

## Synthesis and characterization of RNA containing a rigid and non-perturbing cytidine-derived spin label

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### Supporting Information

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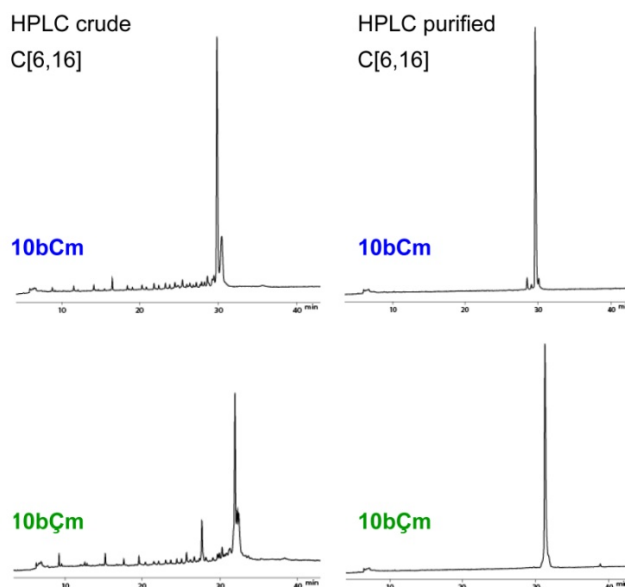
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**List of abbreviations**

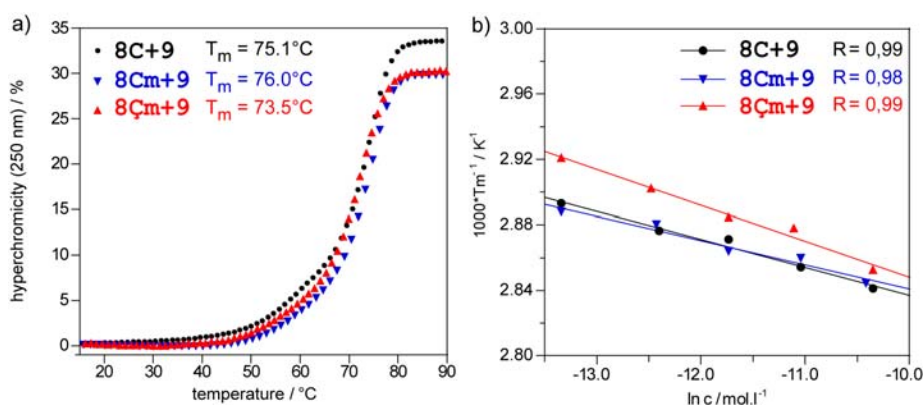
|           |                                                           |
|-----------|-----------------------------------------------------------|
| CD        | circular dichroism                                        |
| CE        | 2-cyanoethyl                                              |
| CW        | continuous-wave                                           |
| DMAP      | 4-dimethylaminopyridine                                   |
| DME       | 1,2-dimethoxyethane                                       |
| DMSO      | dimethylsulfoxide                                         |
| DMTCI     | 4,4'-dimethoxytrityl chloride                             |
| EPR       | electron paramagnetic resonance                           |
| ESI-MS    | electrospray ionization mass spectrometry                 |
| HPLC      | high performance liquid chromatography                    |
| HR-ESI-MS | high resolution electrospray ionization mass spectrometry |
| NBS       | N-Bromosuccinimide                                        |
| NMR       | nuclear magnetic resonance                                |
| TBAF      | tetrabutylammonium fluoride                               |
| tBuOOH    | <i>tert</i> -butylhydroperoxide                           |
| TOM       | (triisopropylsilyloxy)methyl                              |
| UV        | ultraviolet–visible                                       |

**Table S1.** ESI-MS characterization of RNA oligonucleotides.

| Name         | 5'-Sequence-3'                             | spin label | mol.wt. expected | mol.wt. found |
|--------------|--------------------------------------------|------------|------------------|---------------|
| <b>8</b>     | GACCUCGCAUCGUG                             | -          | 4421.7           | 4421.1        |
| <b>8Cm</b>   | GACCUCG <b>Cm</b> AUCGUG                   | -          | 4435.7           | 4435.4        |
| <b>8Çm</b>   | GACCUCG <b>Çm</b> AUCGUG                   | Çm[8]      | 4637.9           | 4638.6        |
| <b>9</b>     | CACGAUGCGAGGUC                             | -          | 4484.8           | 4484.8        |
| <b>10</b>    | GACGUCGGAAGACGUCAGUA                       | -          | 6469.0           | 6469.5        |
| <b>10aCm</b> | GACGUC <b>Cm</b> GGAAGACGUCAGUA            | -          | 6483.0           | 6483.7        |
| <b>10aÇm</b> | GACGUC <b>Çm</b> GGAAGACGUCAGUA            | Çm[6]      | 6685.0           | 6685.8        |
| <b>10bCm</b> | GACGUC <b>Cm</b> GGAAGACGUC <b>Cm</b> AGUA | -          | 6497.0           | 6498.3        |
| <b>10bÇm</b> | GACGUC <b>Çm</b> GGAAGACGUC <b>Çm</b> AGUA | Çm[6,16]   | 6901.5           | 6902.5        |
| <b>11</b>    | UACUGACGUCUUCGACGUC                        | -          | 6279.8           | 6280.6        |
| <b>11aCm</b> | UACUGAC <b>Cm</b> GUCUUCGACGUC             | -          | 6295.8           | 6295.1        |
| <b>11aÇm</b> | UACUGA <b>Çm</b> GUCUUCGACGUC              | Çm[7']     | 6496.0           | 6497.1        |
| <b>12</b>    | GAUGCGCAAGCAUCUACU                         | -          | 5715.5           | 5715.4        |
| <b>13</b>    | AGUAGAUCCGAAAGGAUC                         | -          | 5802.6           | 5802.7        |
| <b>14</b>    | GACGUC                                     | -          | 1874.2           | 1873.6        |
| <b>15</b>    | GACGUCGGA                                  | -          | 2893.8           | 2893.4        |



**Figure S1.** Anion exchange HPLC traces of crude and purified C[6,16]-modified RNAs **10bCm** and **10bÇm**.

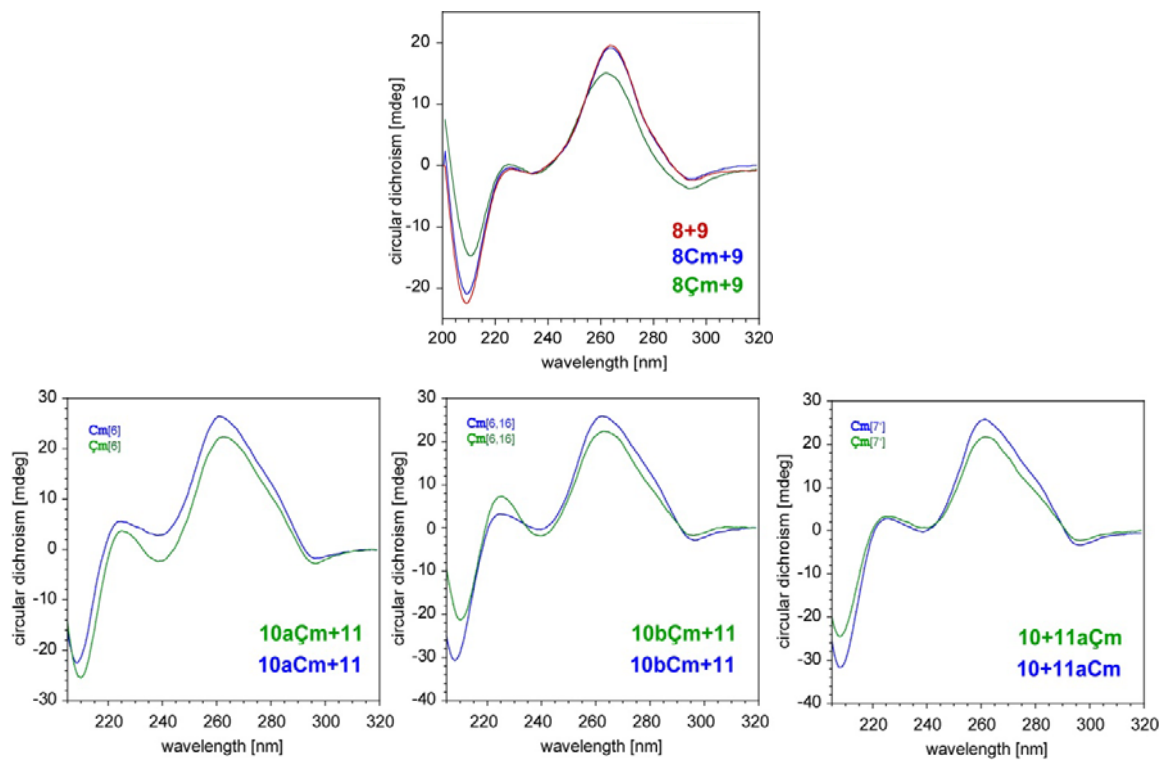


**Figure S2.** a) UV-melting curves of duplexes **8+9** monitored at 250 nm, 8  $\mu$ M strand concentration, 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0. b) Van't Hoff plot ( $\ln(c)$  versus  $1/T_m$ ) for 14-bp duplexes **8+9**. Linear fit parameters and  $\Delta H$  and  $\Delta S$  determined from slope and intercept of the linear fit are in Table S2.

