Supplementary Data

Pyrrolocytosine-pyrene conjugates as fluorescent and CD probes for a fine sensing of ds-DNA/RNA secondary structure and specific recognition of poly G

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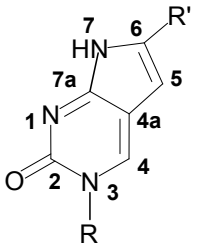


Figure S1. The compound 3*H*-pyrrolo[2,3-*d*] pyrimidin-2(7*H*)-one (pyrrolocytosine) is abbreviated as Pyrr-C.

Physico-chemical properties of 2-4 aqueous solutions:

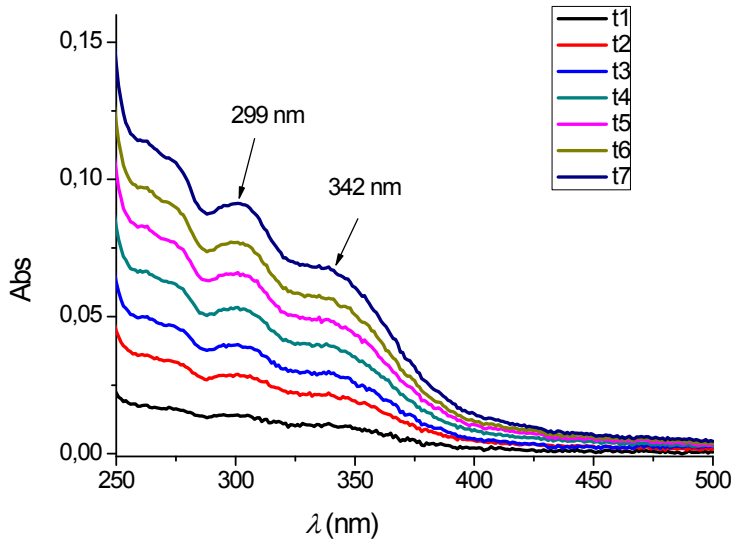
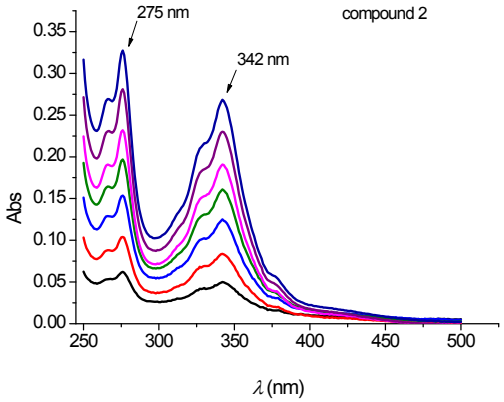
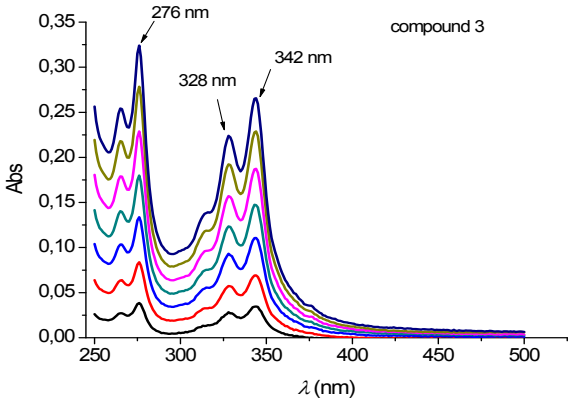
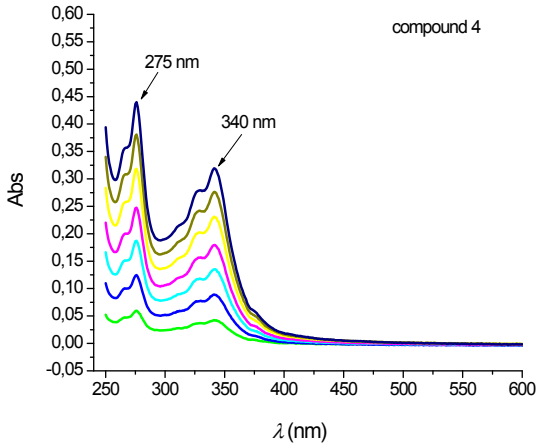


Figure S2. Concentration dependence of the UV/Vis spectrum of **1** (concentration range from 3×10^{-6}– 2×10^{-5} mol/L) in buffered solution pH 7, $I = 0.05$ mol/L.

a)


compound 2
275 nm
342 nm
Abs
λ (nm)


compound 3
276 nm
328 nm
342 nm
Abs
λ (nm)


compound 4
275 nm
340 nm
Abs
λ (nm)

b)

