Supporting Information

# Reversible Photocycloaddition of 8-Pyrenylvinylguanine for Photoreactive Serinol Nucleic Acid (SNA)

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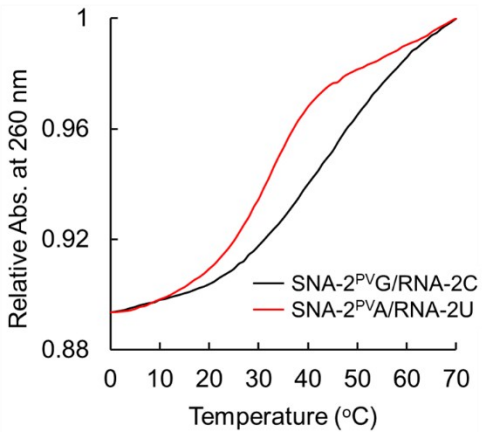



**Fig. S1** Melting profile of duplex between RNA and SNA containing $2^{PV}$G (**SNA-$2^{PV}$G/RNA-2C**, black line, $T_m$ = 40.4 °C) and $2^{PV}$A (**SNA-$2^{PV}$A/RNA-2U**, red line, $T_m$ = 31.0 °C). Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM.

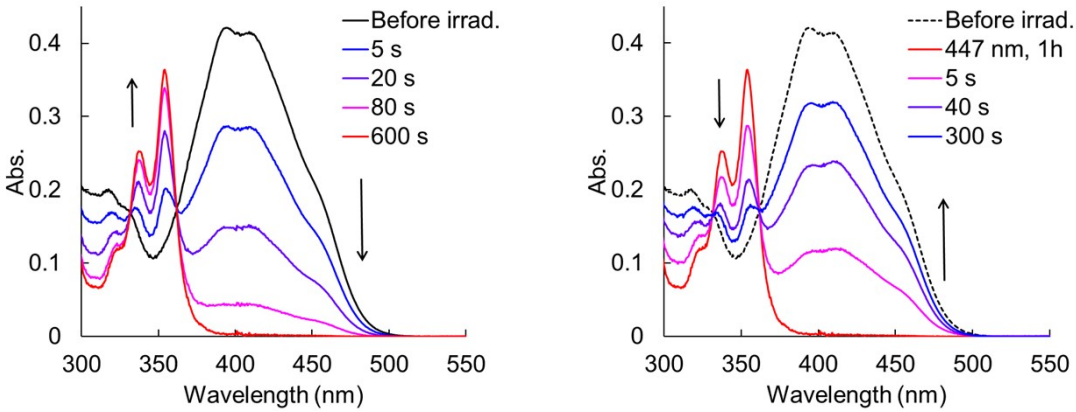



**Fig. S2** (Left) Absorption spectra of **SNA-$2^{PV}$A/RNA-2U** at indicated times of irradiation with 447 nm light. (Right) Absorption spectra of **SNA-$2^{PV}$A/RNA-2U** after 10 min irradiation with 447 nm and after irradiation for indicated times with 350 nm light. Irradiation was performed at 20 °C. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM.

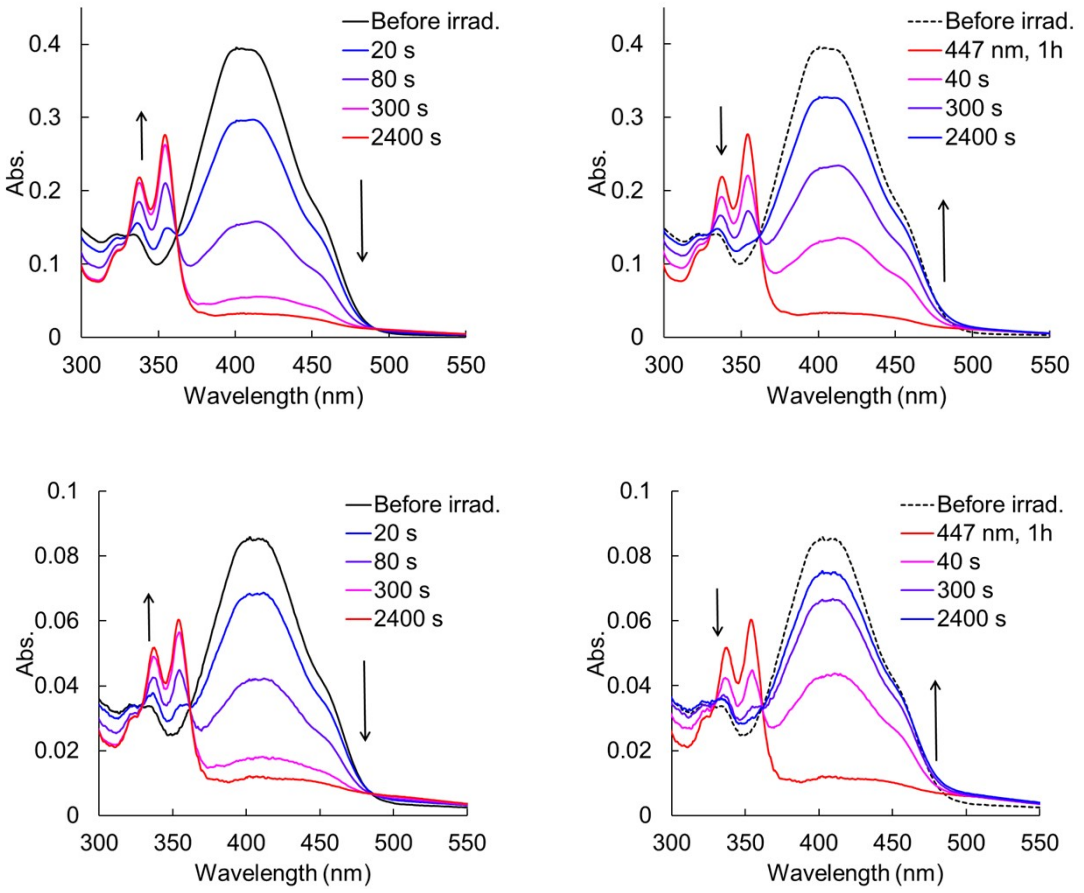



**Fig. S3** (Left) Absorption spectra of **SNA-2$^{PV}$G** single strand at indicated times of irradiation with 447 nm light. (Right) Absorption spectra of **SNA-2 $^{PV}$G** single strand after 1 h irradiation with 447 nm and after irradiation for indicated times with 350 nm light. Irradiation was performed at 20 °C. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM (upper two panels) and 1.0 μM (lower two panels).

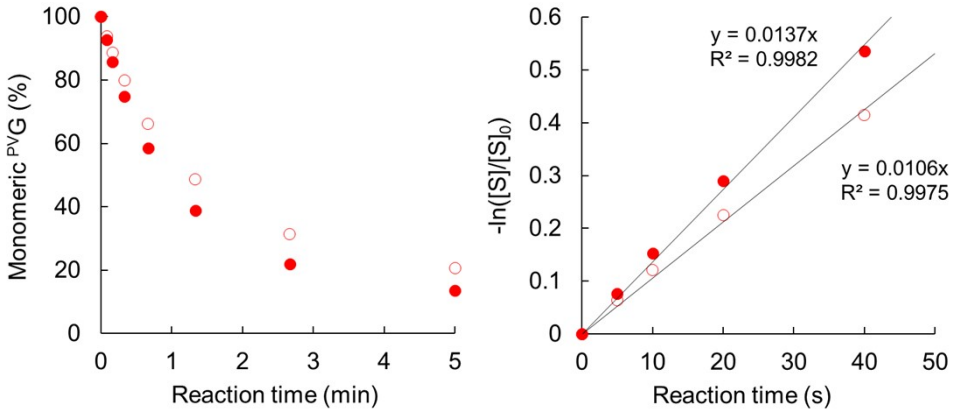



**Fig. S4** (Left) The ratio of monomeric $^{PV}$G in **SNA-2$^{PV}$G** single strand at different concentrations. The ratio of the monomers was calculated from the absorbance at 400 nm. (Right) Plot of -ln([$^{PV}$G]/[$^{PV}$G]$_0$) as a function of time at the initial stage of the reaction. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM (closed circles) and 1.0 μM (open circles).

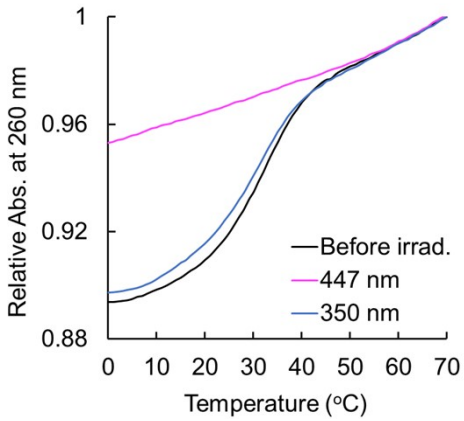



**Fig. S5** Melting profile of **SNA-2$^{PV}$A/RNA-2U** before (black line) and after irradiation with 447 nm light (purple line) and 350 nm light (blue line). Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM.