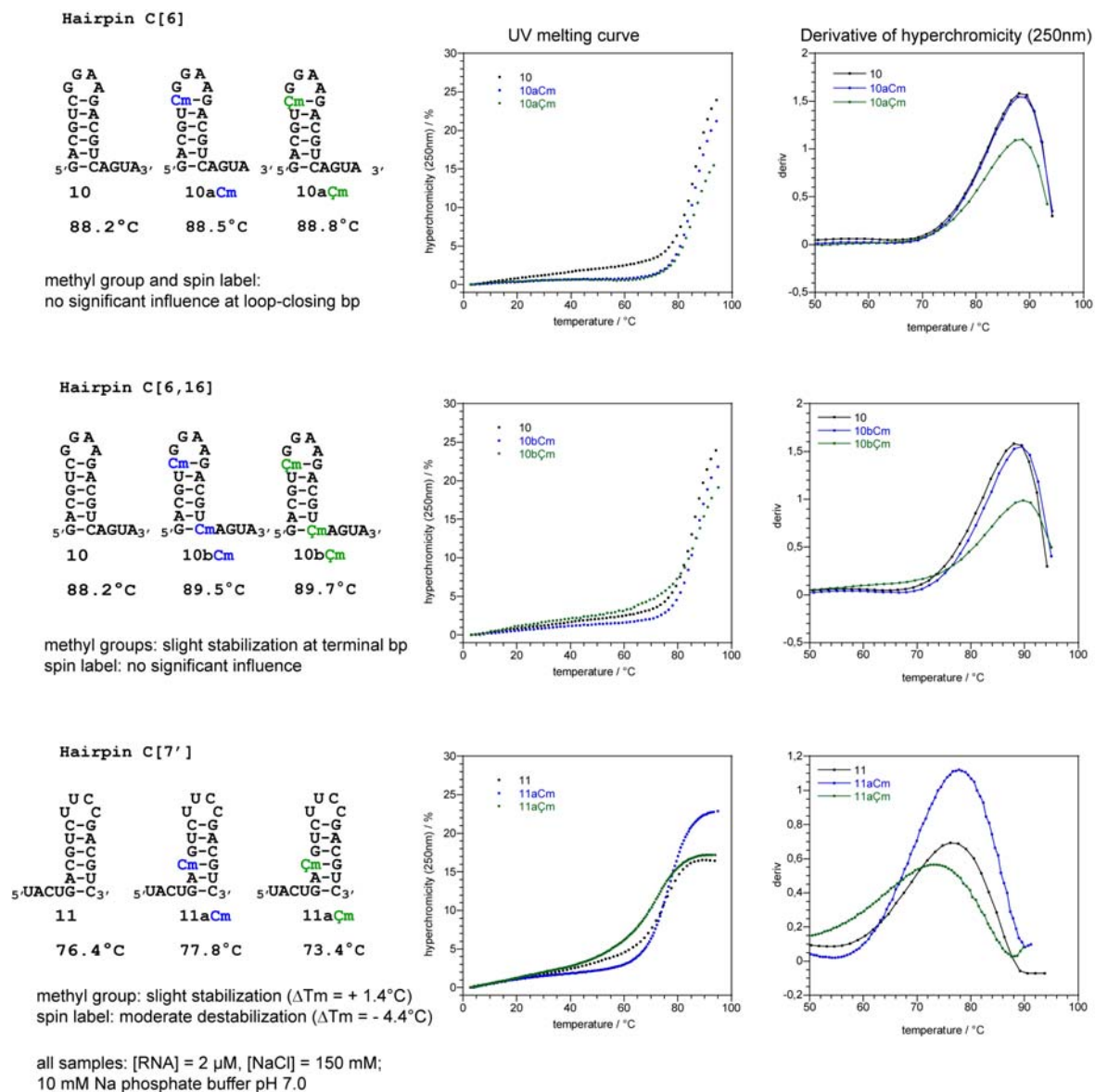
**Table S2.** Thermodynamic data for 14-bp duplexes **8+9** (van't Hoff plot  $\ln(c)$  versus  $1/T_m$  in Figure S2)

| Modification  | slope<br>( $R/\Delta H$ )<br>( $10^{-3}$ ) | intercept<br>( $\frac{\Delta S - R \ln 4}{\Delta H}$ )<br>( $10^{-3}$ ) | $R^2$ | $\Delta H$<br>[kcal/mol] | $\Delta S$<br>[cal/mol.K] | $\Delta G^{298}$<br>[kcal/mol] | $ \Delta \Delta G^{298} ^a$<br>[kcal/mol] |
|---------------|--------------------------------------------|-------------------------------------------------------------------------|-------|--------------------------|---------------------------|--------------------------------|-------------------------------------------|
| C(unmodified) | -0.0171                                    | 2.666                                                                   | 0.991 | -114                     | -302                      | -24.4                          |                                           |
| Cm[8]         | -0.0147                                    | 2.664                                                                   | 0.981 | -141                     | -378                      | -28.2                          | 3.8                                       |
| Çm[8]         | -0.0219                                    | 2.629                                                                   | 0.992 | -90.5                    | -235                      | -20.4                          | 4.0                                       |

<sup>a</sup> difference to unmodified RNA

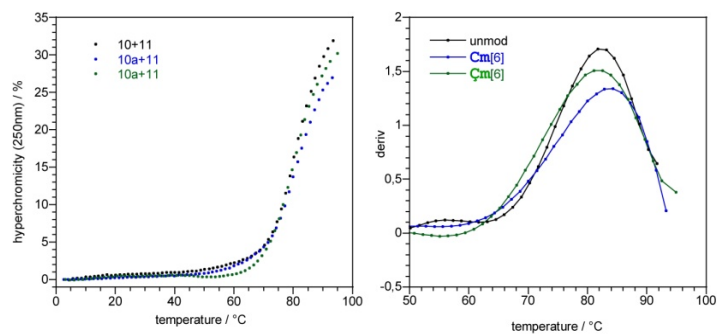
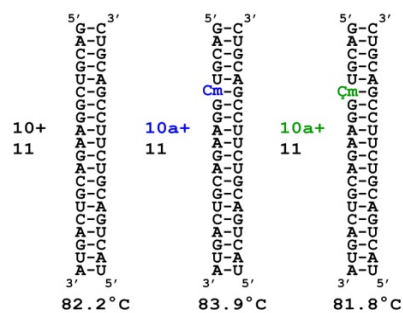


**Figure S3.** top: CD spectra of model duplex **8+9**, 10  $\mu$ M duplex in 10 mM potassium phosphate buffer, pH 7.0, 150 mM NaCl, at 25°C. bottom row: CD spectra of **Cm** and **Çm** modified 20bp-duplex RNAs, 40  $\mu$ M duplex in 10 mM potassium phosphate buffer, pH 7.0, 150 mM NaCl, at 25°C.

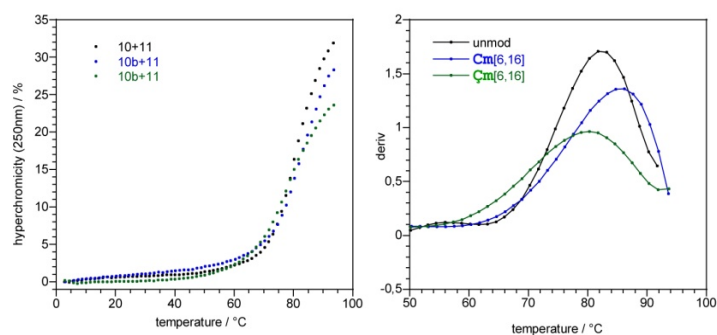
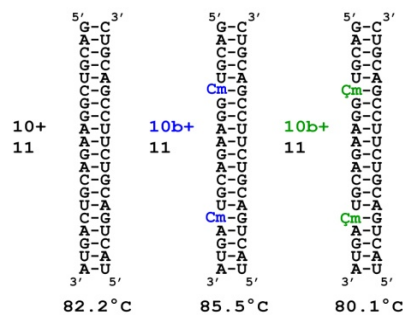


**Figure S4.** UV melting curve analysis of hairpin RNAs.

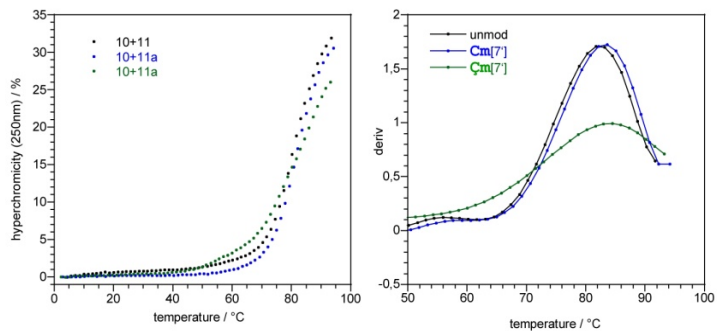
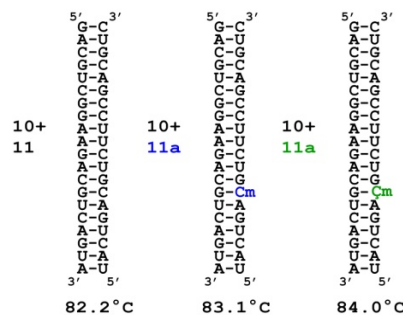
## Duplex C[6]



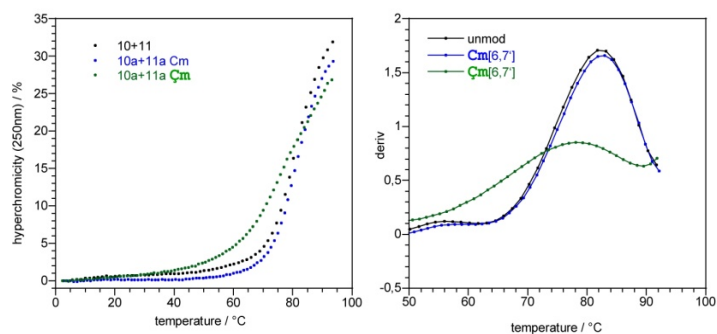
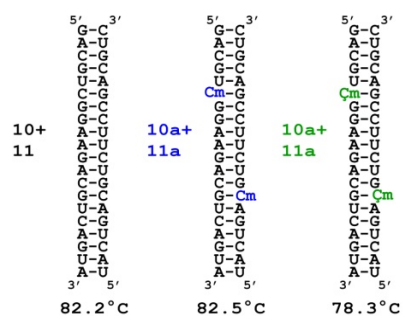
## Duplex C[6,16]