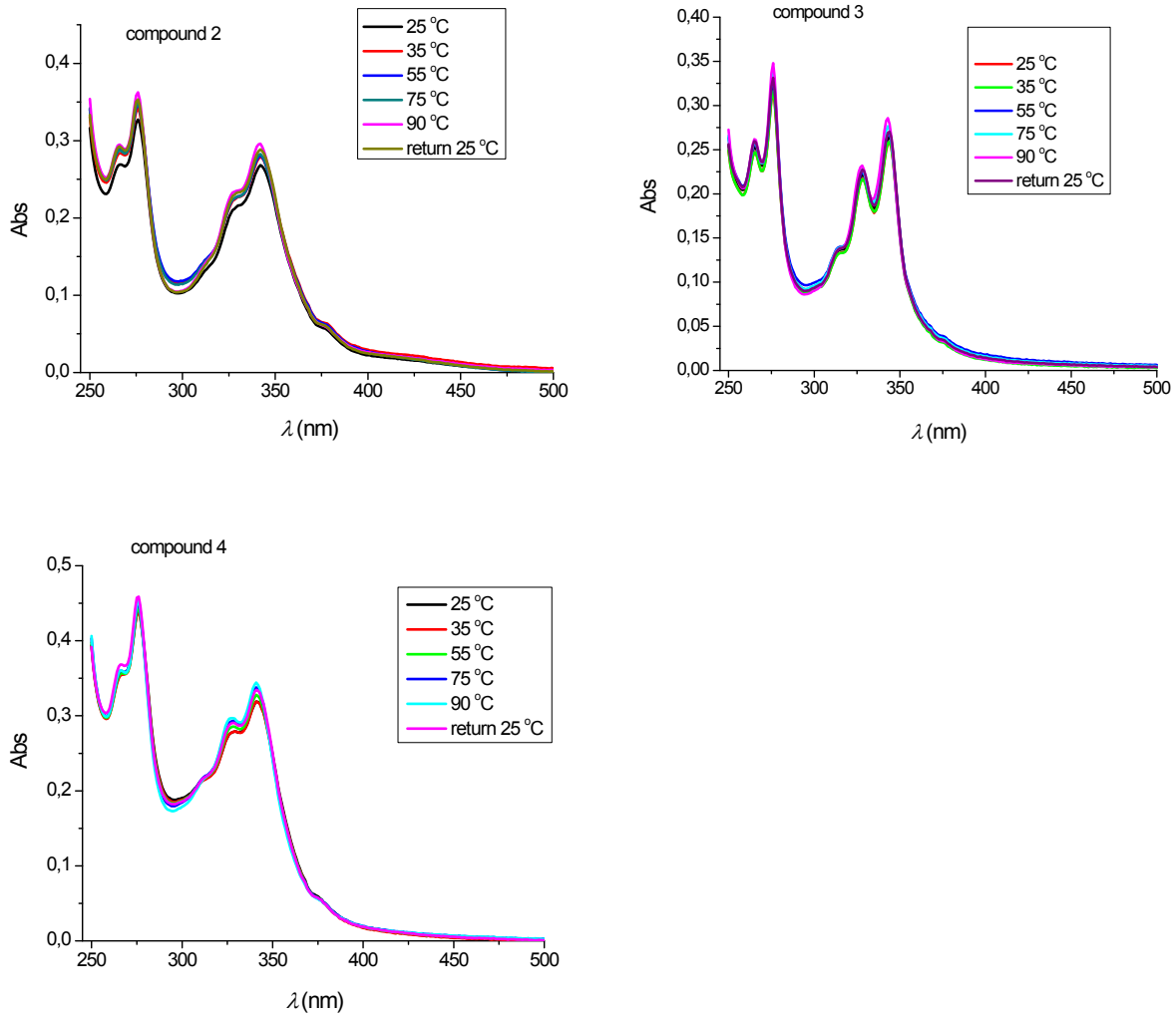


Figure S3. a) Concentration dependence of the UV/Vis spectrum of **2 - 4** (concentration range from 3×10^{-6} – 2×10^{-5} mol/L) in buffered solution (pH 7.0, I = 0.05 mol/L); b) temperature dependence of the UV/Vis spectrum of **2 - 4** (c= 2×10^{-5} mol/L) in buffered solution (pH 7.0, I = 0.05 mol/L).

Table S1. Electronic absorbance data of **1 - 4**

Compound	λ_{max}/nm	$\varepsilon\times10^3$/Lmol^{-1}cm^{-1}
1	299 342	4.3±0.1 3.1±0.1
2	275 342	12.2±0.2 14.5±0.2
3	276 328 342	16.1±0.9 11±0.1 13.1±0.1
4	275 340	21.3 ±0.2 15.4 ±0.1