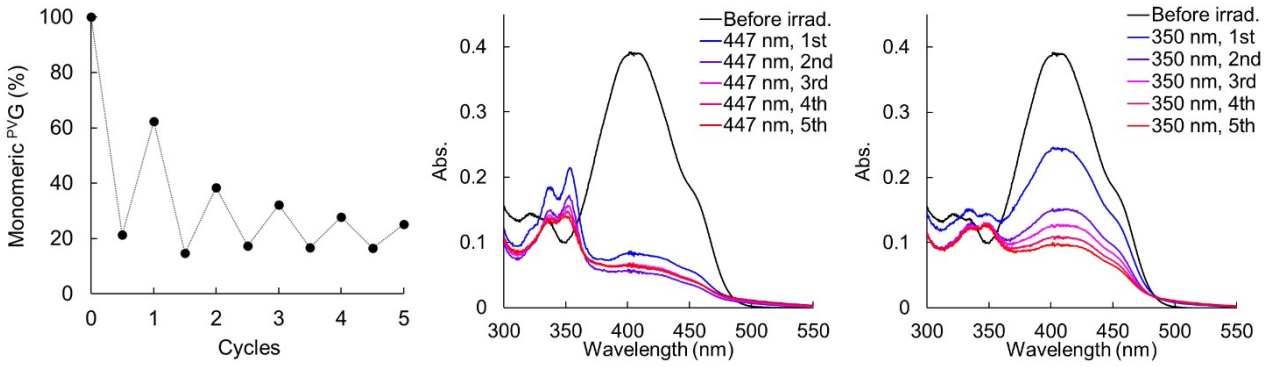



**Fig. S6** (Left) Ratios of remaining monomeric $^{PV}$G after multiple photoswitching cycles with 447 nm and 350 nm to **SNA-2$^{PV}$G/RNA-2C** duplex. The concentration of remaining monomers was calculated by using absorption spectra recorded after each irradiation. (Middle and right) Absorption spectra of **SNA-2$^{PV}$G/RNA-C** after repeated irradiation cycles. Samples were irradiated at 447 nm for 1 h and at 350 nm for 1 h, alternatively. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0) 20 °C. The concentration of oligonucleotides was 5.0 μM.

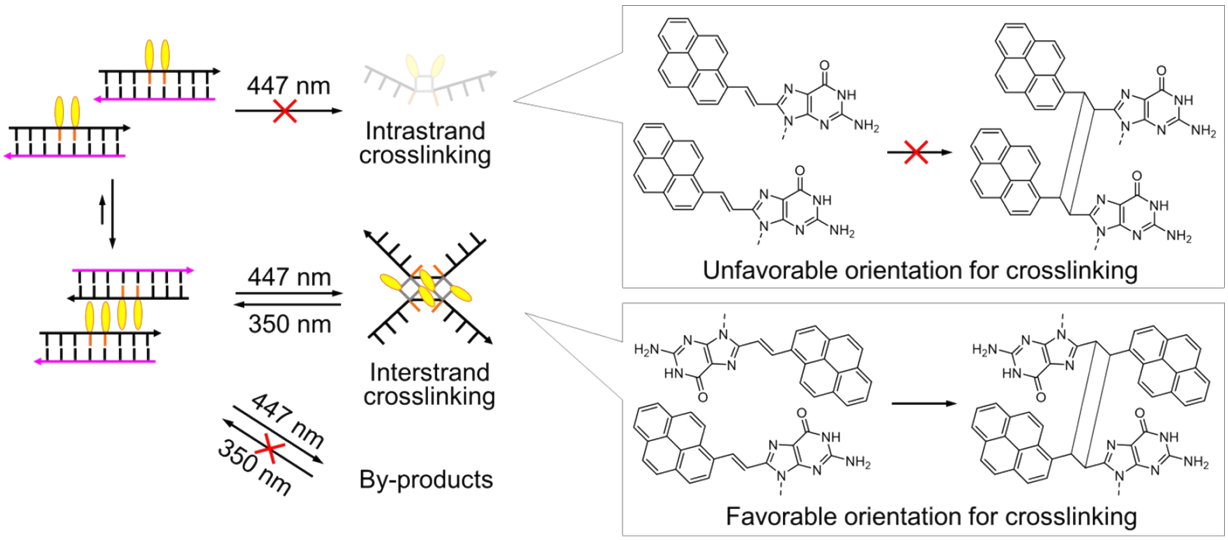



**Fig. S7** Schematics of the predicted mechanism and favorable orientation for interstrand photocrosslinking reaction of $^{PV}$Gs.

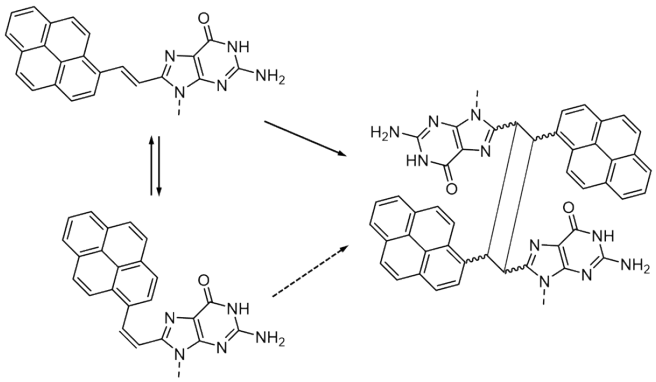


**Fig. S8** Schematics of photocycloaddition of $^{PV}$Gs via *trans-cis* isomerization in single stranded SNA-1$^{PV}$G.

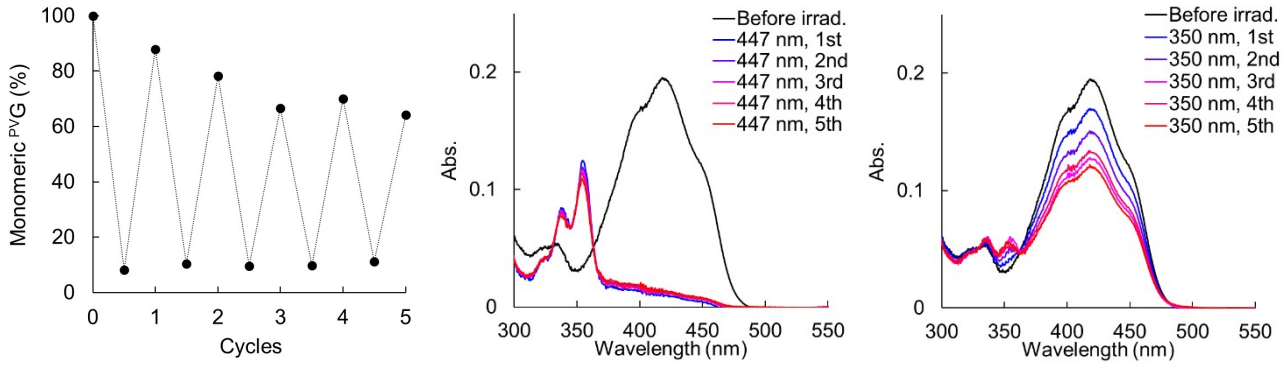



**Fig. S9** (Left) The %ratios of remaining monomeric $^{PV}$G after multiple photoswitching cycles with 447 nm and 350 nm to **SNA-1$^{PV}$G** single strand. The concentration of remaining monomers was calculated by using absorption spectra recorded after each irradiation. (Middle and right) Absorption spectra of **SNA-1$^{PV}$G** after repeated irradiation cycles. Samples were irradiated at 447 nm for 20 min and at 350 nm for 10 min, alternatively. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0) 20 °C. The concentration of oligonucleotide was 5.0 μM.

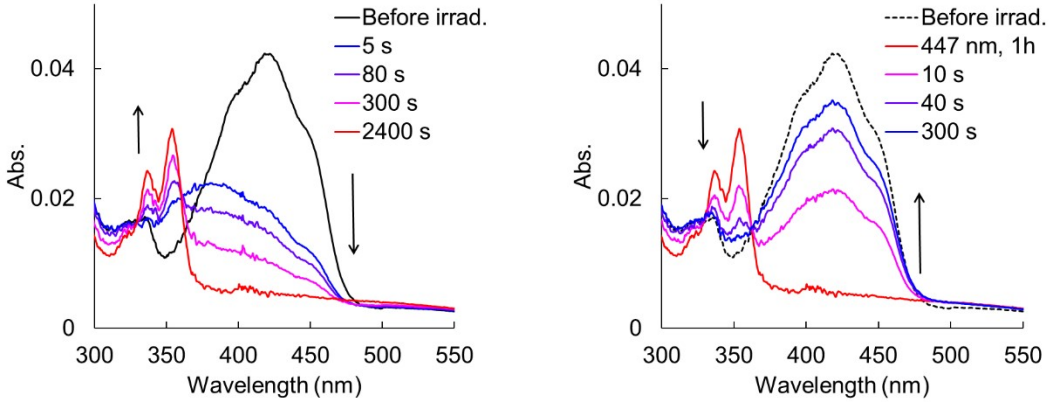



**Fig. S10** (Left) Absorption spectra of **SNA-1$^{PV}$G** single strand at indicated times of irradiation with 447 nm light. (Right) Absorption spectra of **SNA-1$^{PV}$G** single strand after 1 h irradiation with 447 nm and after irradiation for indicated times with 350 nm light. Irradiation was performed at 20 °C. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 1.0 μM.