## Duplex C[7']

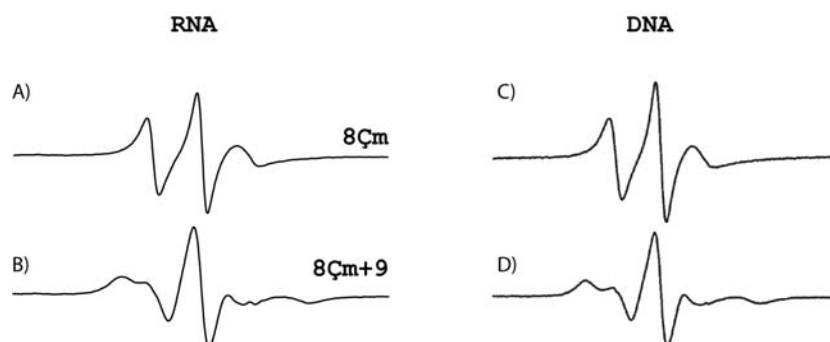


## Duplex C[6,7']

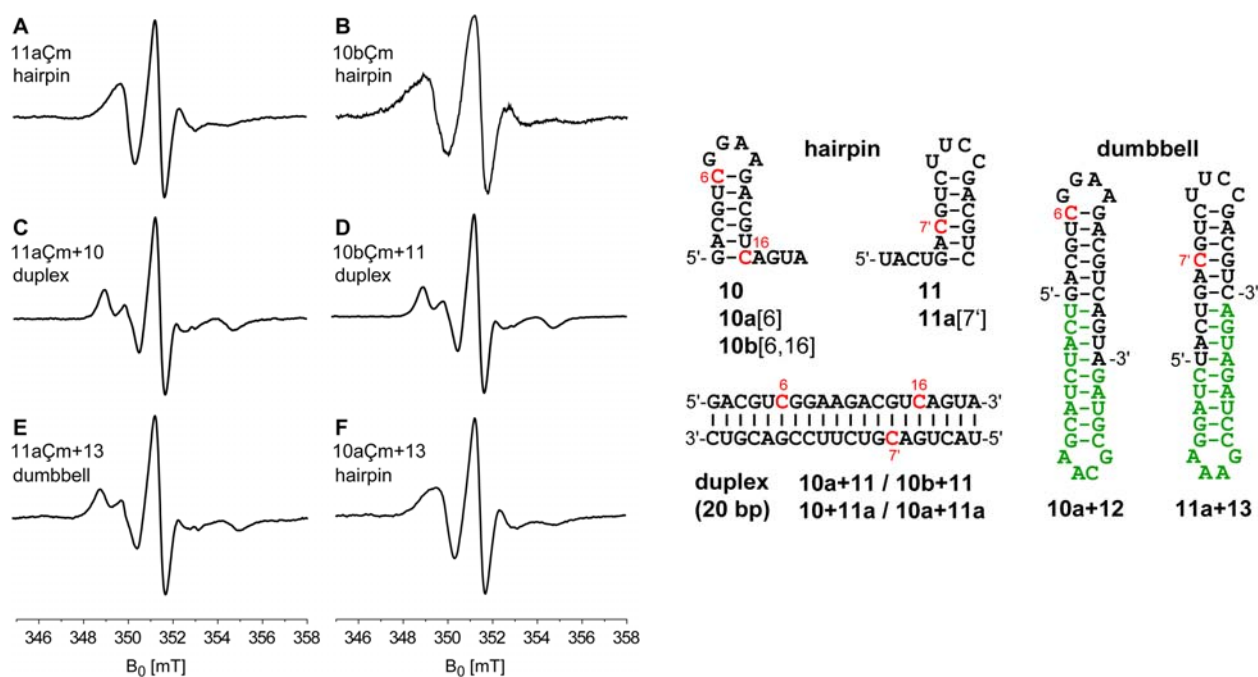


all samples: [RNA] = 10  $\mu$ M, [NaCl] = 150 mM;  
10 mM Na phosphate buffer pH 7.0

Figure S5. UV melting curve analysis of 20bp-duplex RNAs.



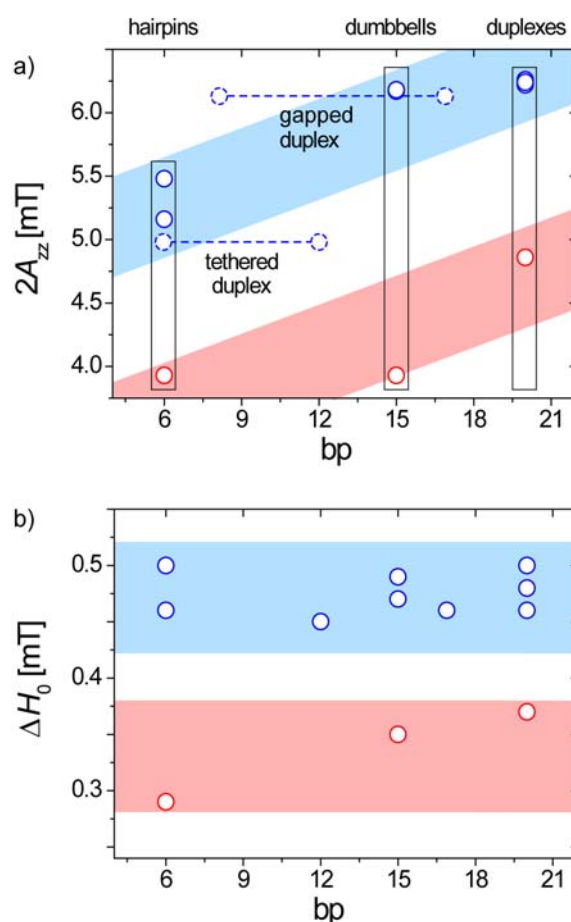
**Figure S6.** CW-EPR spectra of A) single-strand **8Çm** and B) duplex **8Çm+9**; 25  $\mu\text{M}$  **Çm**-labeled RNA, 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0, 23°C. For comparison, CW-EPR spectra of analogous **Ç**-labeled DNA samples are shown. D) single-strand GACCTCGÇATCGTG, D) duplex (GACCTCGÇATCGTG).(CACGATGCGAGGTC). Spectra in A) and B) are same as in Figure 2b; spectra in C) and D) are reproduced from Barhate et al., *Angew. Chem. Int. Ed.* 2007, 46, 2655.



**Figure S7.** Additional CW-EPR spectra of **Çm**-labeled hairpin, duplex and dumbbell RNAs. Spectra were recorded at X-band (9 GHz) over 160 G at 23°C with RNA conc. of 10-25  $\mu\text{M}$  in 10 mM sodium phosphate buffer, pH 7.0 containing 150 mM NaCl. For convenience, Figure 3 of the manuscript is reproduced to the right, showing RNA sequences and secondary structure contexts.

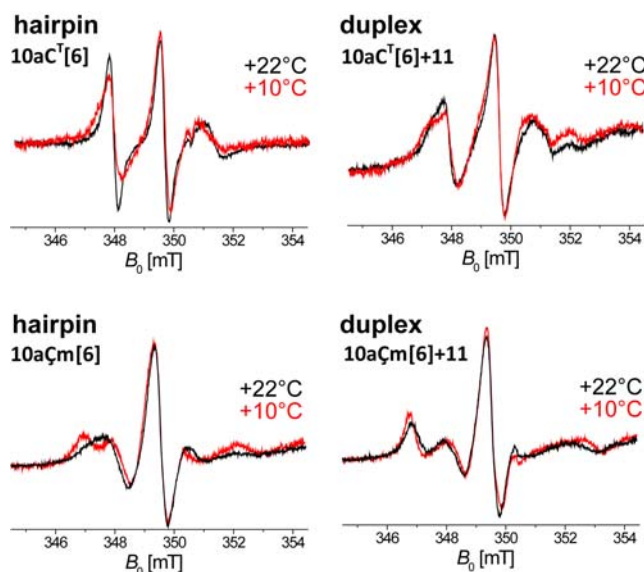
**Table S3.** Hyperfine values and central line widths of CW-EPR spectra depicted in Figure 4, S7, S10.

| Number  | label      | structure [label position] | base-pairs | central line width<br>$\Delta H_0$ [mT] | hyperfine value<br>$2A_{zz}$ [mT] |
|---------|------------|----------------------------|------------|-----------------------------------------|-----------------------------------|
| 10a     | $C^T$      | hairpin [6]                | 6          | 0.29                                    | 3.93                              |
| 10a+11  | $C^T$      | duplex [6]                 | 20         | 0.37                                    | 4.86                              |
| 10a+12  | $C^T$      | dumbbell [6]               | 15         | 0.35                                    | 3.93                              |
| 10a     | $\dot{C}m$ | hairpin [6]                | 6          | 0.50                                    | 5.48                              |
| 10a+11  | $\dot{C}m$ | duplex [6]                 | 20         | 0.50                                    | 6.26                              |
| 10a+12  | $\dot{C}m$ | dumbbell [6]               | 15         | 0.49                                    | 6.17                              |
| 11a     | $\dot{C}m$ | hairpin [7']               | 6          | 0.46                                    | 5.16                              |
| 10+11a  | $\dot{C}m$ | duplex [7']                | 20         | 0.48                                    | 6.22                              |
| 11a+13  | $\dot{C}m$ | dumbbell [7']              | 15         | 0.47                                    | 6.18                              |
| 11a+14  | $\dot{C}m$ | tethered duplex [7']       | 6/12       | 0.45                                    | 4.98                              |
| 11a+15  | $\dot{C}m$ | gapped duplex [7']         | 8/17       | 0.46                                    | 6.13                              |
| 10a+11a | $\dot{C}m$ | duplex [6,7']              | 20         | 0.46                                    | 6.24                              |



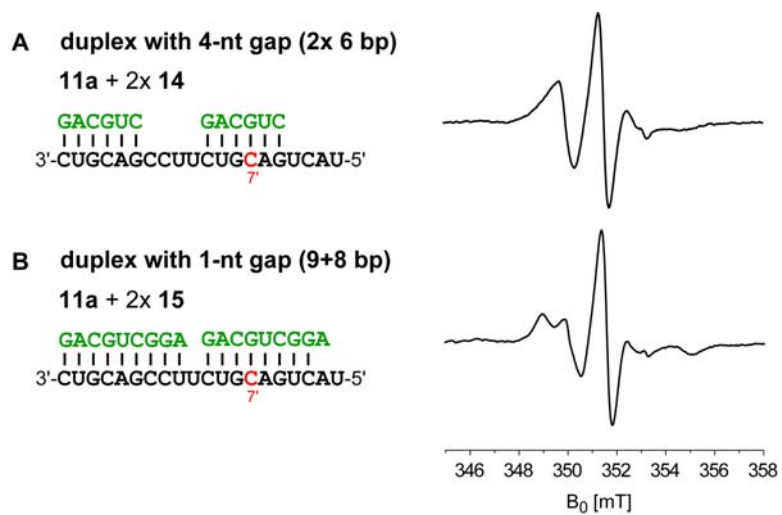
**Figure S8.** Graphic depiction of a) hyperfine values ( $2A_{zz}$ ) and b) width of the central line ( $\Delta H_0$ ) as a function of the number of base-pairs in  $\dot{C}m$  (blue)- and  $C^T$  (red)-labeled RNA structures. Tri-molecular structures are represented using outlined symbols with connecting lines between the number of base-pairs in the spin-labeled stem (i.e., 6 bp in the tethered duplex **11+14**, and 8 bp in the gapped duplex **11+15**) and the total number of base-pairs in each construct. This representation helps to visualize that the mobility of the tethered duplex is more closely related to the hairpin structure (which also contains a 6-bp stem), while the  $2A_{zz}$  value for the gapped duplex fits better into the region for a duplex containing 17 base-pairs. The numerical data are given in Table S3.