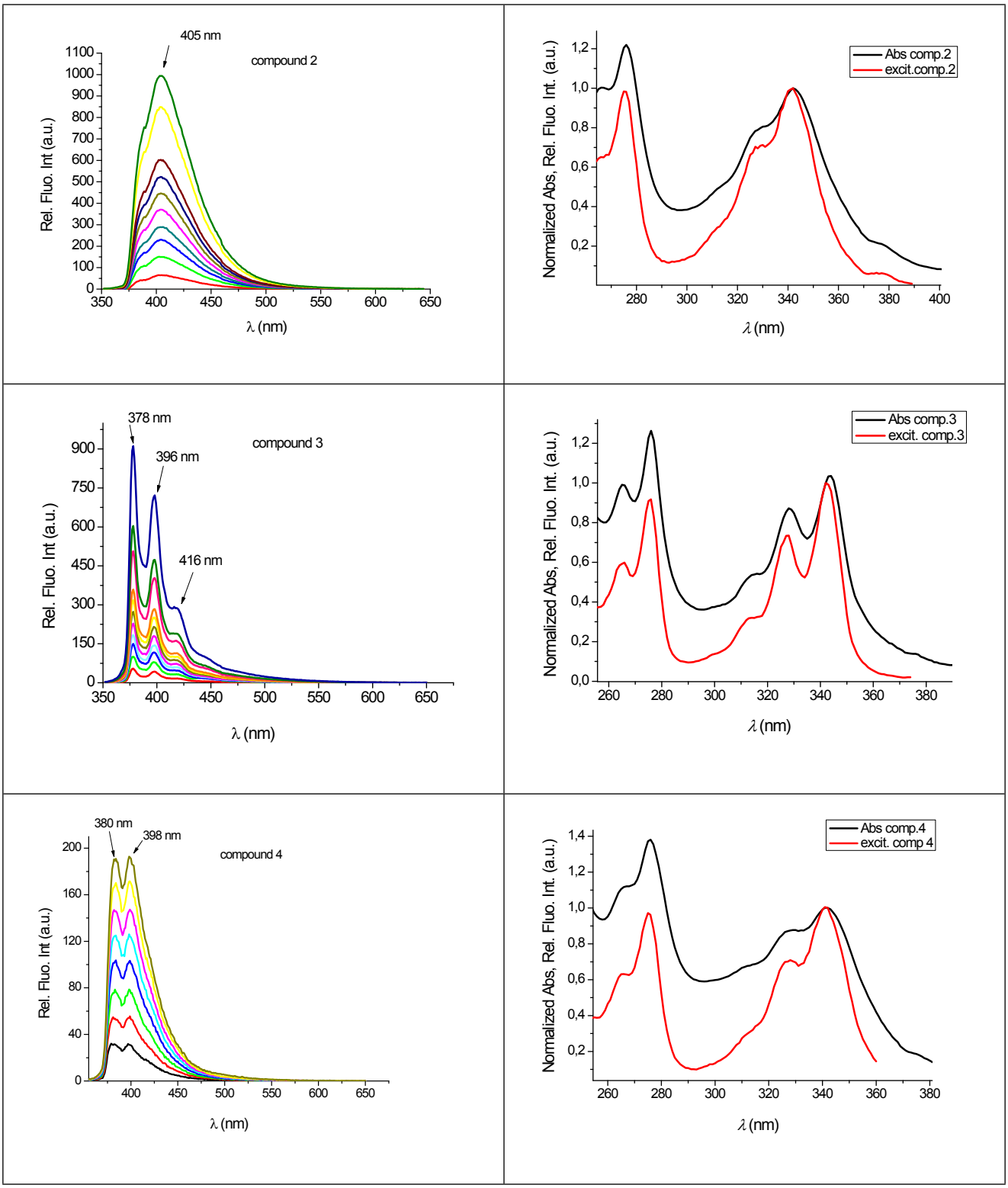


Figure S4. **LEFT**: Fluorescence spectra changes of **2** - **4** at different concentrations (1×10^{-7}–1×10^{-6} mol/L); **RIGHT**: Comparison of normalized absorption (c = 2×10^{-5} mol/L) and excitation spectra (c= 1×10^{-6} mol/L; λ_{em} = 400 nm) of **2** - **4**. Done at pH = 7.0, sodium cacodylate buffer, I = 0.05 mol/L.

DNA, RNA binding studies:

Table S2. Structural properties of studied DNA and RNA.[1]

Structure type	Groove width [Å] major	minor	Groove depth [Å] major	minor
A_nU_n	3.8	10.9	-	-
[a]B-DNA	11.7	5.7	8.5	7.5
$(dGdC)_n$	13.5	9.5	10.0	7.2
$(dAdT)_n$	11.2	6.3	-	-

[a] Calf Thymus (ct)-DNA

Thermal denaturation data:

Here shown are normalized thermal denaturation data, focused on inflection point. All experiments are done at least twice, and for more accurate reading of thermal denaturation points 1st derivation of melting curves was used (maximum at inflection point of the curve).