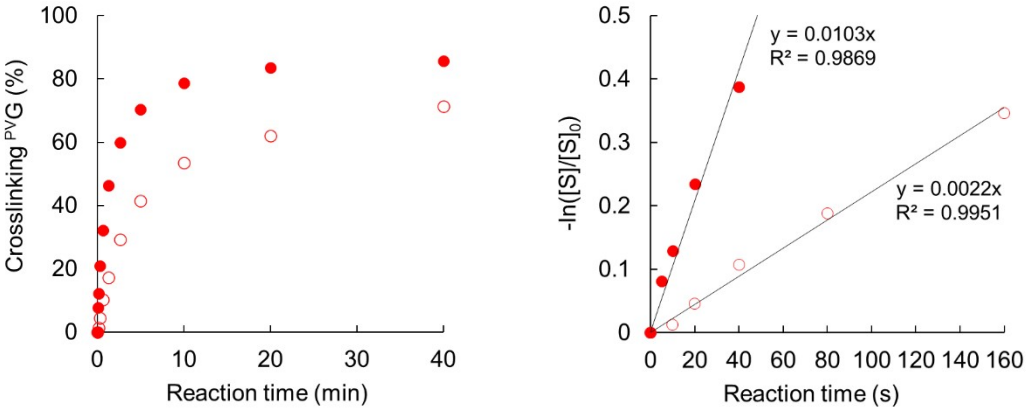



**Fig. S11** (Left) The ratio of crosslinking $^{PV}$G in **SNA-1$^{PV}$G** single strand at different concentration. In order to ignore spectral changes caused by *cis*-isomerization, calculations of crosslinking ratio were performed at 376 nm, which is near the isosbestic point of *trans-cis* isomerization and where there is no absorption of the cross-linked $^{PV}$Gs. (Right) Plot of -ln([$^{PV}$G]/[$^{PV}$G]$_0$) as a function of time at the initial stage of the reaction. [$^{PV}$G] is a concentration of un-crosslinked $^{PV}$Gs. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM (closed circles) and 1.0 μM (open circles).

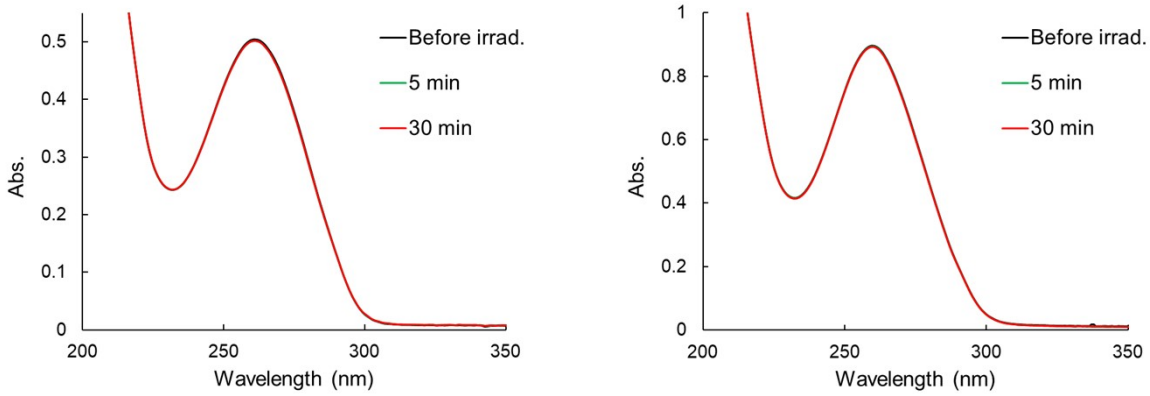



**Fig. S12** (Left) The absorption spectra of single-stranded **SNA-0$^{PV}$G** ((*S*)-ACTGGTCA-(*R*)) before and after irradiation with 350 nm light. (Right) The absorption spectra of **SNA-0$^{PV}$G/RNA-2C** duplex before and after irradiation with 350 nm light. Change in the absorbance at 260 nm was less than 1%. Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM.

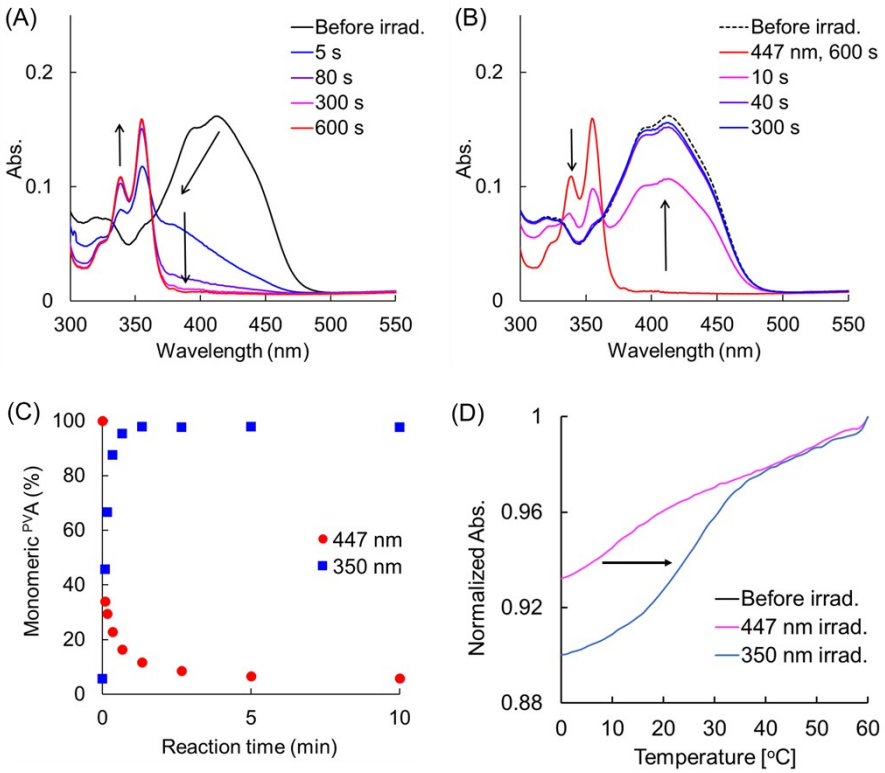
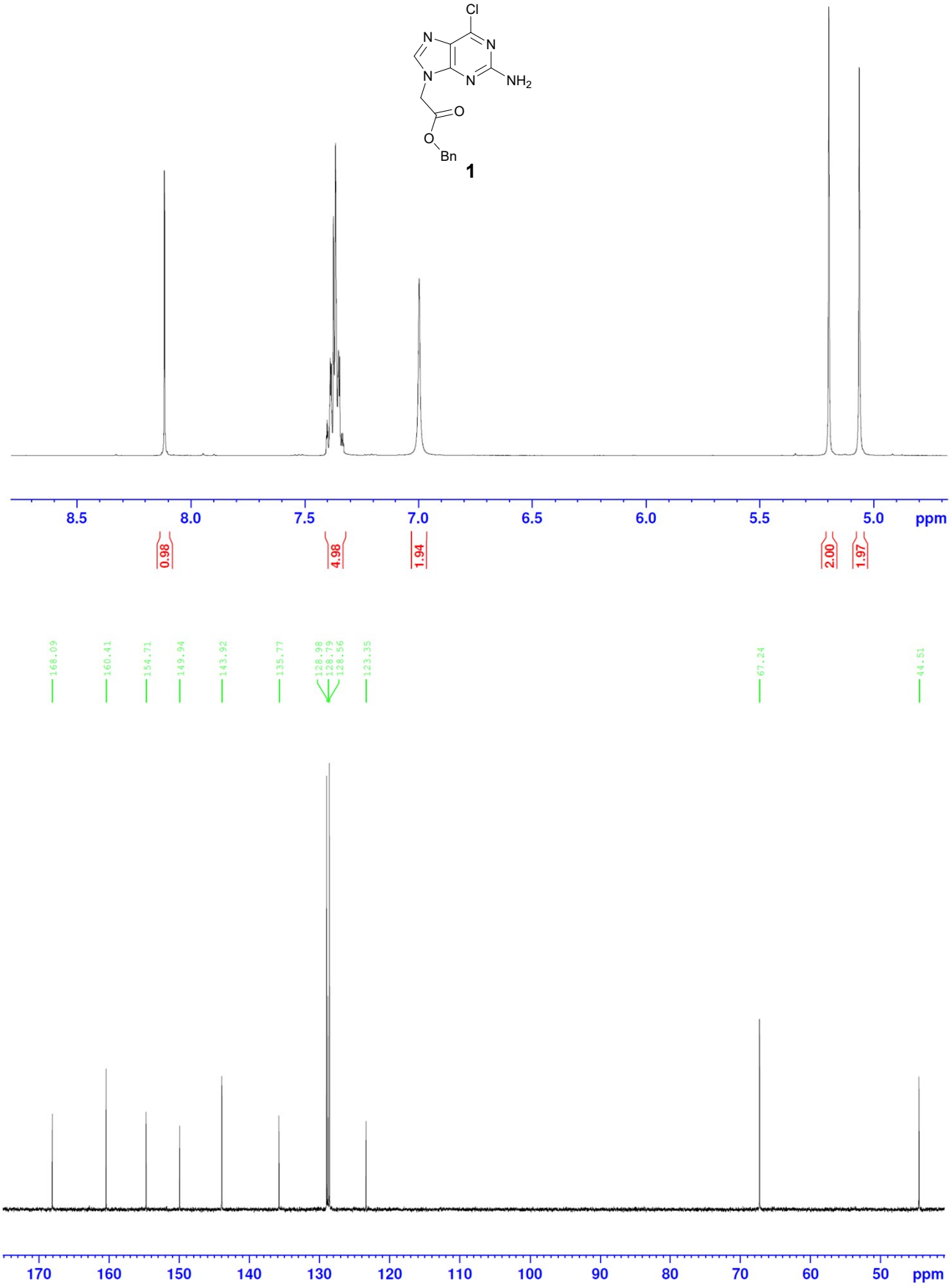
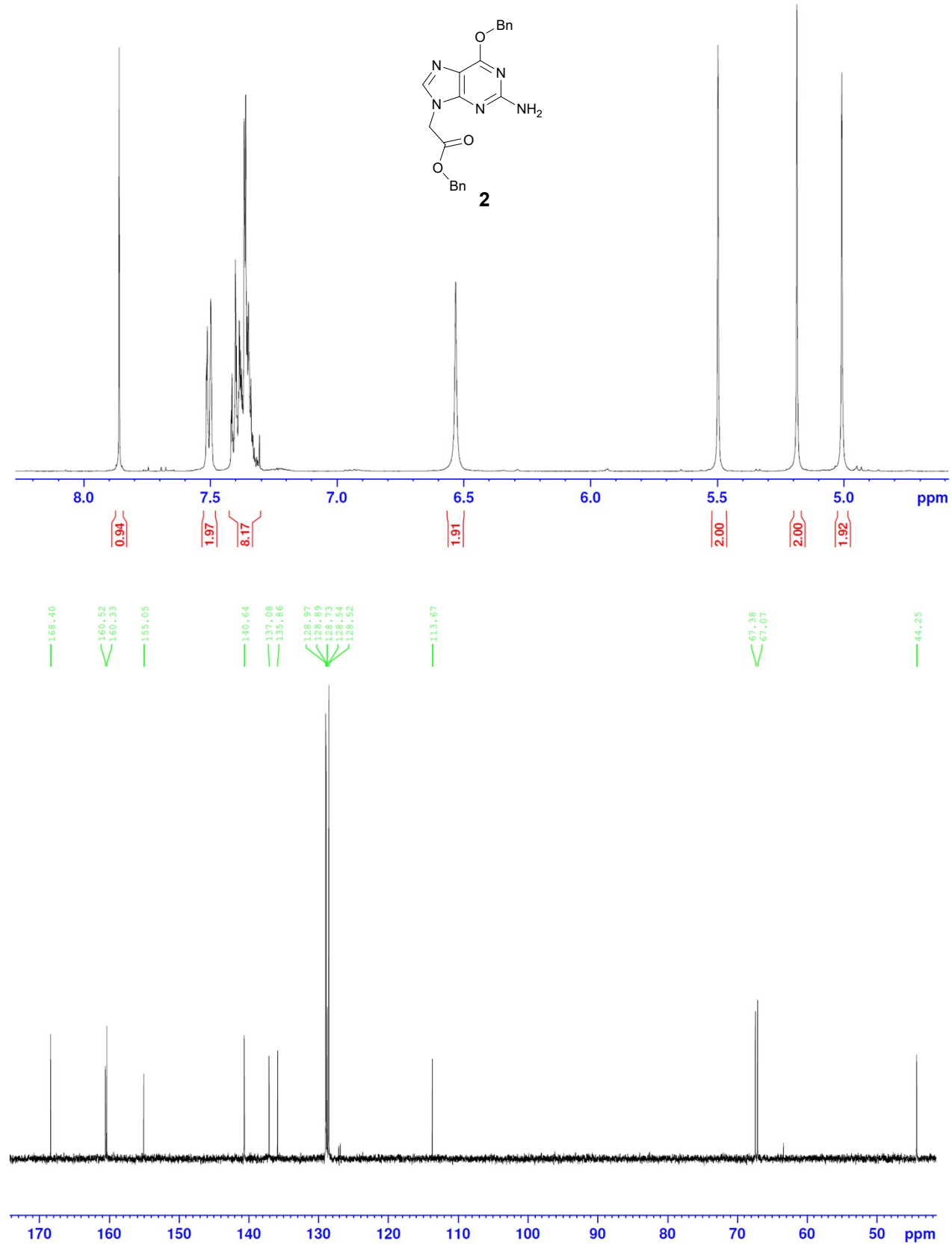



