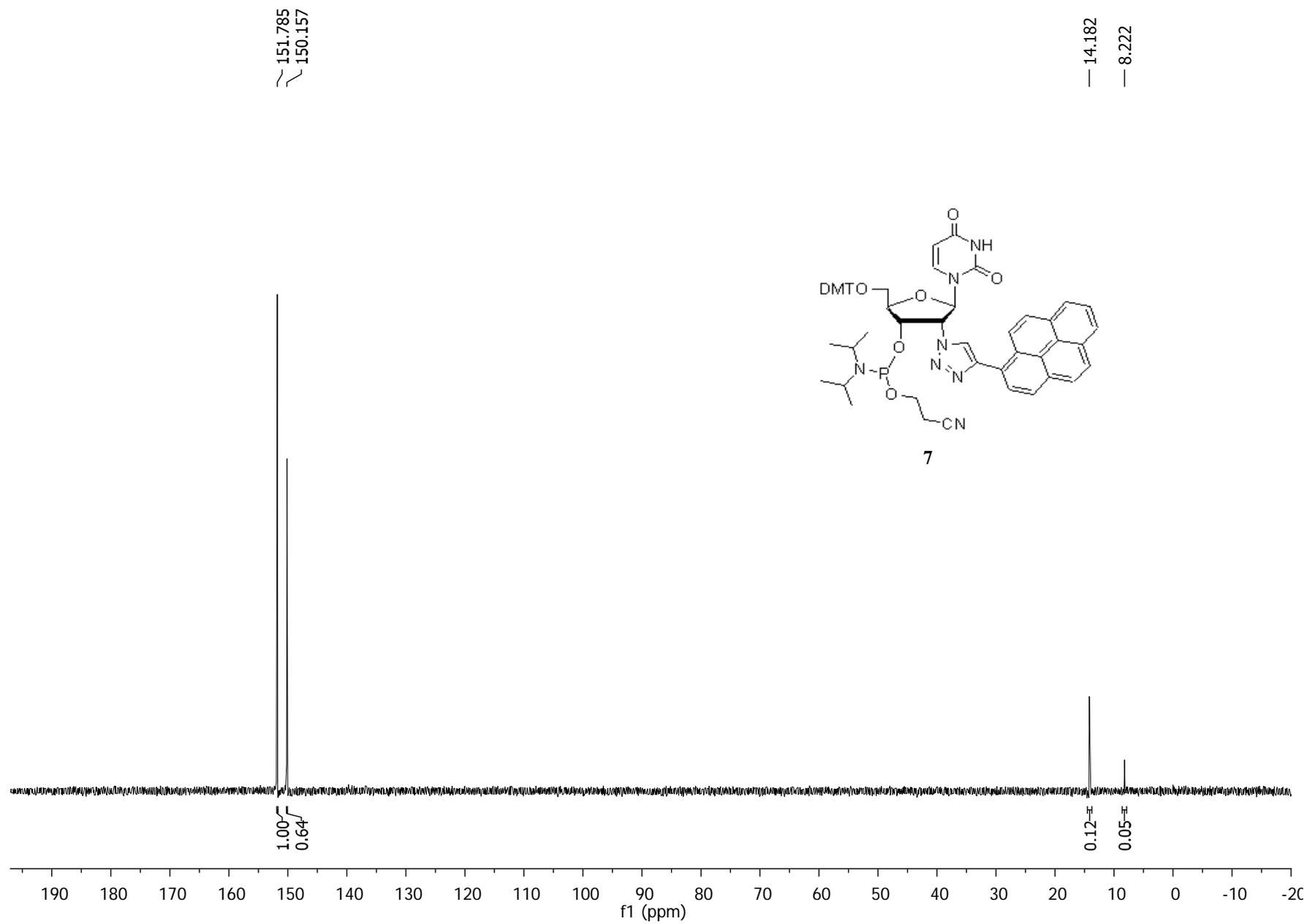


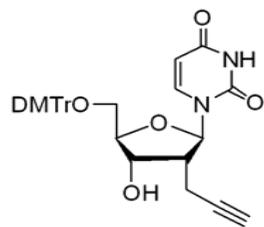