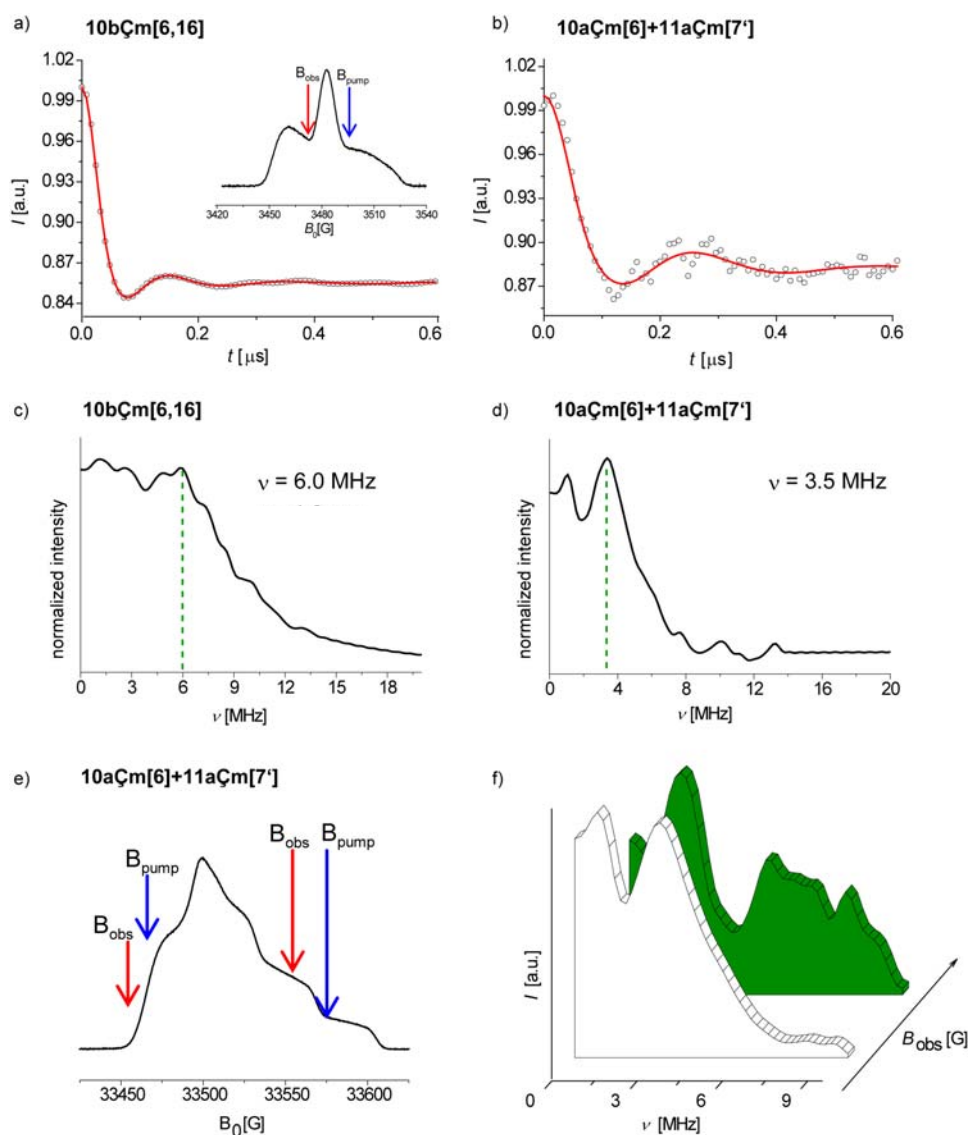


**Figure S9.** CW-EPR spectra of  $C^T$ - (top) and  $\mathcal{C}m$ - (bottom) labeled hairpin RNAs **10a** (left) and duplex **10a+11** (right). Spectra are compared at 22°C (black line) and 10°C (red line). CW EPR measurements at variable temperatures were carried out at 9 GHz using a Varian E-12 ESR spectrometer with nitrogen gas flow temperature regulation. Samples were contained in 1-mm i.d. glass capillaries which were immersed in silicone oil to improve thermal stability. The sample temperature was measured with a thermocouple placed inside the quartz tube just above the top of the ESR cavity.

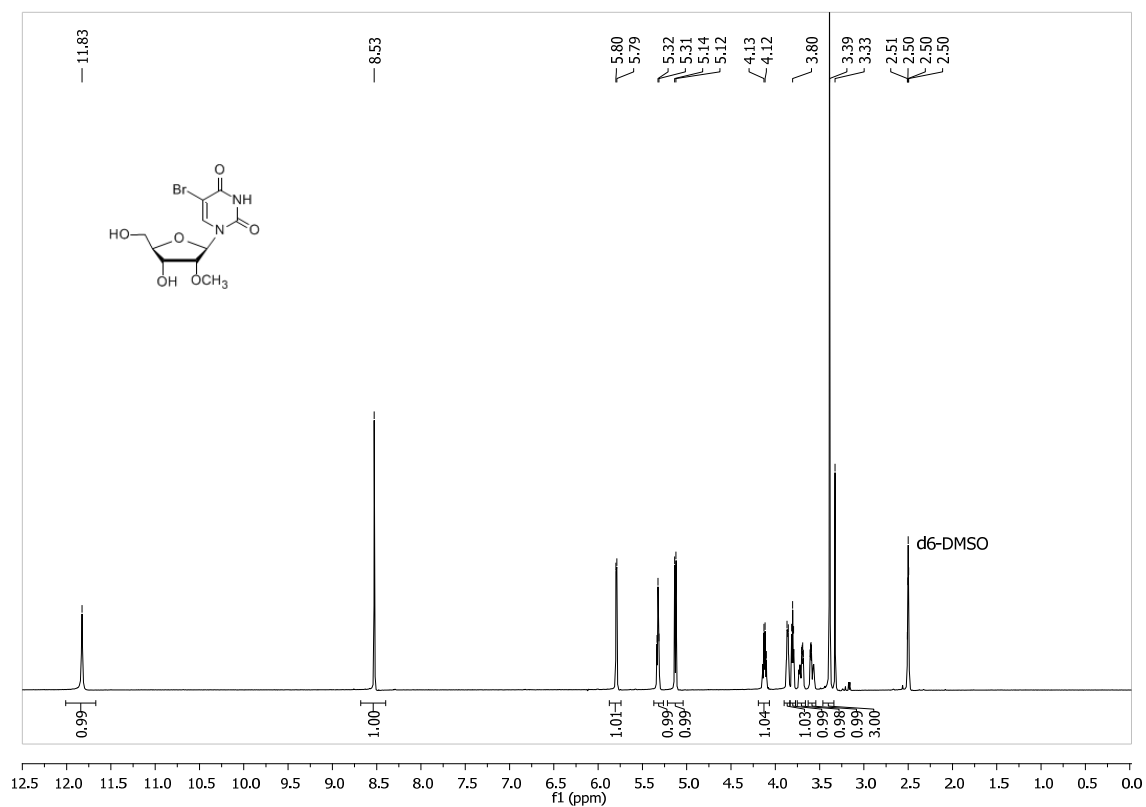
The spectrum of the  $C^T$ -labeled hairpin RNA **10a** was not significantly affected by lowering the temperature, which suggests a low energy barrier for spin-label rotation. Also in the duplex **10aC<sup>T</sup>+11**, only a minor change in line width was observed. In contrast, spectral broadening and significant splitting of the low field component was observed for the  $\mathcal{C}m$ -labeled hairpin structure **10aÇm**. This result also supports the finding that the internal local motion of the  $C^T$  label is much higher than the mobility of the  $\mathcal{C}m$  label. At a temperature difference of only 12°C, this pronounced effect is not due to changes in sample viscosity, and therefore likely reflects decreased local dynamics of the loop-closing base-pair in the hairpin, which can only be monitored by the new label  $\mathcal{C}m$ .