**Fig. S13** The absorption spectra of single-stranded **SNA-1$^{PV}$A** ((*S*)-GCT$^{PV}$AATGC -(*R*)) before and after irradiation with (A) 447 nm light and (B) 350 nm light. (C) The ratio of monomeric $^{PV}$A in SNA-1$^{PV}$A single strands after irradiation at 447 nm (red circles) and 350 nm (blue squares). (D) Melting profile of the duplex of pre-crosslinked SNA-1$^{PV}$A and RNA-2U (purple line) and after irradiation with 350 nm light (blue line). Conditions: 100 mM NaCl, 10 mM phosphate buffer (pH 7.0). The concentration of oligonucleotides was 5.0 μM.

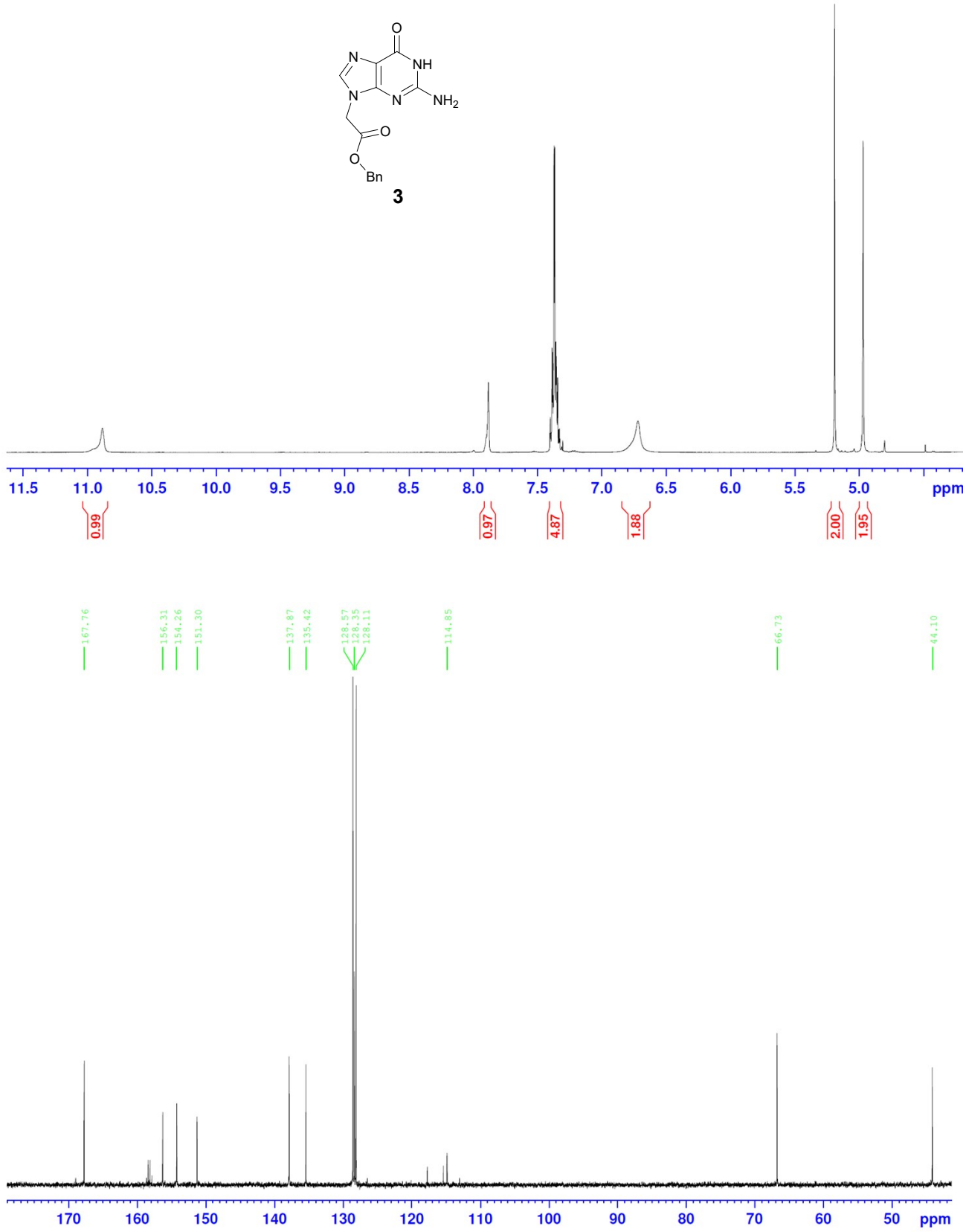
$^{1}$H-NMR and $^{13}$C-NMR spectrum of **1**