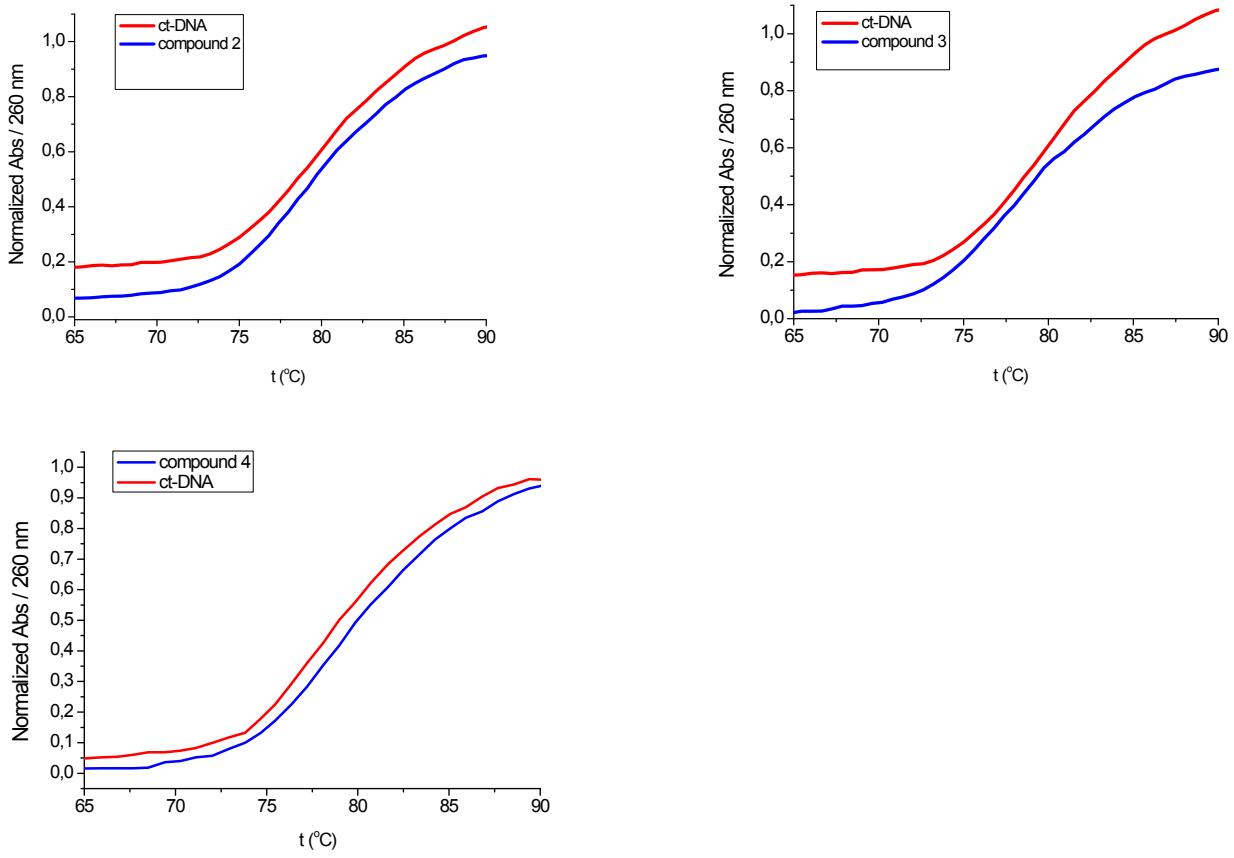


Figure S5. Normalized thermal denaturation spectra of **ctDNA** upon addition of **2 - 4** (r = 0.3[compound]/ [polynucleotide]) at pH 7.0 (buffer sodium cacodylate, I = 0.05 mol/L).