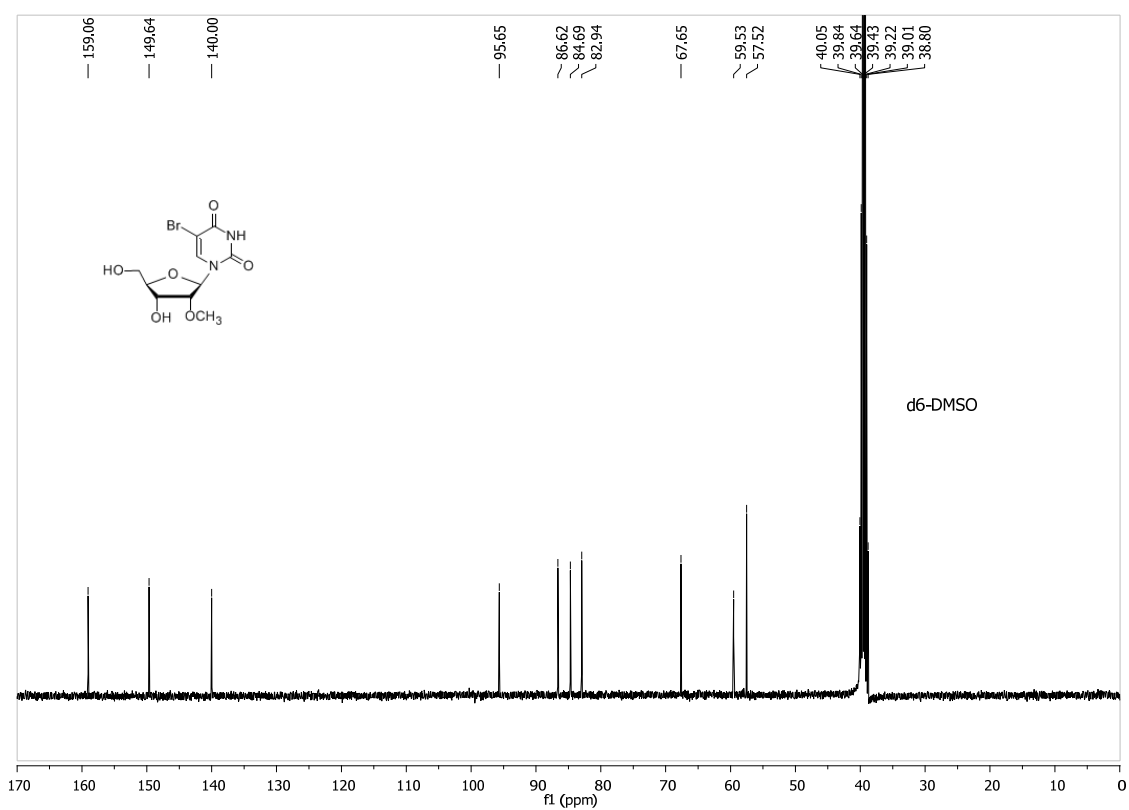
**Figure S10.** Tri-molecular RNA structures investigated. Secondary structures and CW-EPR spectra of gapped duplexes. Spectra were recorded at X-band (9 GHz) over 160 G at 23°C with RNA conc. of 25  $\mu$ M in 10 mM sodium phosphate buffer, pH 7.0 containing 150 mM NaCl.



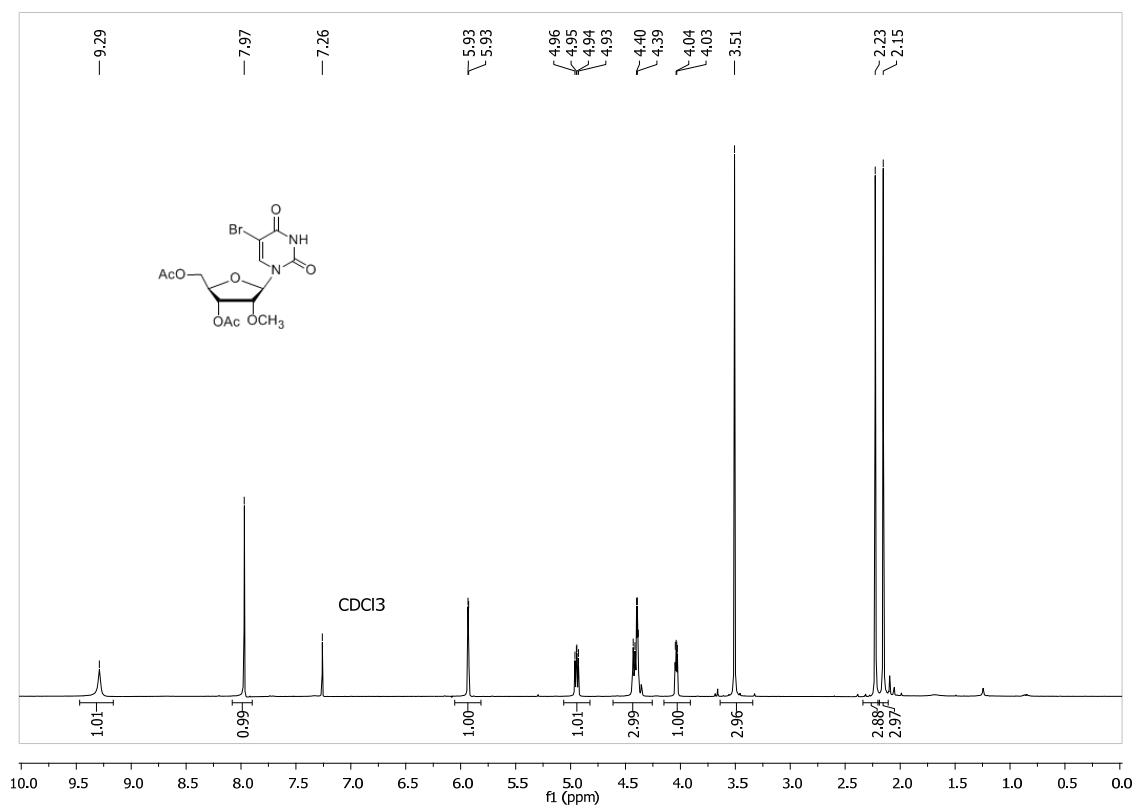
**Figure S11.** Pulsed electron double resonance (PELDOR) experiments for double-labeled RNA. Dipolar evolution functions at 9 GHz of a) double-Çm-labeled hairpin **10bÇm** and b) double-labeled duplex **10aÇm+11aÇm**. The inset in a) shows the 9 GHz ESE spectrum, and indicates the difference between pump and observe pulses (65 MHz). All experiments were performed using the four-pulse PELDOR sequence  $\pi/2(\nu_1) - \tau_1 - \pi(\nu_1) - \tau_0 - \pi(\nu_2) - (\tau_1 + \tau_2 - \tau_0) - \pi(\nu_1) - \tau_2 - \text{echo}$ . Parameters (9 GHz):  $T = 50$  K,  $\pi/2 = 16$  ns,  $\pi = 32$  ns,  $\pi_{\text{ELDOR}} = 36$  ns, SPP = 20, SRT = 5 ms, 100 scans;  $[c] = 50$   $\mu$ M. c) and d) Fourier-transformed dipolar spectra at X-band (Pake patterns) of experiments shown in a) and b), respectively. The green lines indicate the frequencies  $\nu_{\perp}$ , corresponding to inter-spin distances of 2.0 nm and 2.5 nm, respectively. e) 94 GHz ESE spectrum of double-labeled duplex **10aÇm+11aÇm**. The arrows indicate two sets of pump and observe pulses, used for detection of PELDOR data shown in f). The parameters for the 94 GHz PELDOR experiment were:  $T = 40$  K,  $\pi/2 = 24$  ns,  $\pi_{\text{ELDOR}} = 56$  ns, SRT = 15 ms, 30 scans/trace;  $[c] = 60$   $\mu$ M. The Pake patterns in c), d), and f) display clear orientation selection, which is currently being analyzed in detail using orientation selection PELDOR experiments at high-field with fixed and variable frequency separation (G. Sicoli et al, manuscript in preparation).



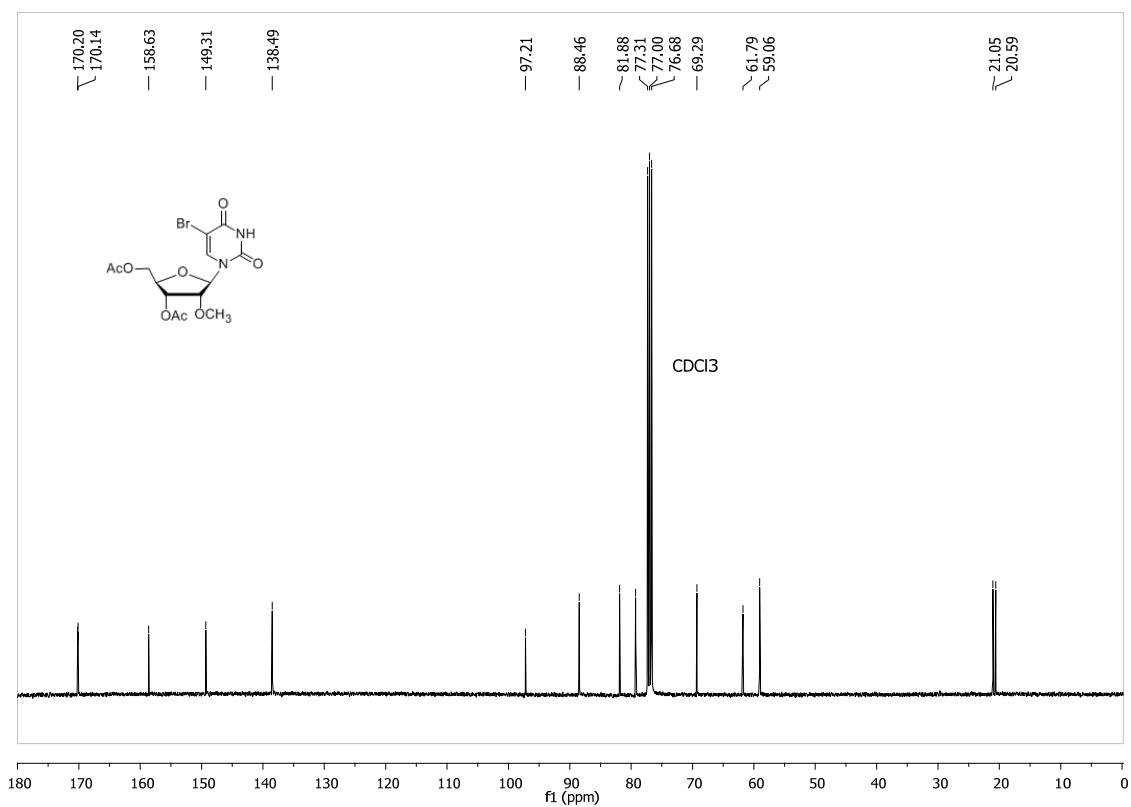
**Figure S12.**  $^1\text{H}$  NMR spectrum of **2**