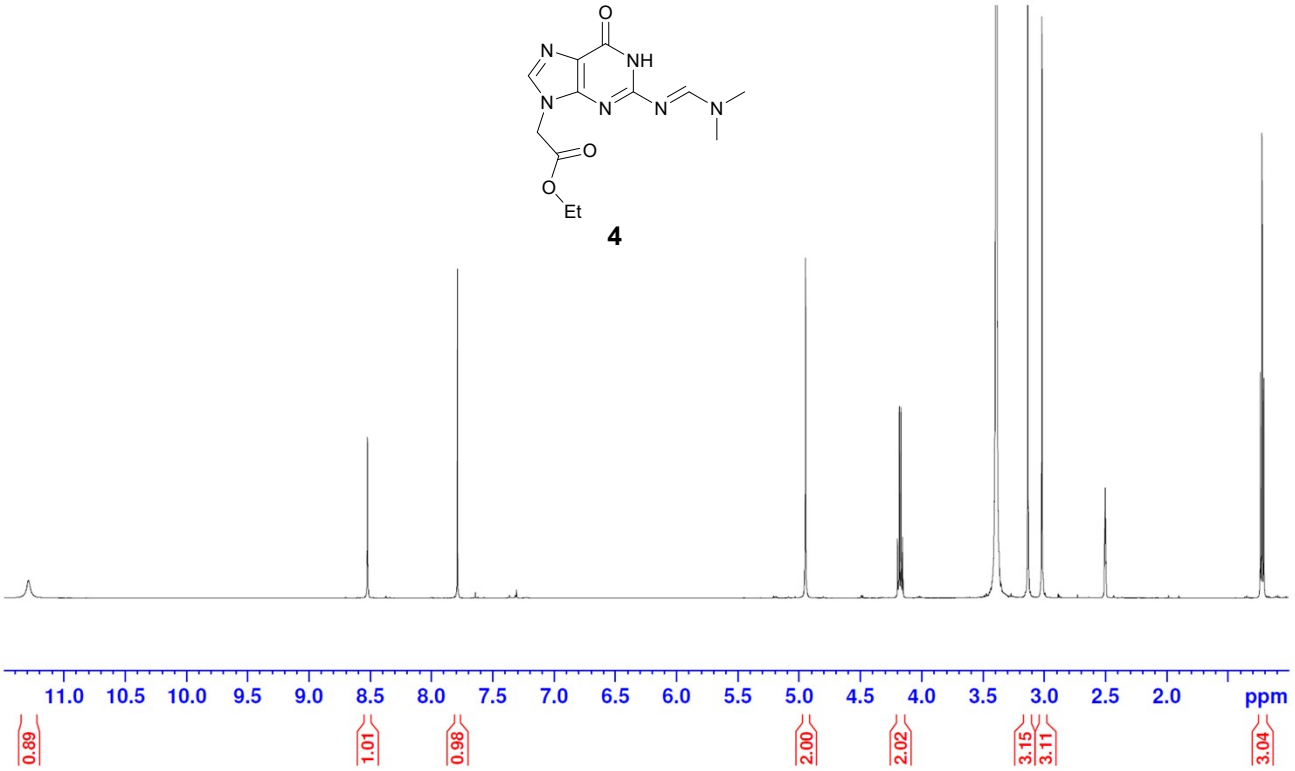
$^{1}$H-NMR and $^{13}$C-NMR spectrum of **2**