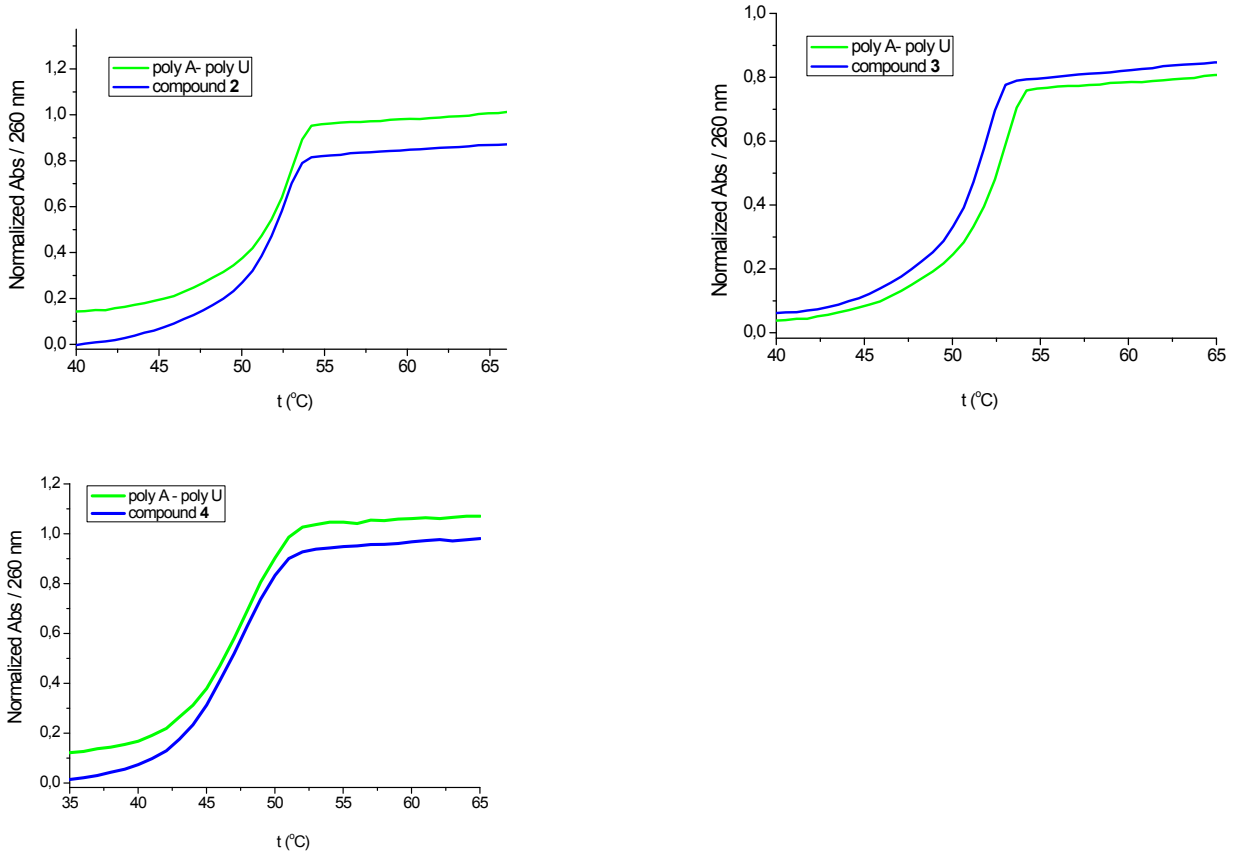


Figure S6. Normalized thermal denaturation spectra of **poly A – poly U,** upon addition of **2** - **4** (*r* = 0.3[compound]/ [polynucleotide]) at pH 7.0 (buffer sodium cacodylate, *I* = 0.05 mol/L).

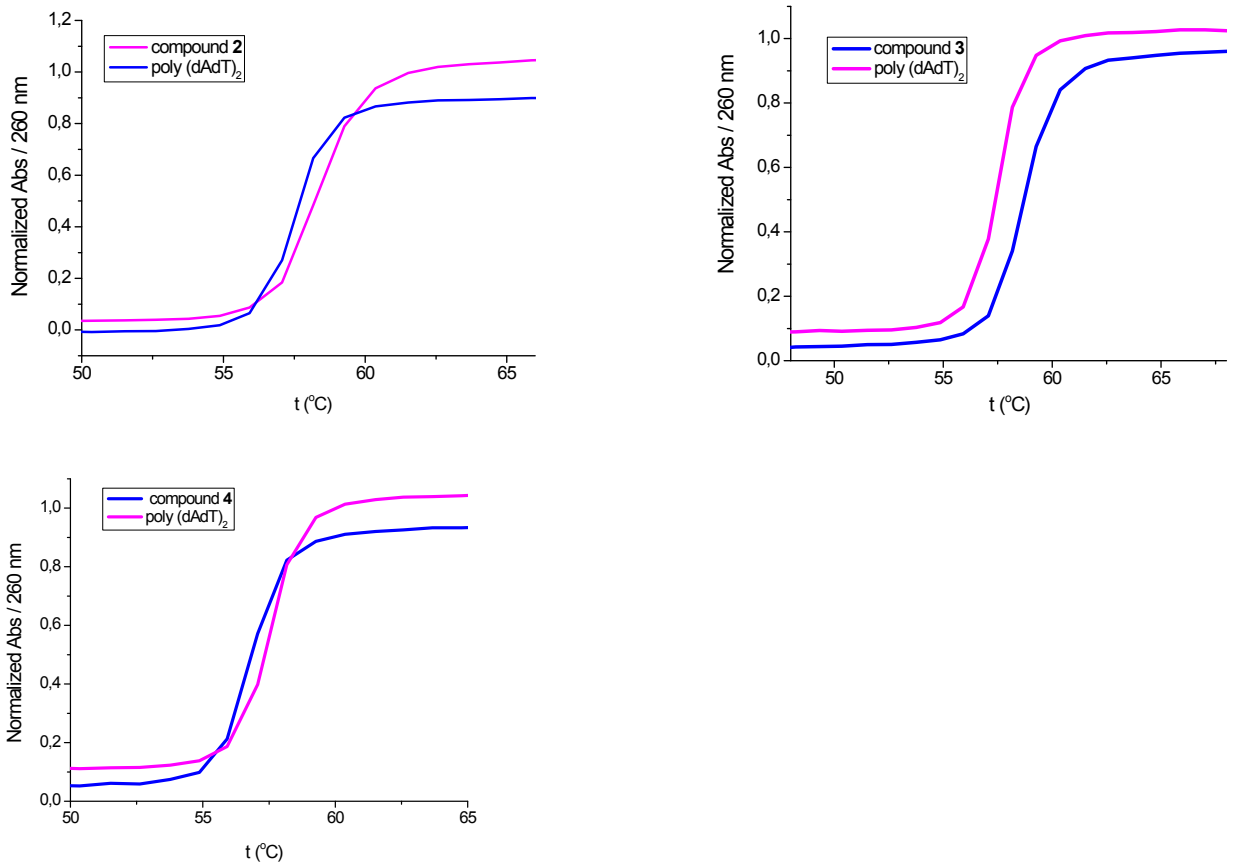


Figure S7. Normalized thermal denaturation spectra of **poly (dAdT)$_2$,** upon addition of **2 - 4** (r = 0.3[compound]/ [polynucleotide]) at pH 7.0 (buffer sodium cacodylate, I = 0.05 mol/L).