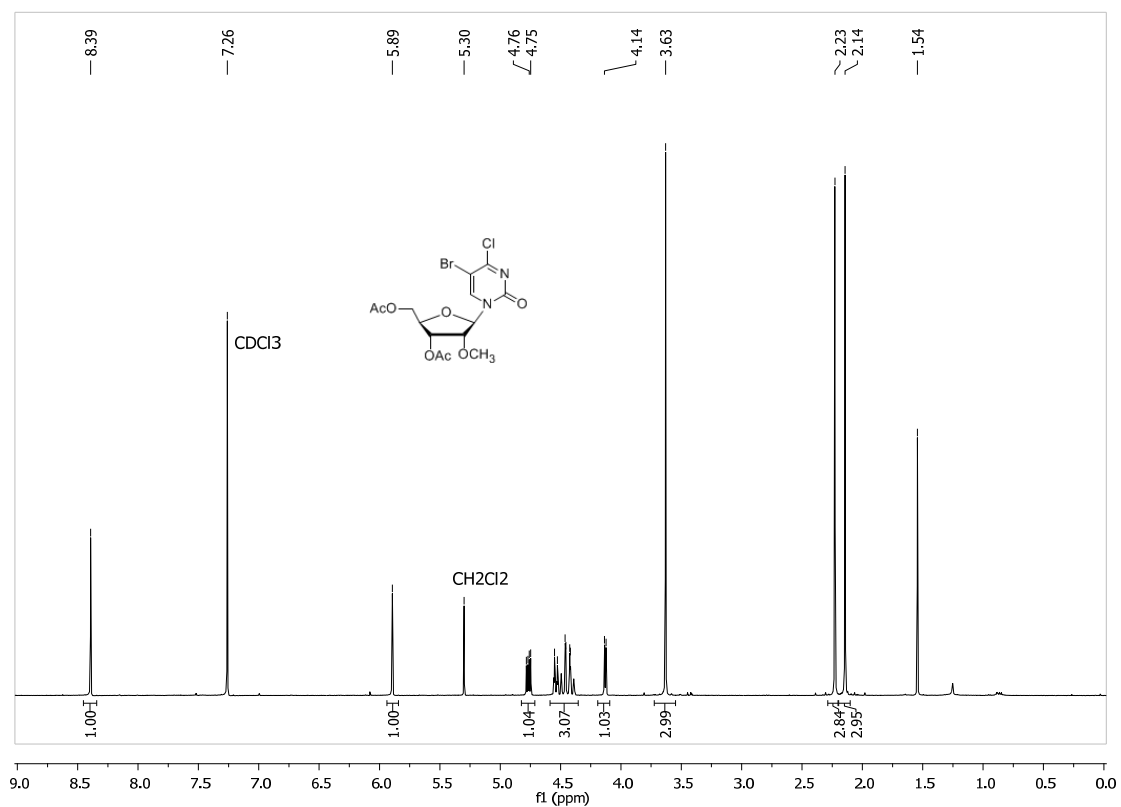
**Figure S13.**  $^{13}\text{C}$  NMR spectrum of **2**



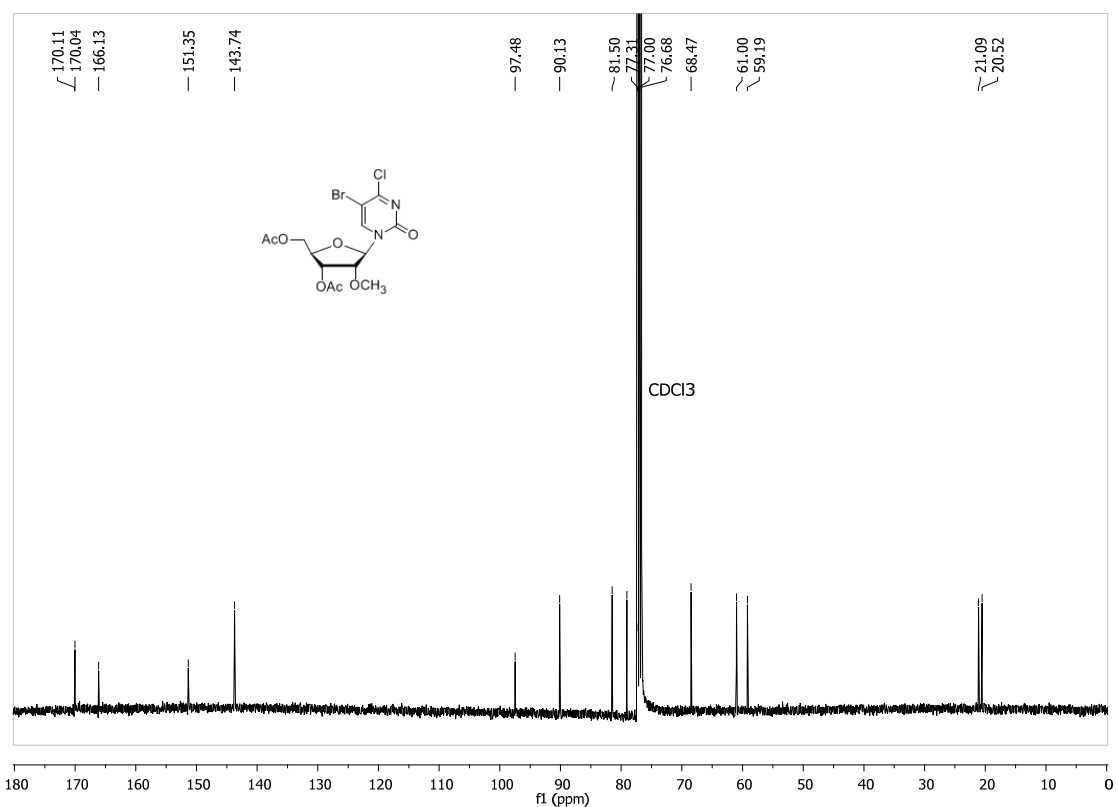
**Figure S14.**  $^1\text{H}$  NMR spectrum of **3**



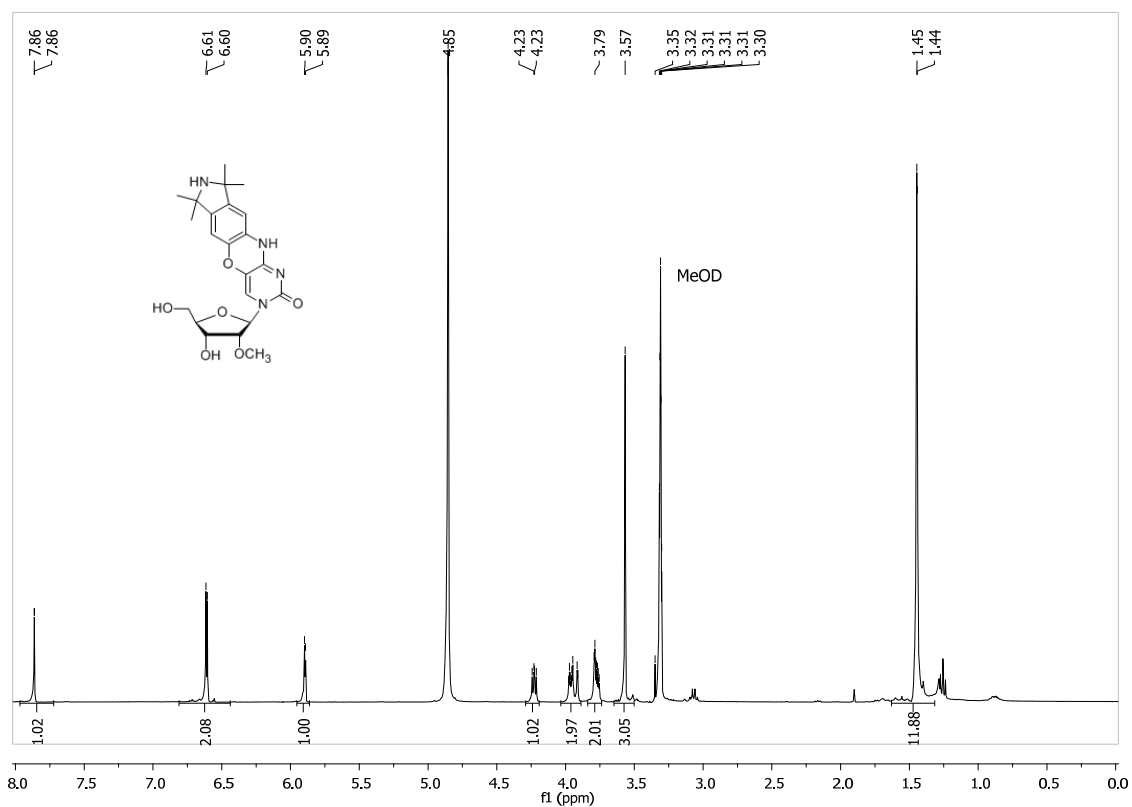
**Figure S15.**  $^{13}\text{C}$  NMR spectrum of **3**