$^{1}$H-NMR and $^{13}$C-NMR spectrum of **3**

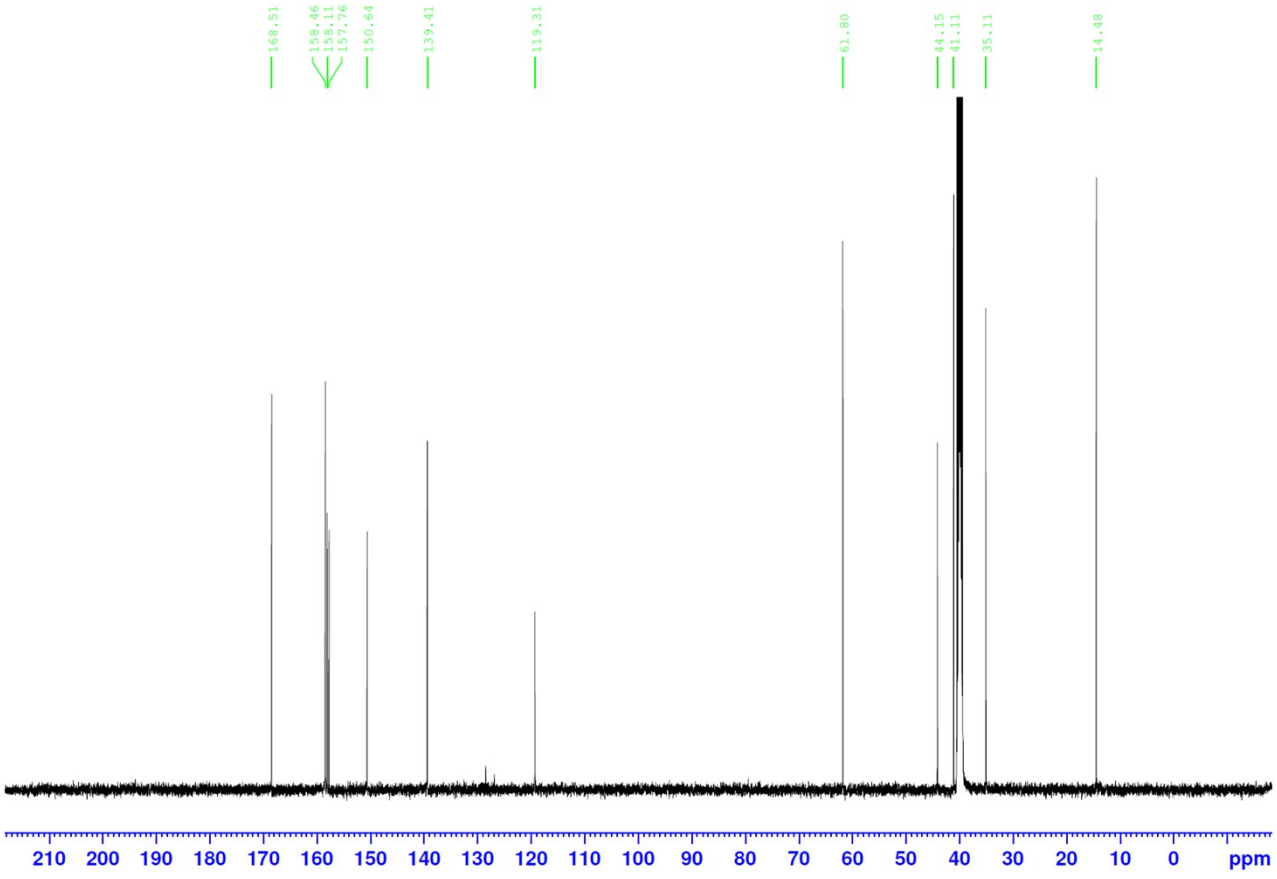


$^{1}$H-NMR and $^{13}$C-NMR spectrum of **4**

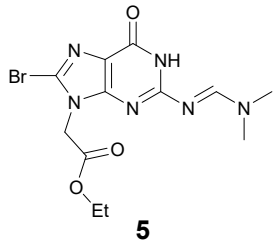



**4**



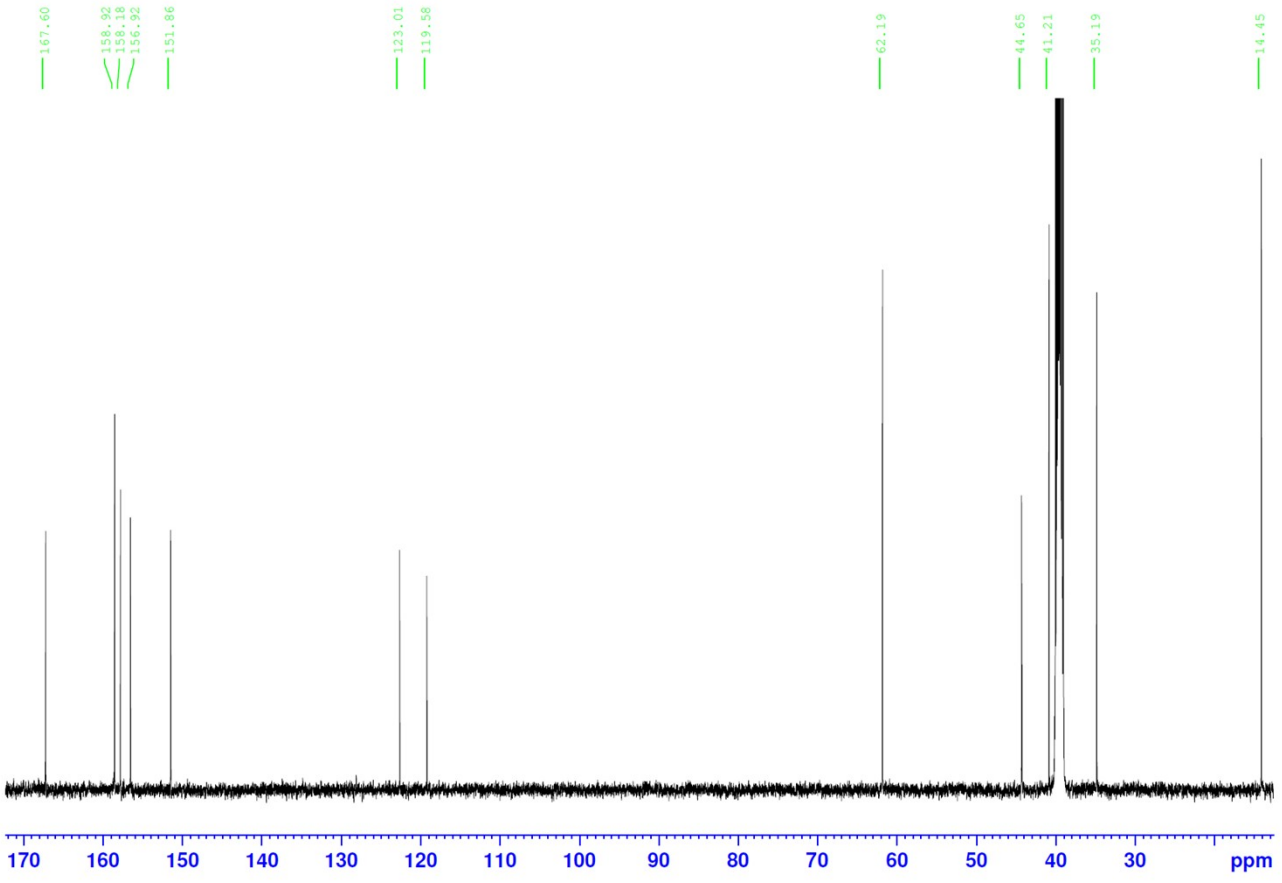



$^{1}$H-NMR and $^{13}$C-NMR spectrum of **5**

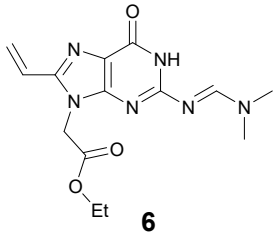