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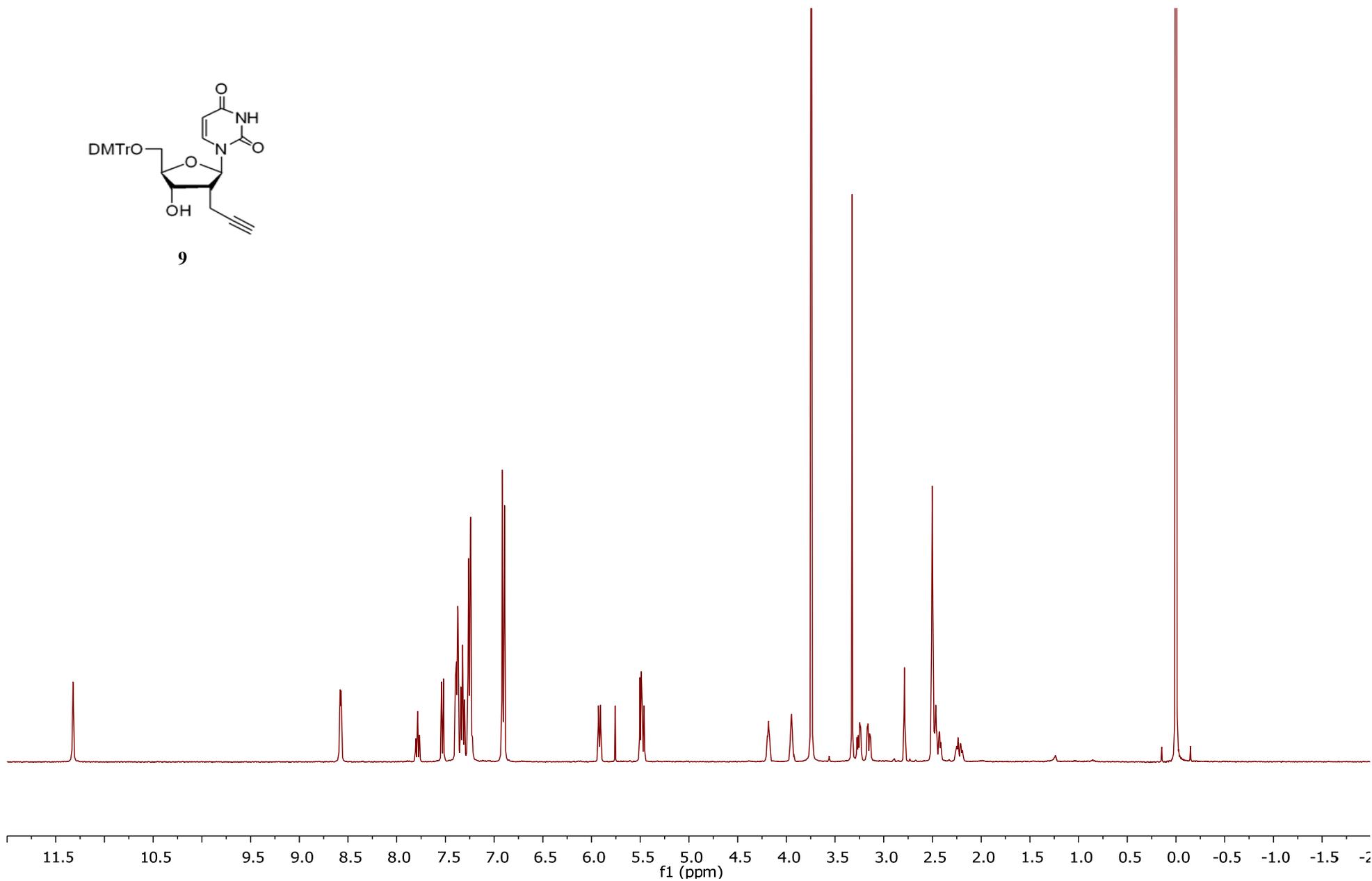