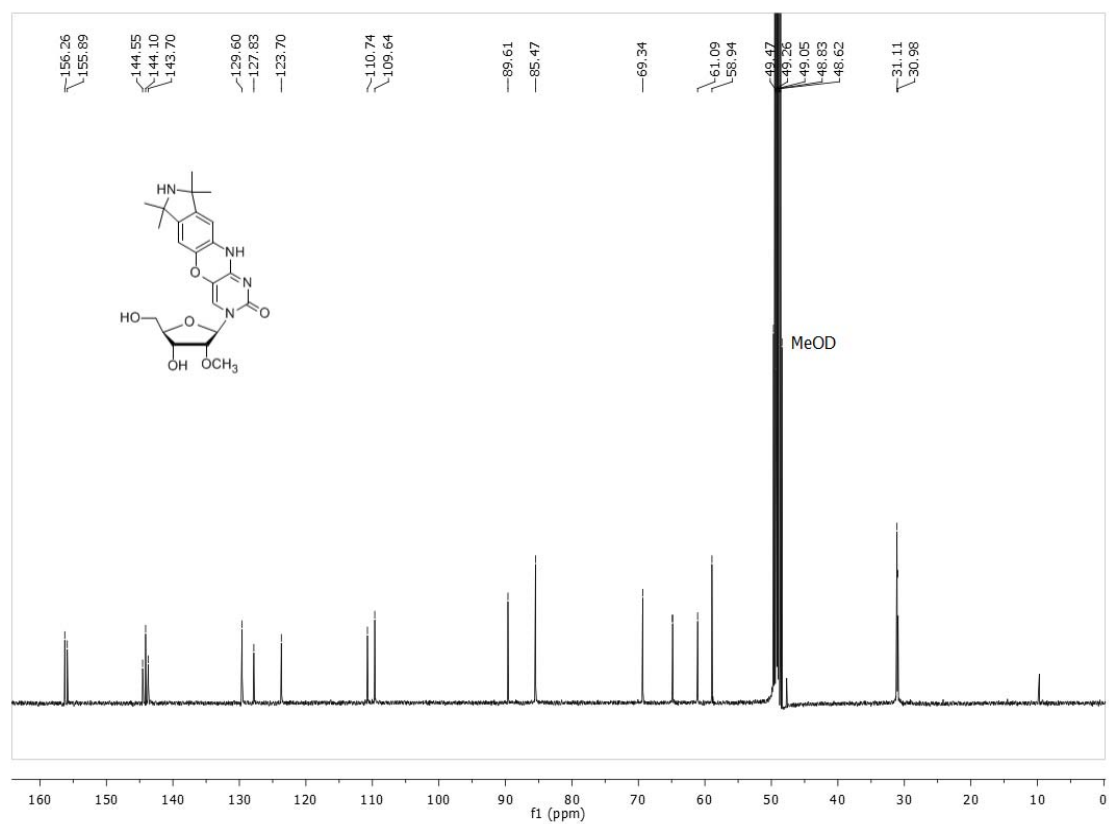
**Figure S16.**  $^1\text{H}$  NMR spectrum of **4**



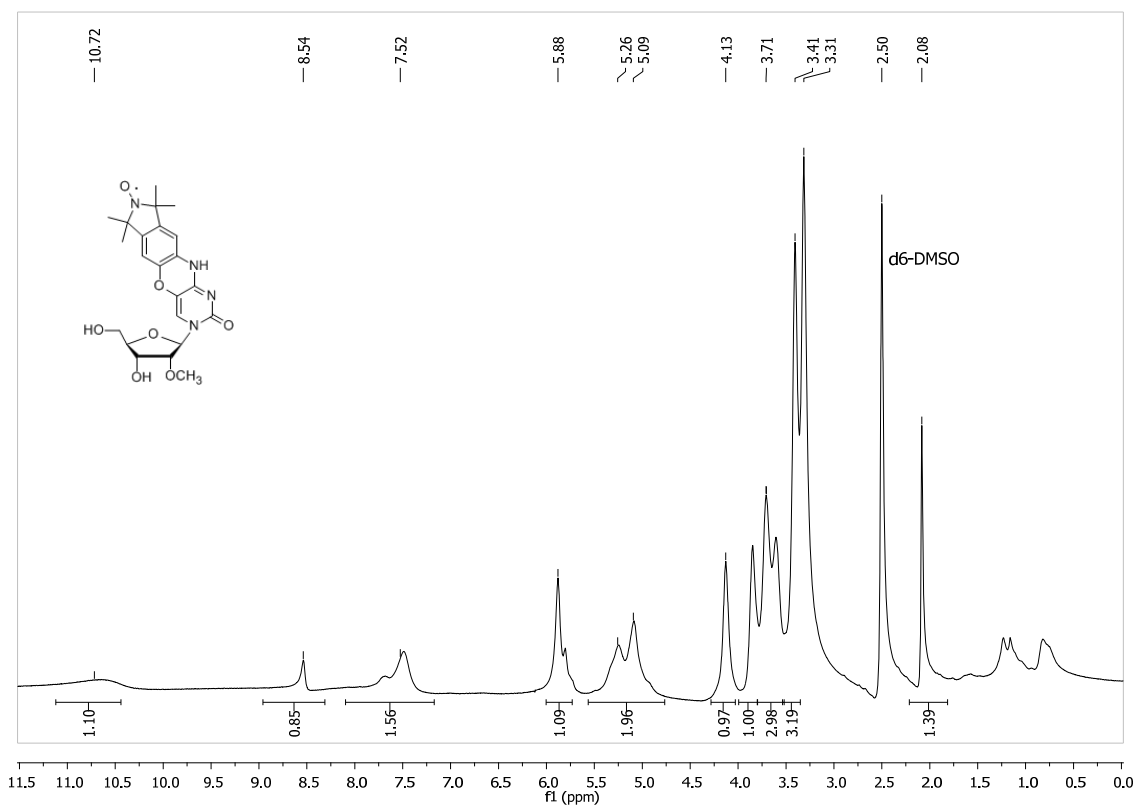
**Figure S17.**  $^{13}\text{C}$  NMR spectrum of **4**



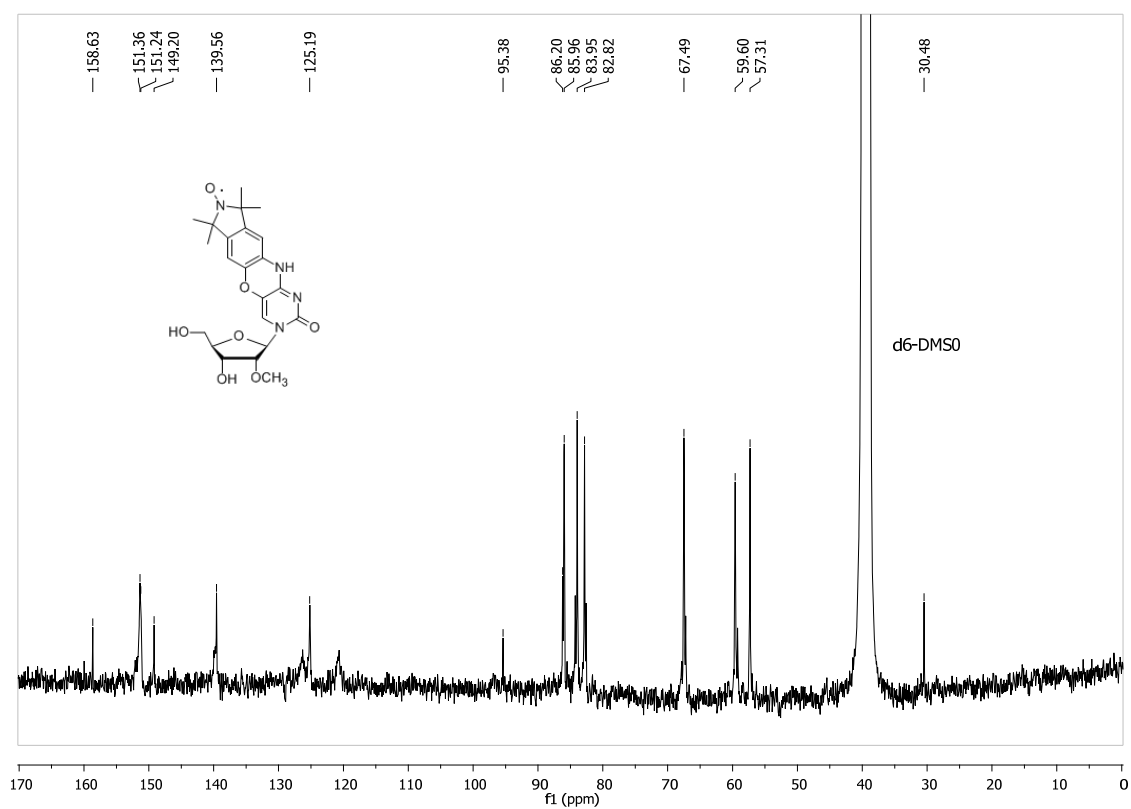
**Figure S18.**  $^1\text{H}$  NMR spectrum of **7**