**5**



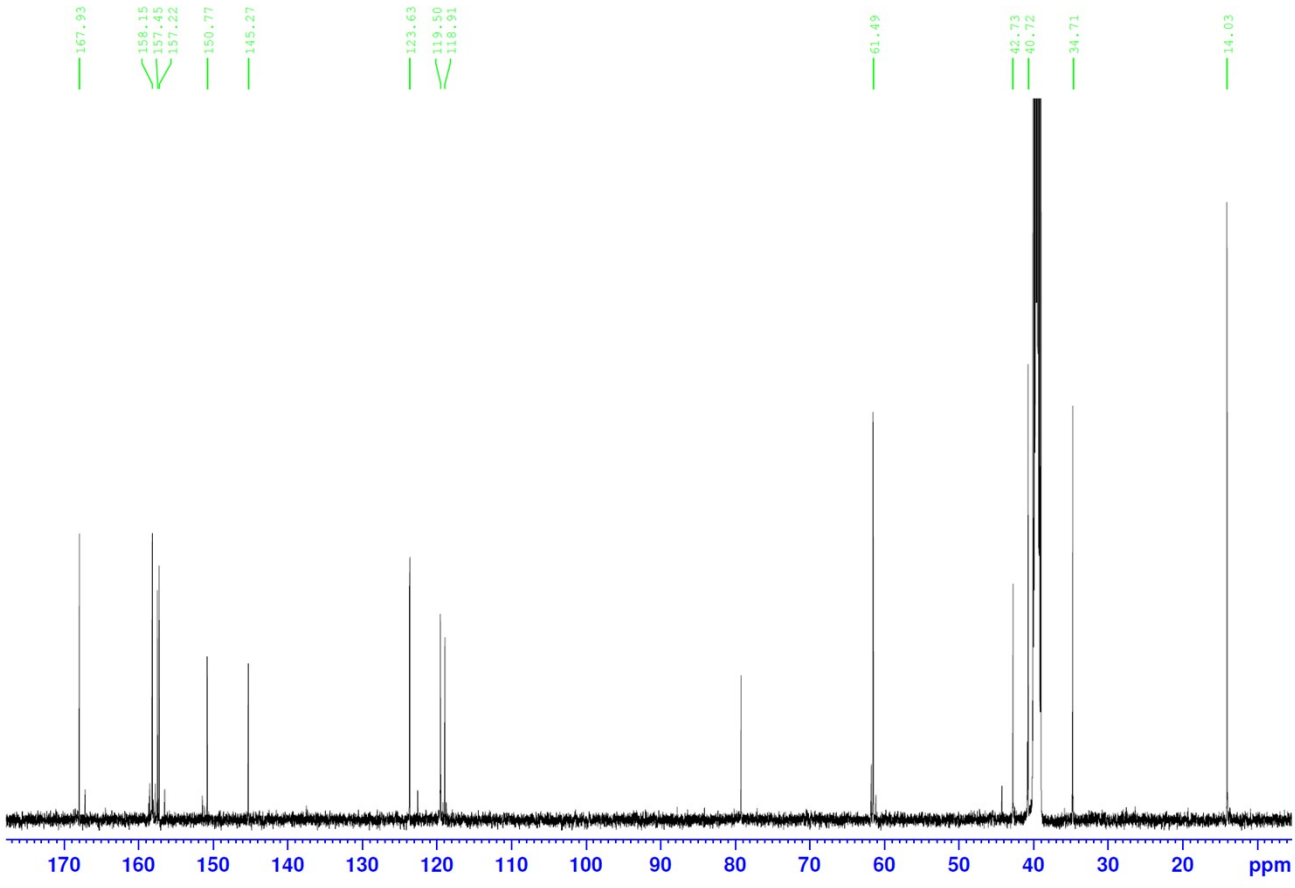



$^{1}$H-NMR and $^{13}$C-NMR spectrum of **6**

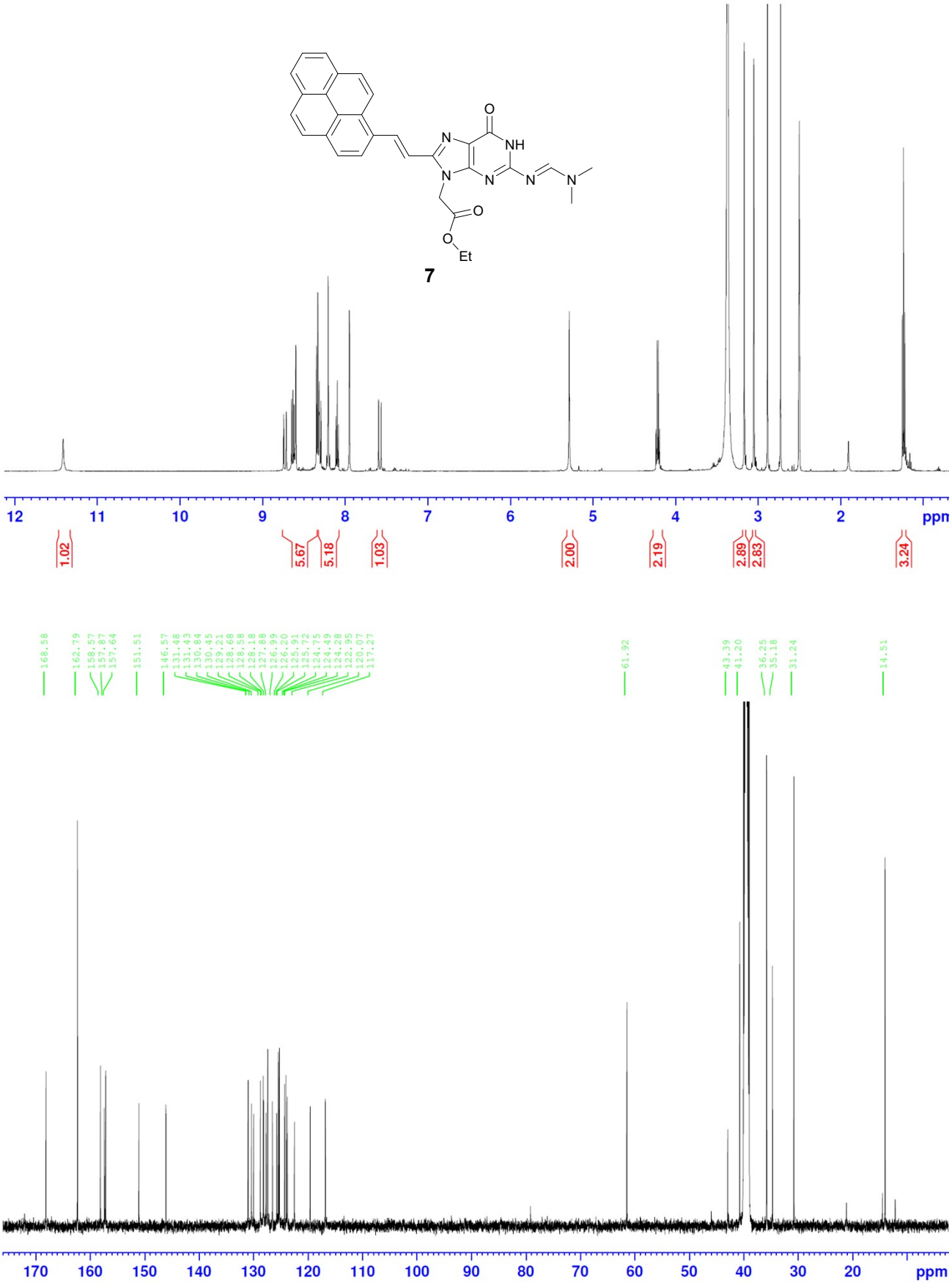



**6**

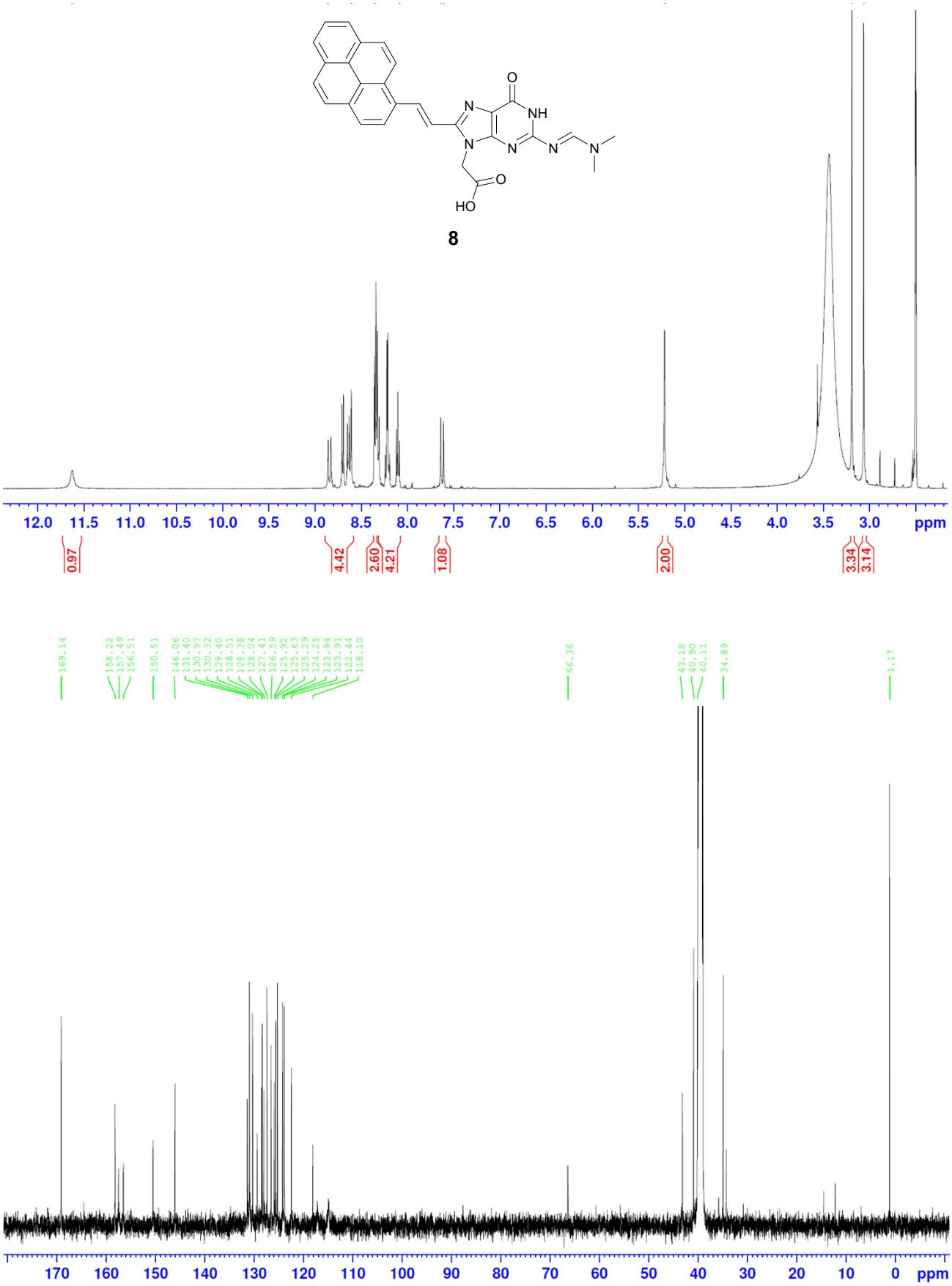


$^{1}$H-NMR and $^{13}$C-NMR spectrum of **7**

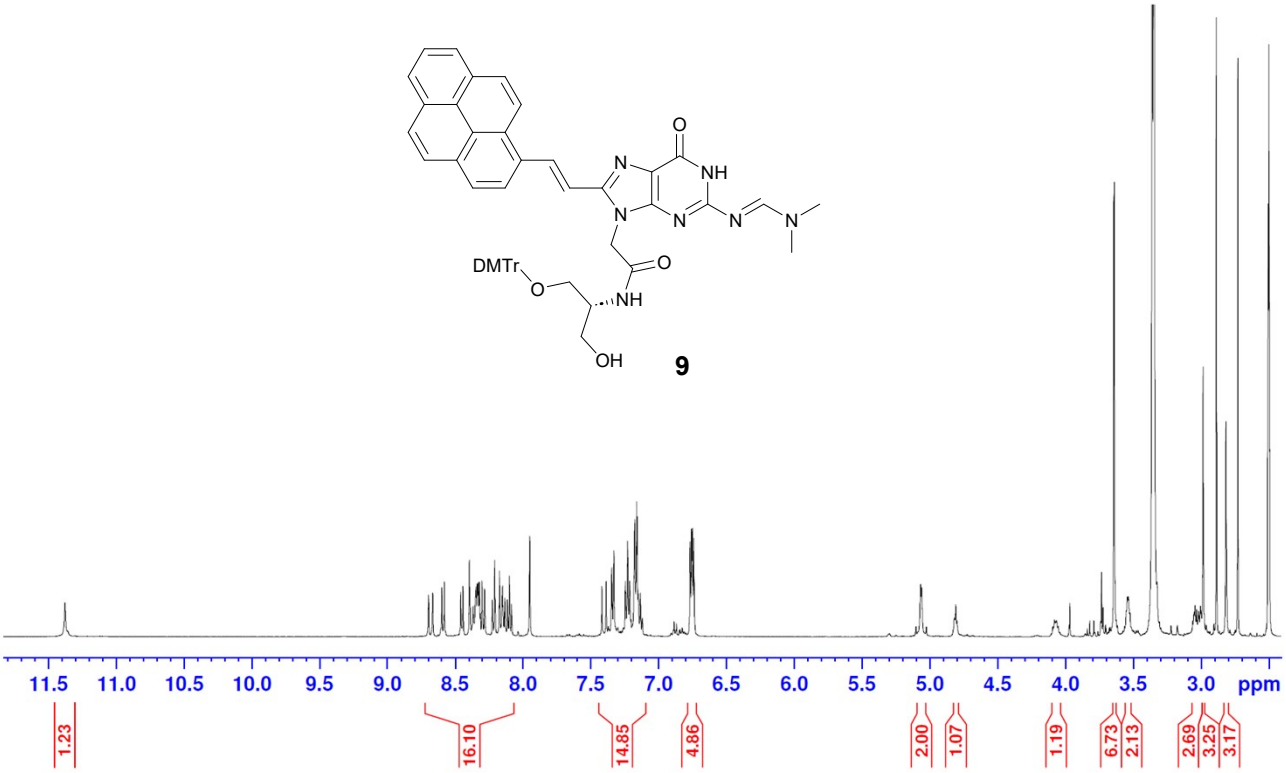


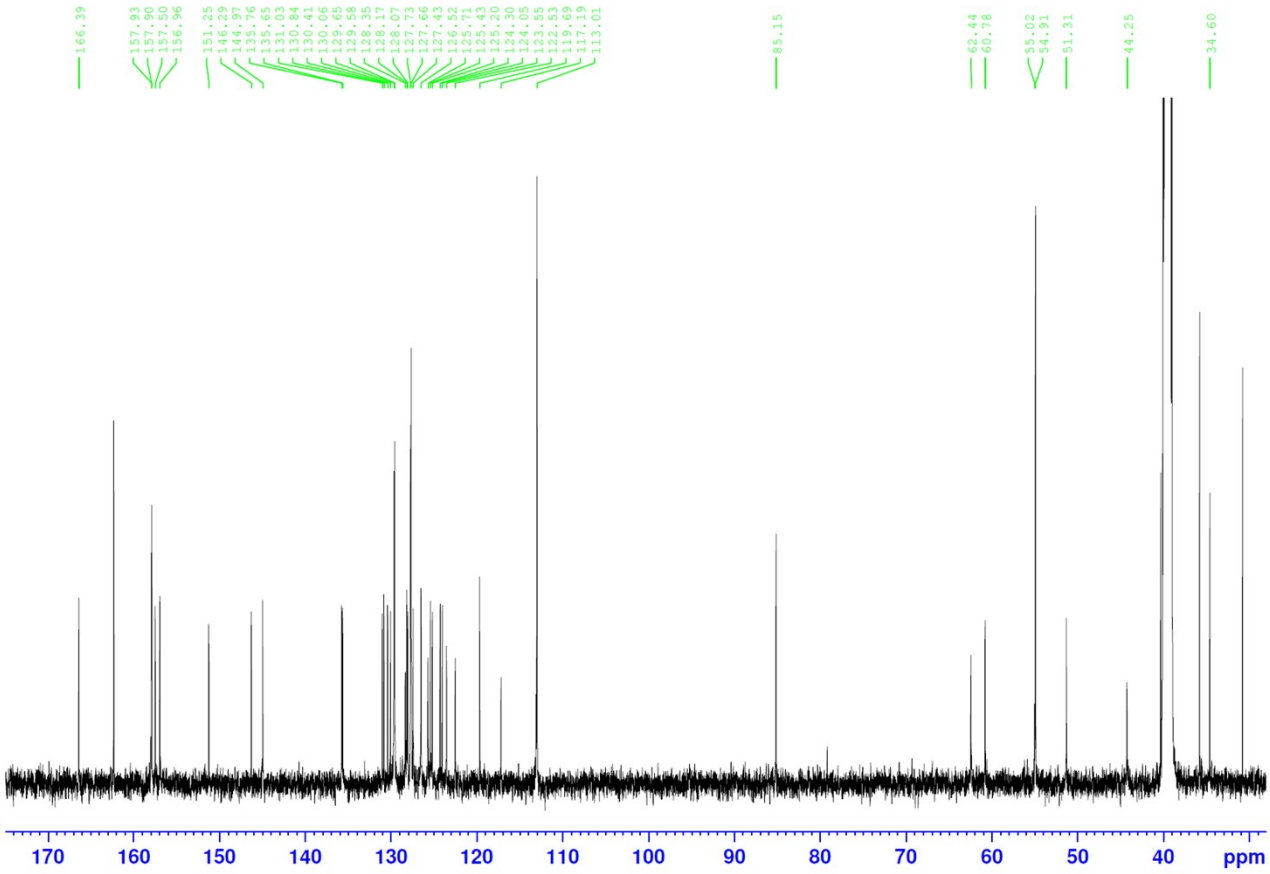
$^{1}$H-NMR and $^{13}$C-NMR spectrum of **8**