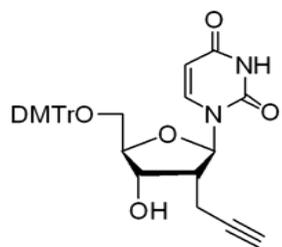




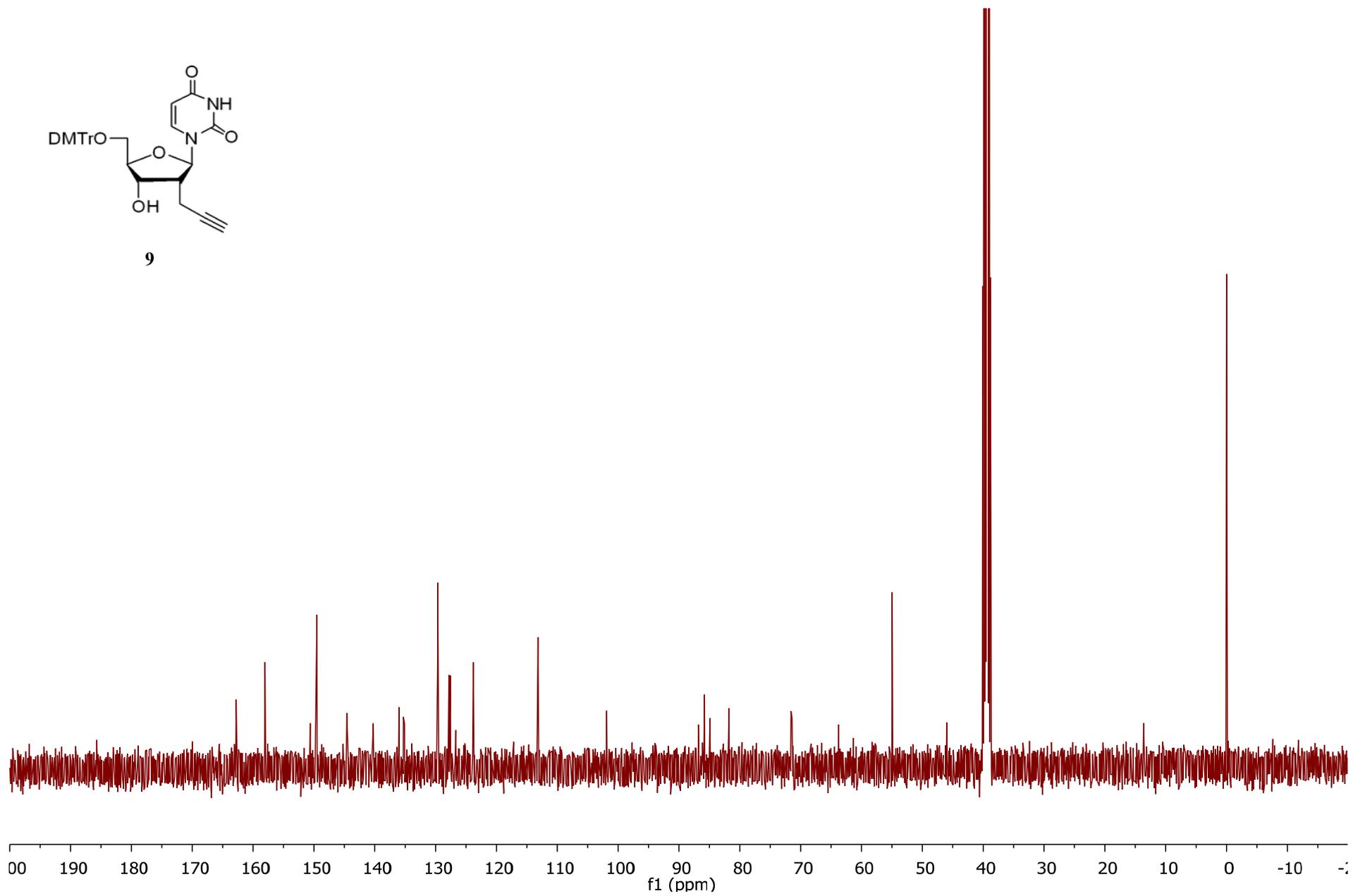


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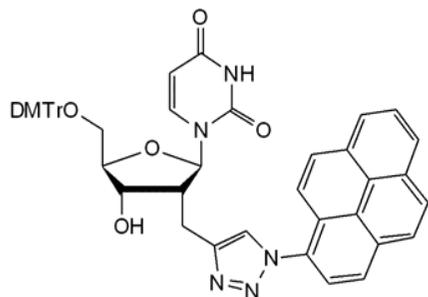