**Figure S19.**  $^{13}\text{C}$  NMR spectrum of **7**

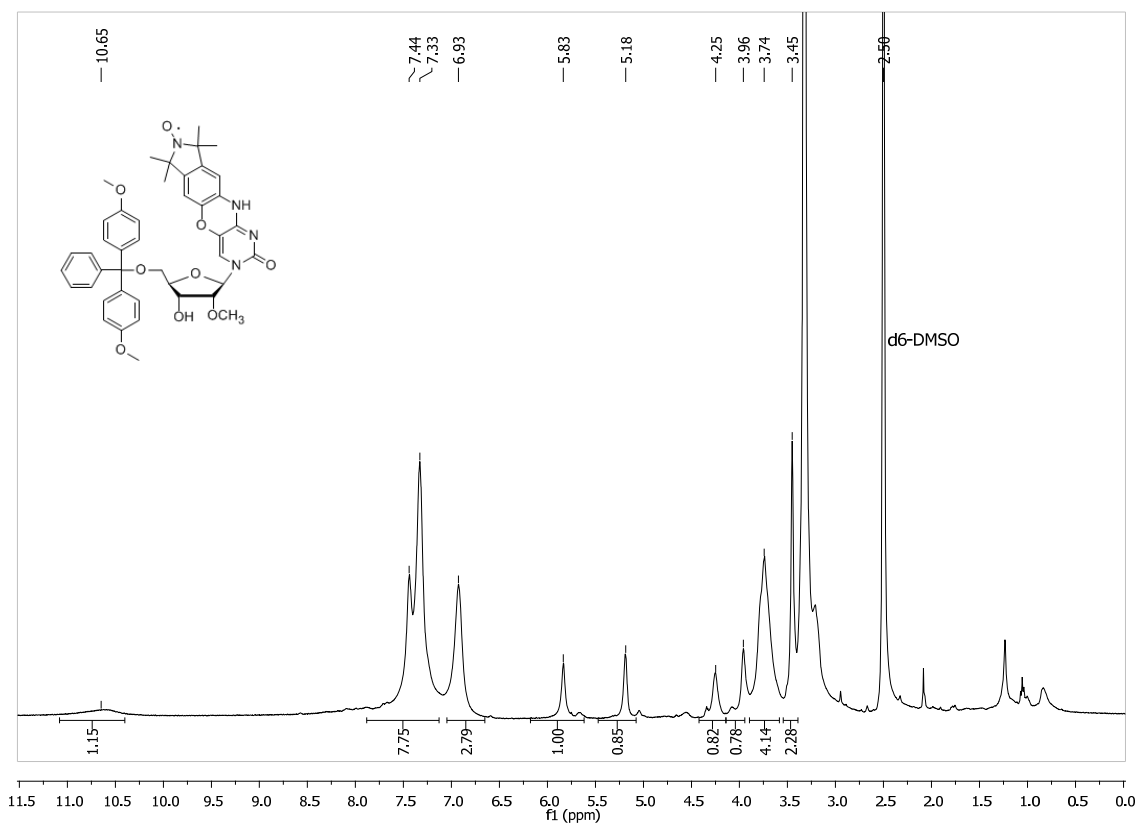


**Figure S20.**  $^1\text{H}$  NMR spectrum of **Cm**

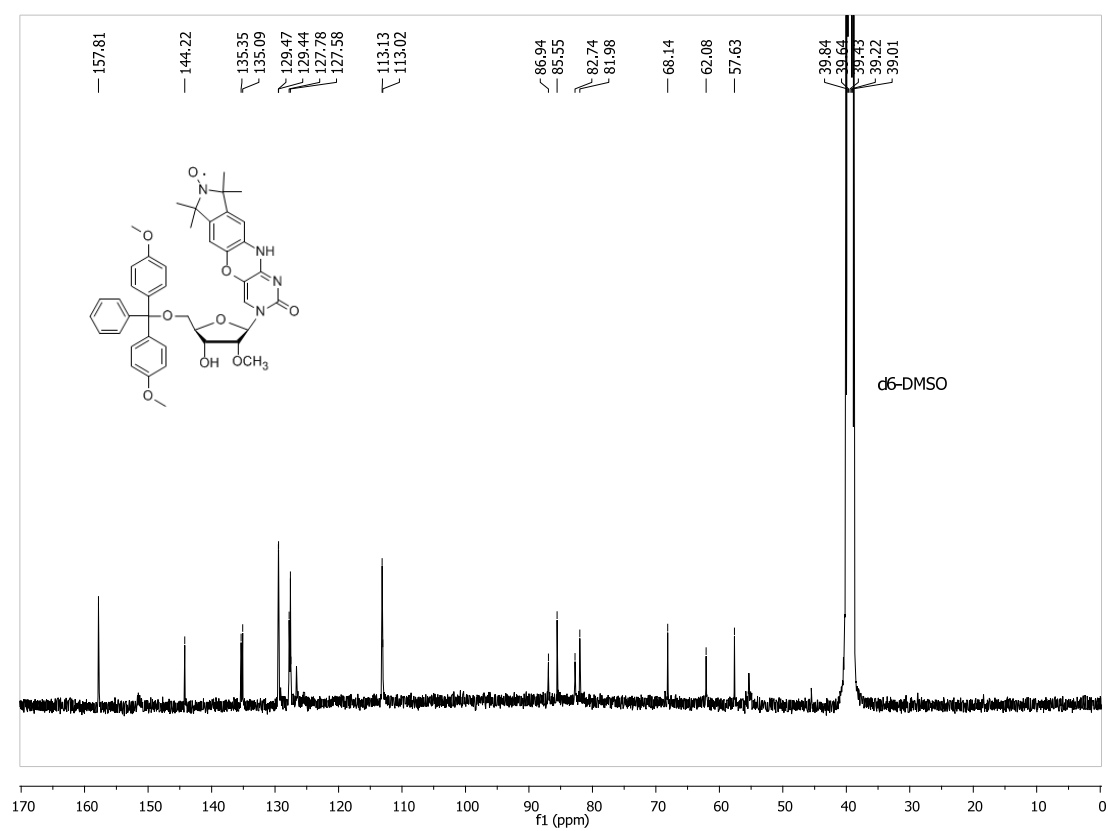


**Figure S21.**  $^{13}\text{C}$  NMR spectrum of **Cm**

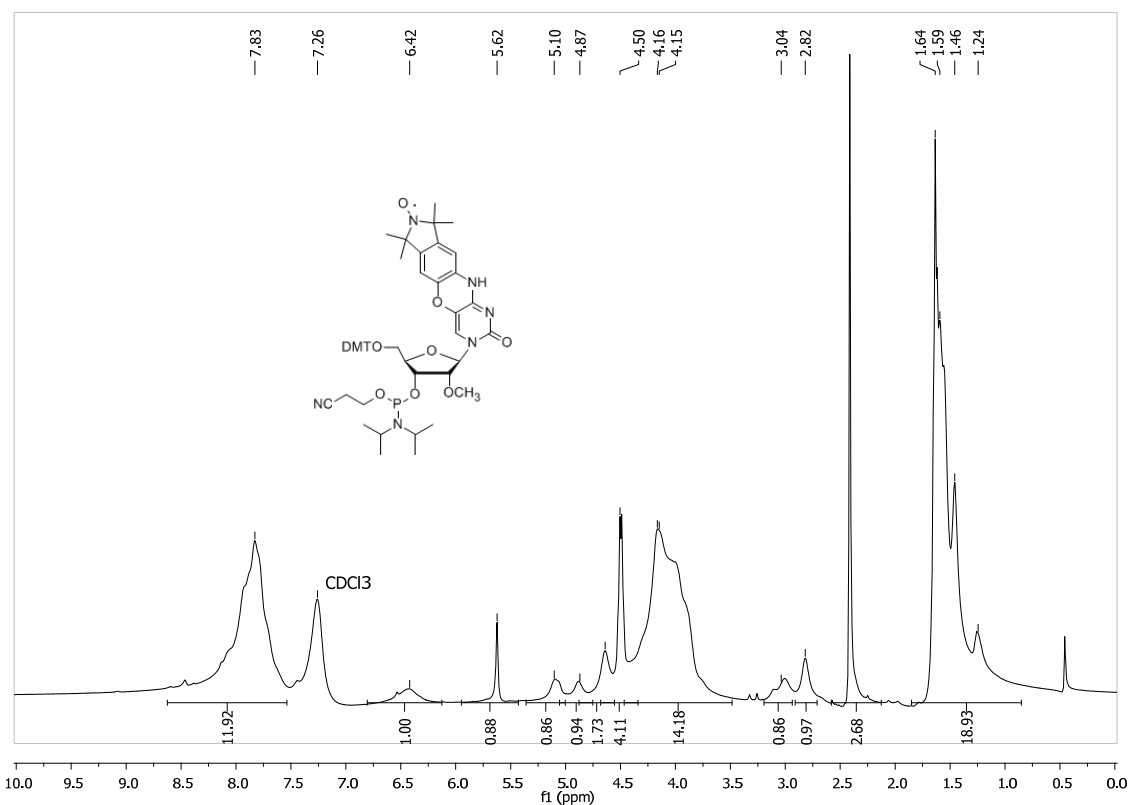
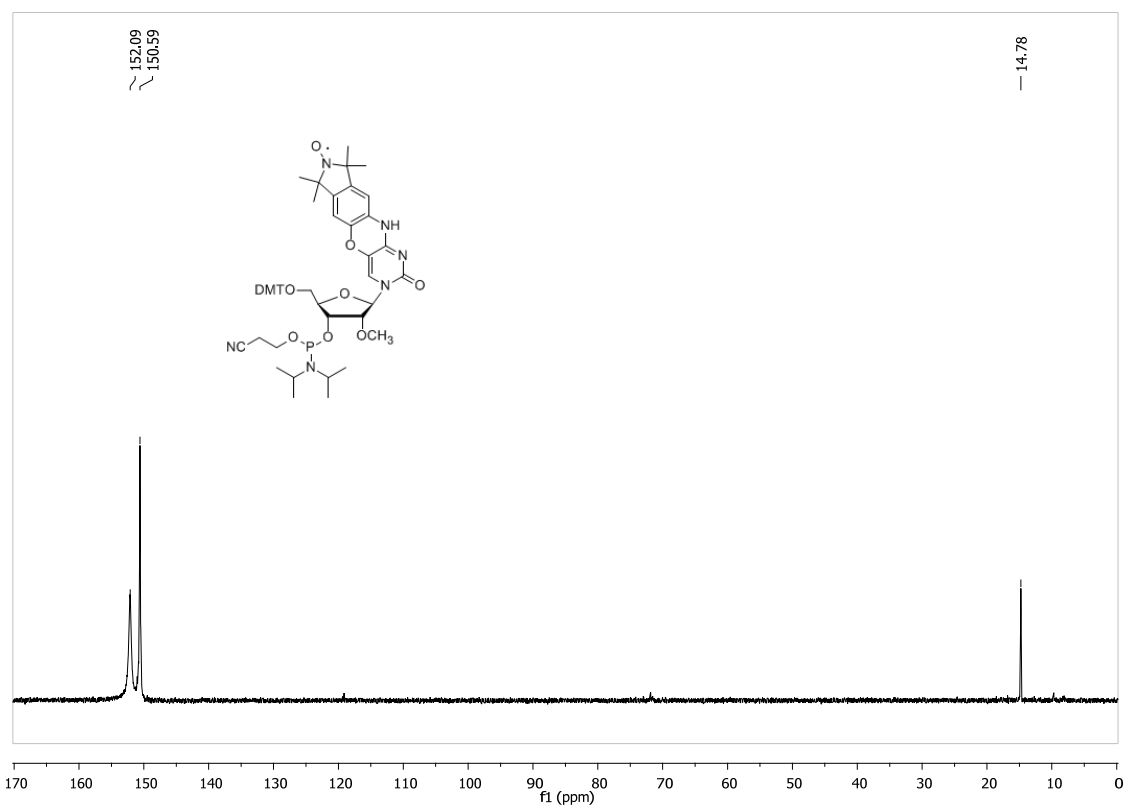




**Figure S22.**  $^1\text{H}$  NMR spectrum of tritylated  $\text{Cm}$



**Figure S23.**  $^{13}\text{C}$  NMR spectrum of tritylated  $\text{Cm}$

**Figure S24.** <sup>1</sup>H NMR spectrum of **Çm phosphoramidite 1****Figure S25.** <sup>31</sup>P NMR spectrum of **Çm phosphoramidite 1**