Table S3. The [a]ΔT_m values (°C) of studied ds- polynucleotides upon addition of ratio [b]***r*** = 0.3 of **2-4** at pH 7.0 (sodium cacodylate buffer, I = 0.05 mol/L).

	ctDNA	poly A – poly U	poly (dAdT)$_2$
2	0.8	-0.4	1.0
3	-0.1	-0.7	1.1
4	0.9	0.5	1.0

[a] Error in ΔT_m : ± 0.5°C;
[b] ***r*** = [compound] / [polynucleotide];

Fluorimetric titrations of compounds 1 -4 with ds-DNA/RNA:

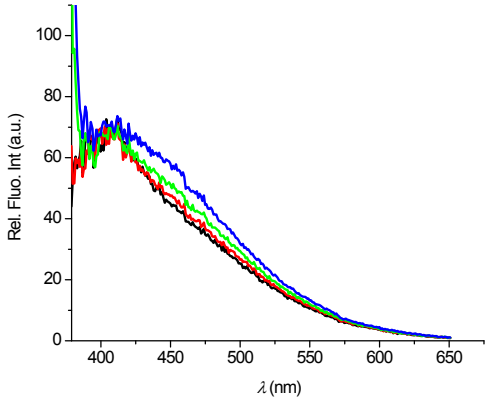


Figure S8. Fluorescence spectra changes of **1** (c = 2×10^{-6} mol/L) upon addition of **ct-DNA** (concentration range from 2×10^{-5}–2×10^{-4} mol/L; λ_{exc} = 355 nm) at pH 7.0, sodium cacodylate buffer, I = 0.05 mol/L.

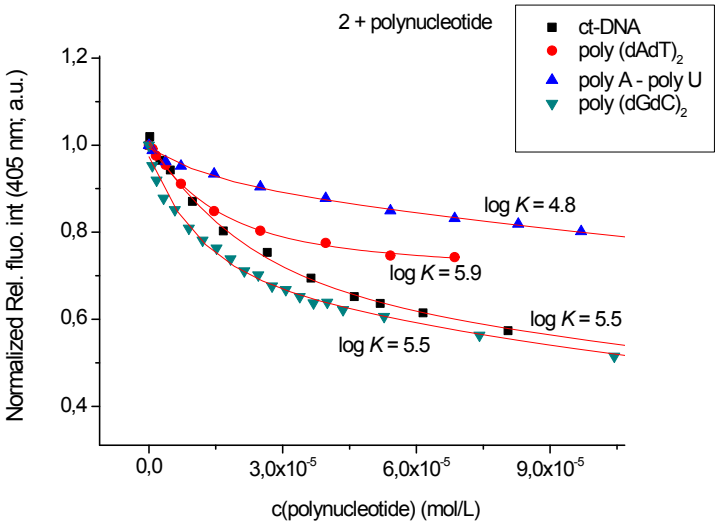


Figure S9. Fluorimetric titrations normalized to starting fluorescence intensity of **2** (c = 4×10^{-7} mol/L; λ_{exc} = 342 nm, λ_{em} = 405 nm) with all ds-polynucleotides. Done at pH = 7.0, sodium cacodylate buffer, I = 0.05 mol/L.

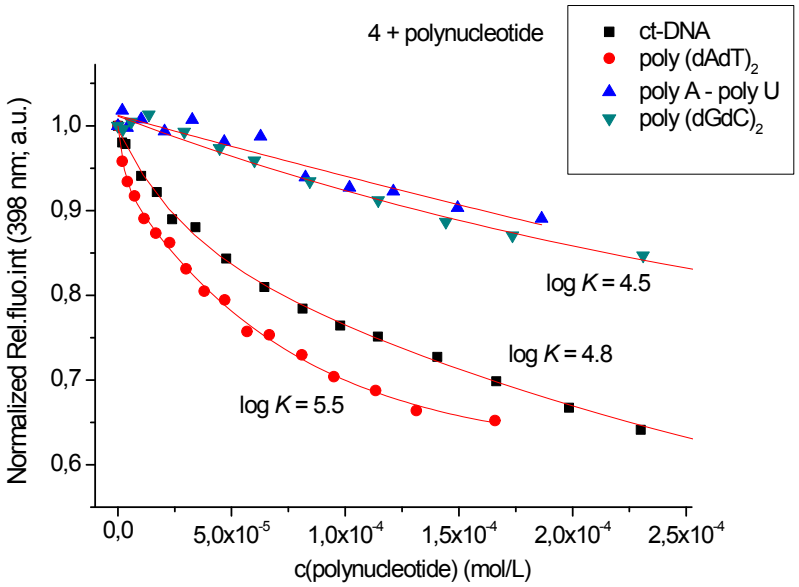


Figure S10. Fluorimetric titrations normalized to starting fluorescence intensity of **4** ($c = 1\times10^{-6}$ mol/L; $\lambda_{exc} = 340$ nm) with all ds-polynucleotides. Done at pH = 7.0, sodium cacodylate buffer, I = 0.05 mol/L.

Fluorimetric titrations of compounds 1 -4 with ss-RNA:

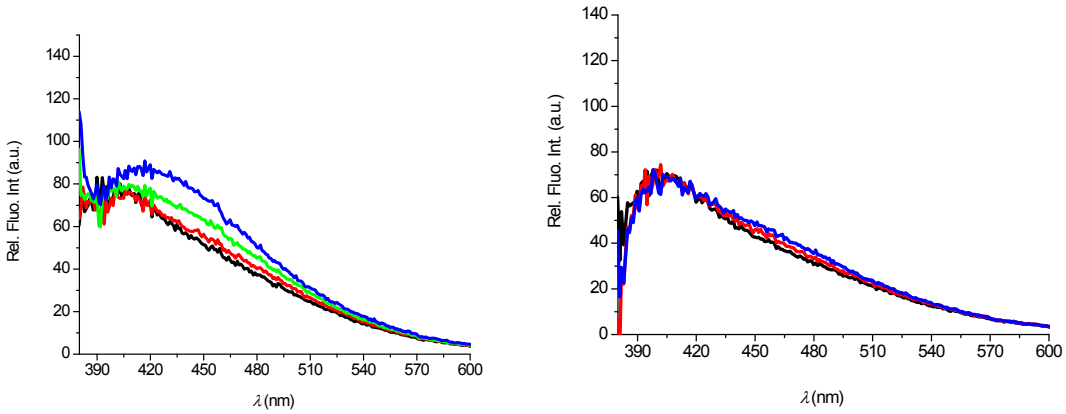


Figure S11. Fluorescence spectra changes of **1** (c=2×10^{-6} mol/L; λ_{exc}= 355 nm) upon addition of **poly A** (LEFT) and **poly C** (RIGHT) at pH 7.0, sodium cacodylate buffer, I=0.05 mol/L.

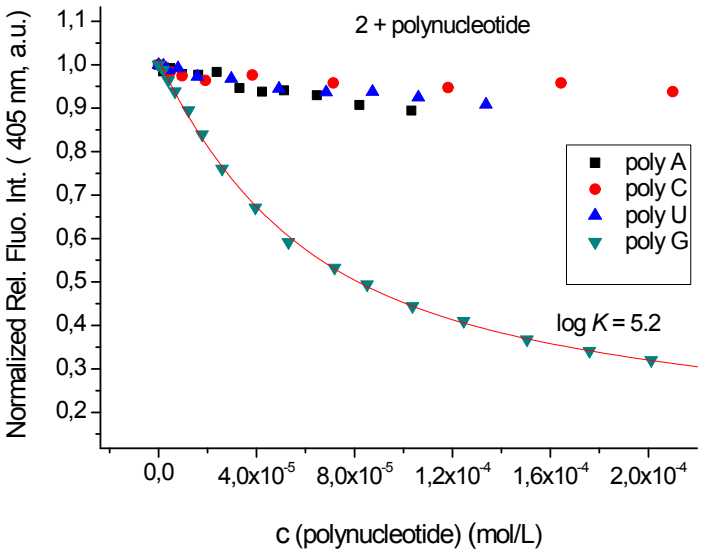


Figure S12. Fluorimetric titrations normalized to starting fluorescence intensity of **2** (c = 1×10^{-6} mol/L, λ_{exc} = 342 nm, λ_{em} = 405 nm) upon titration with all ss-polynucleotides (sodium cacodylate buffer, pH = 7.0, I = 0.05 mol/L).

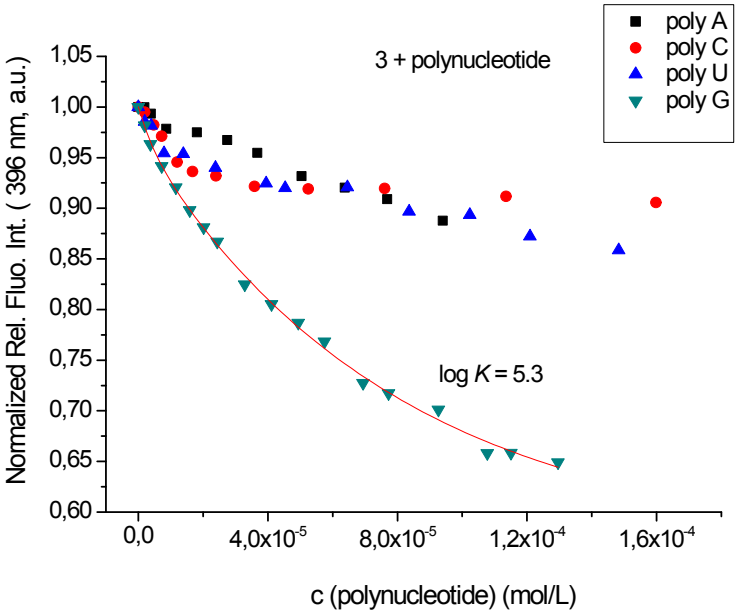


Figure S13. Fluorimetric titrations normalized to starting fluorescence intensity of **3** ($c = 1\times10^{-6}$ mol/L; $\lambda_{exc} = 342$ nm, $\lambda_{em} = 396$ nm) upon titration with all ss-polynucleotides. Done at pH = 7.0, sodium cacodylate buffer, $I = 0.05$ mol/L.