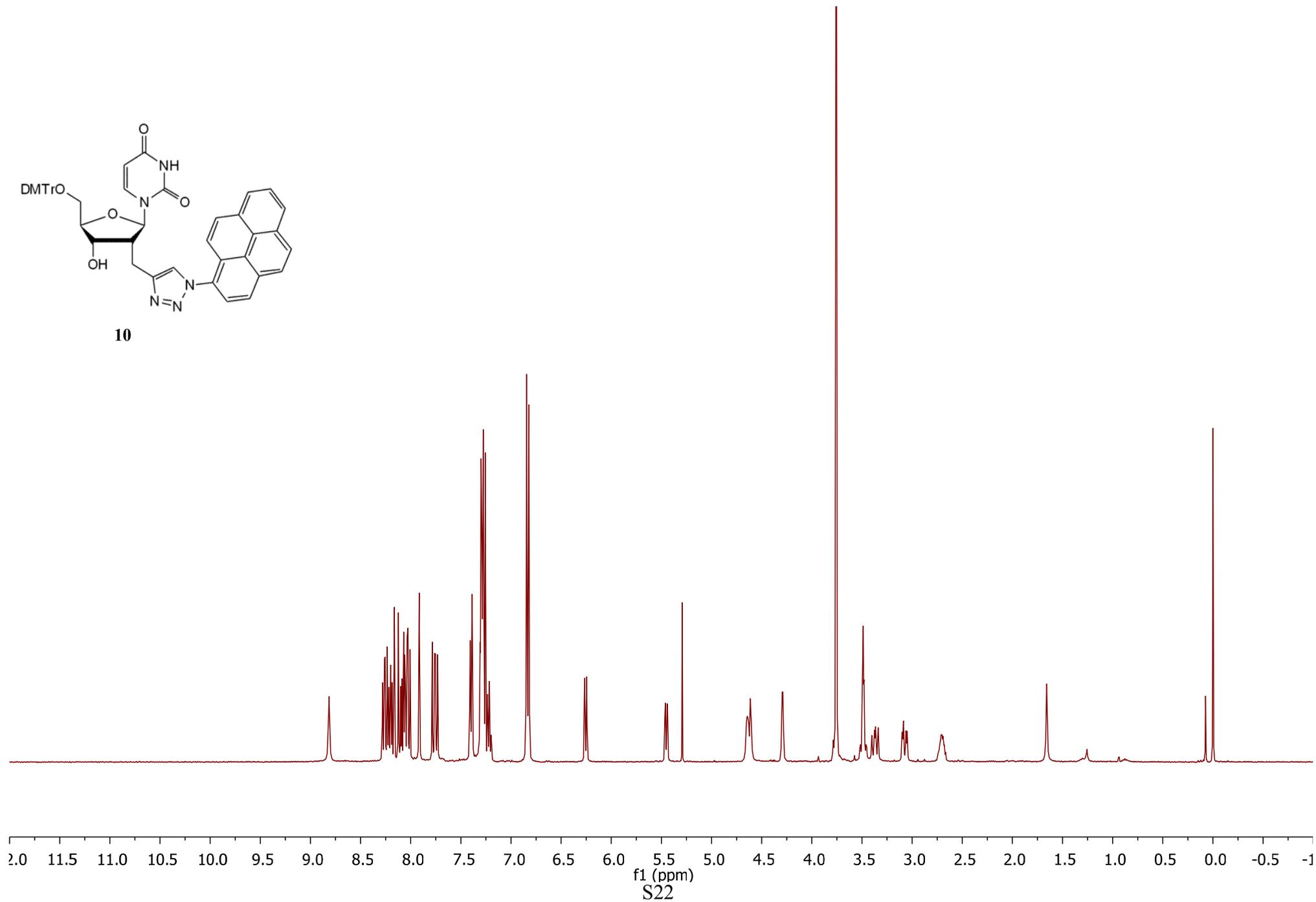
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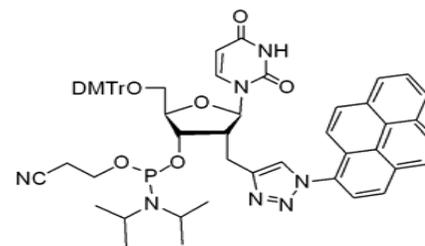


S21



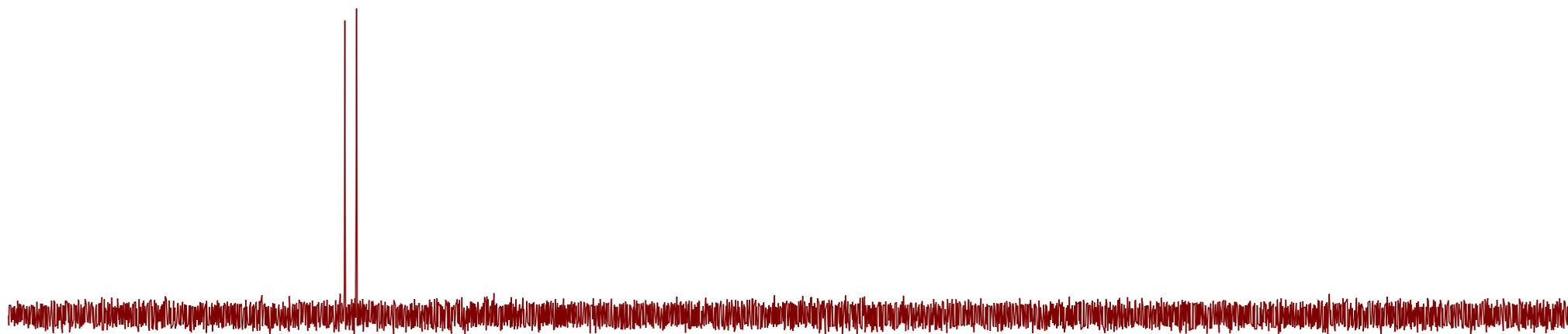
10





11

150.52
149.36



f1 (ppm)

S24