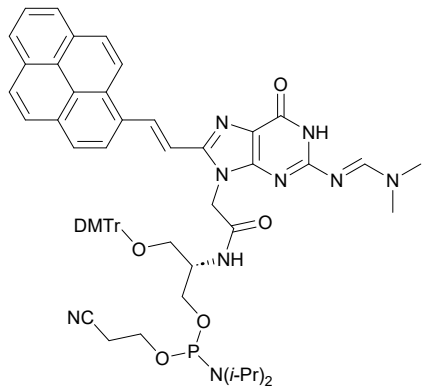

**8**

$^{1}$H-NMR and $^{13}$C-NMR spectrum of **9**



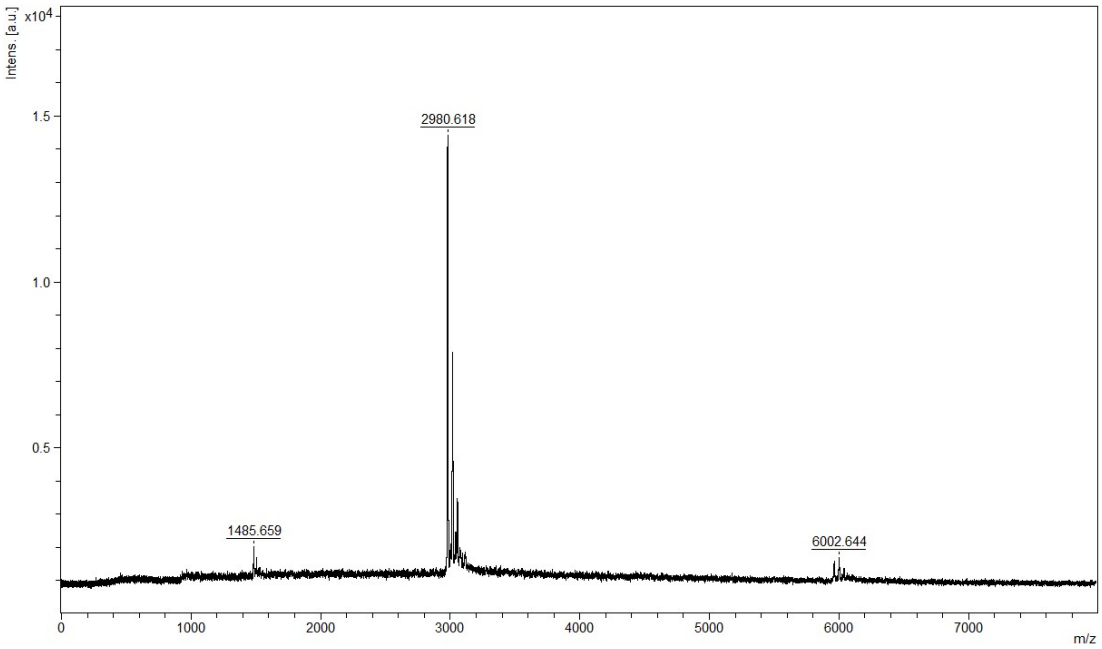
$^{1}$H-NMR, $^{13}$C-NMR, and $^{31}$P-NMR spectrum of **10**



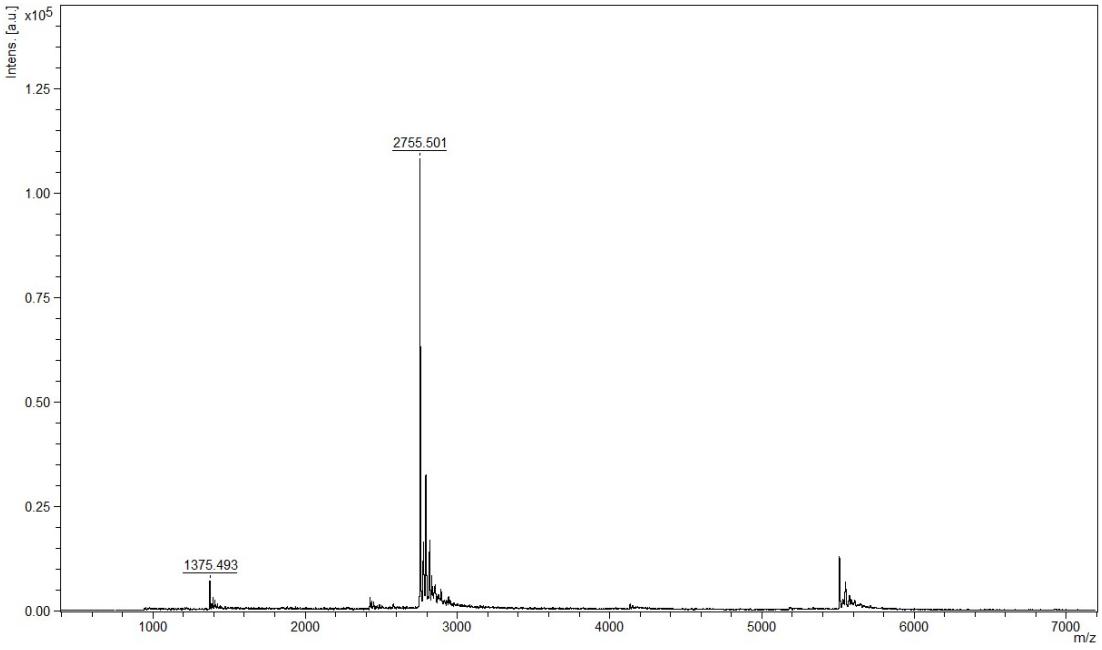

**10**

**Results of MALDI-TOF MS:**

**SNA-2$^{PV}$G**: Obsd. m/z 2980.6 (Calcd. for [M + H$^+$]: m/z 2981.7)




**SNA-1$^{PV}$G**: Obsd. m/z 2755.5 (Calcd. for [M + H$^+$]: m/z 2755.6)



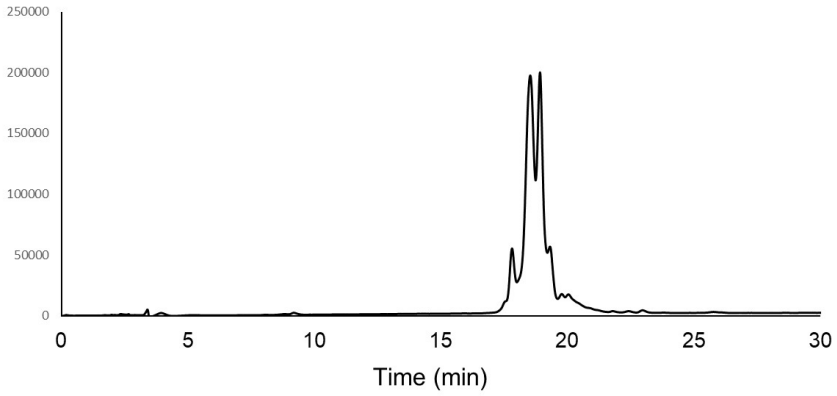
**Results of HPLC:**

Buffer A: 50 mM ammonium formate

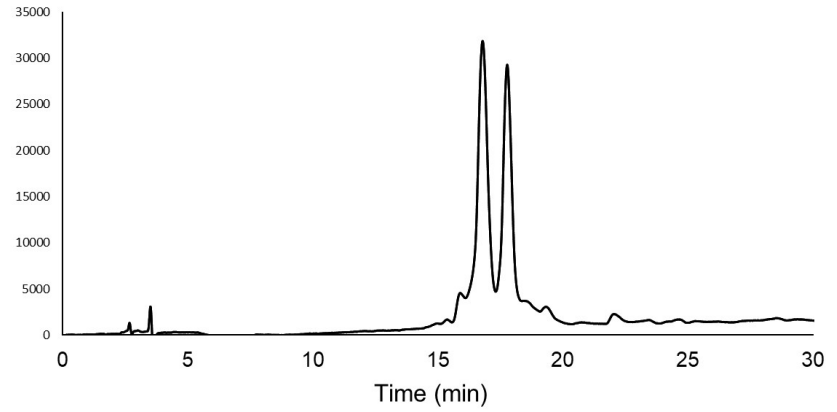
Buffer B: mixture of 50 mm ammonium formate and acetonitrile (50:50, v/v)

A Mightysil RP-18GP II column heated to 65 $^{o}$C was used for HPLC analyses. The flow rate was 0.5 mL $min^{-1}$. A buffer A and buffer B were used as mobile phases. HPLC chromatograms were monitored at 260 nm. Spectra of peaks at each retention time were recorded on JASCO EXTREMA HPLC system.

**SNA-2$^{PV}$G:** From 25% buffer B to 60% buffer B.




**SNA-1$^{PV}$G**: From 15% buffer B to 50% buffer B.




Even after purification, two SNA sequences showed multiple peaks in the HPLC chart. We checked MS of all fractions of these peaks by MALDI-TOF and found only the desired MS peak. Additionally, re-subjection of the collected samples at different retention times produced the same HPLC chart with multiple peaks. Furthermore, denaturing PAGE of the purified sample indicated a single pure band (Fig. 4B and 5E). Therefore, we concluded that the multiple HPLC peaks were derived from the higher-order structures of the SNA strand, despite sufficient purity.