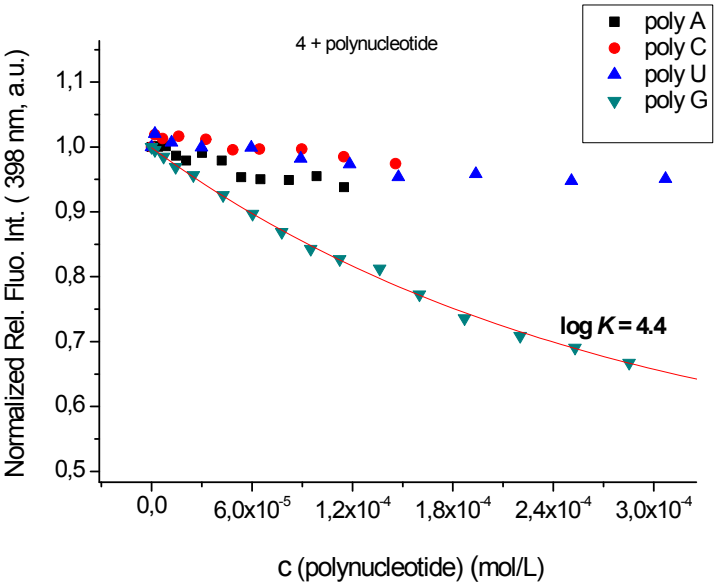
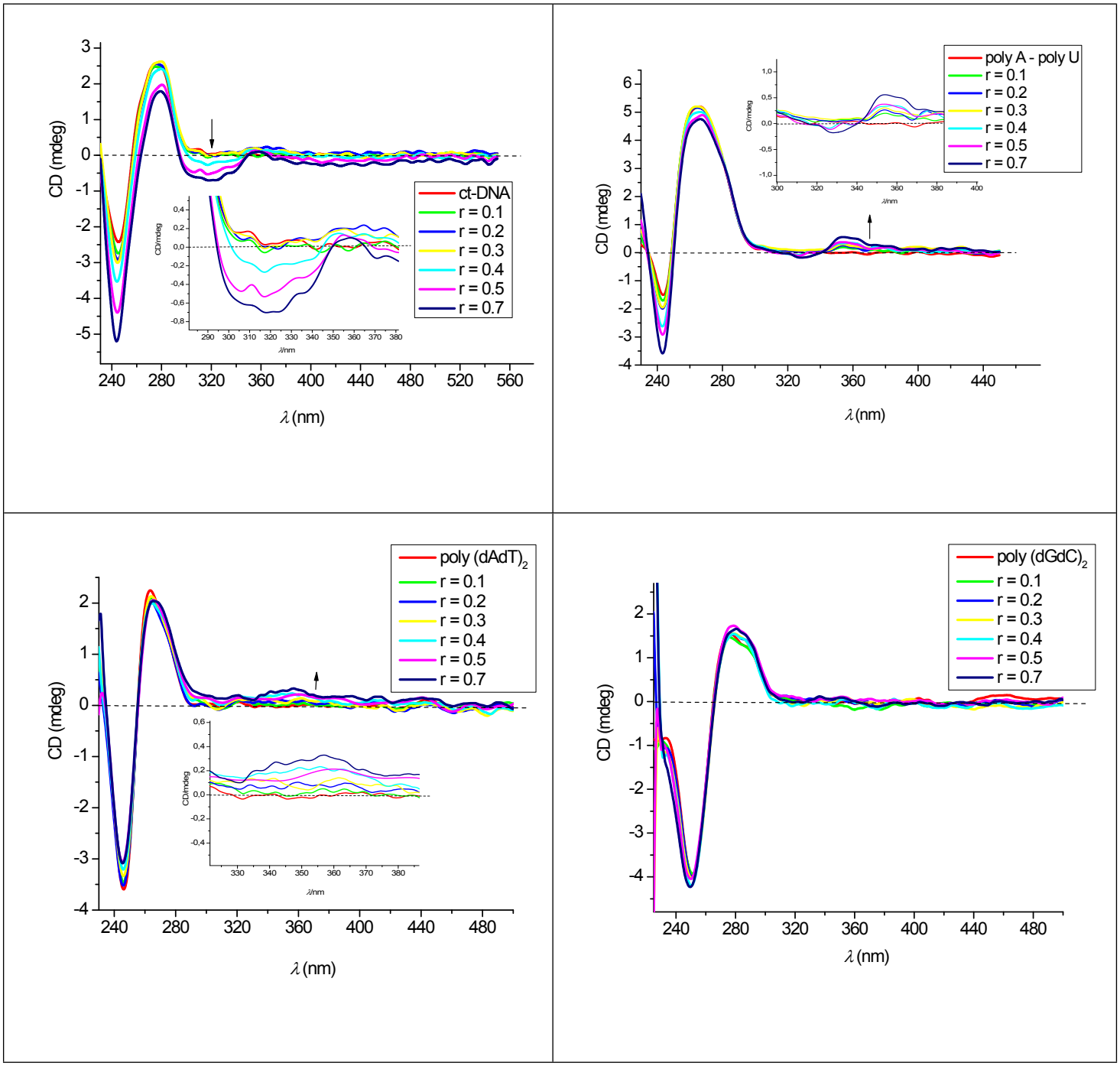


Figure S14. Fluorimetric titrations normalized to starting fluorescence intensity of **4** ($c = 1\times10^{-6}$ mol/L; $\lambda_{exc} = 340$ nm, $\lambda_{em} = 398$ nm) upon titration with all ss-polynucleotides. Done at pH = 7.0, sodium cacodylate buffer, $I = 0.05$ mol/L.

Circular dichroism (CD) experiments

So far, non-covalent interactions at 25 °C were studied by monitoring the spectroscopic properties of studied compound upon addition of the polynucleotides. In order to get insight into the changes of polynucleotide properties induced by small molecule binding, we have chosen CD spectroscopy as a highly sensitive method toward conformational changes in the secondary structure of polynucleotides.[2] In addition, achiral small molecules can eventually acquire induced CD spectrum (ICD) upon binding to polynucleotides, which could give useful information about modes of interaction.[2]



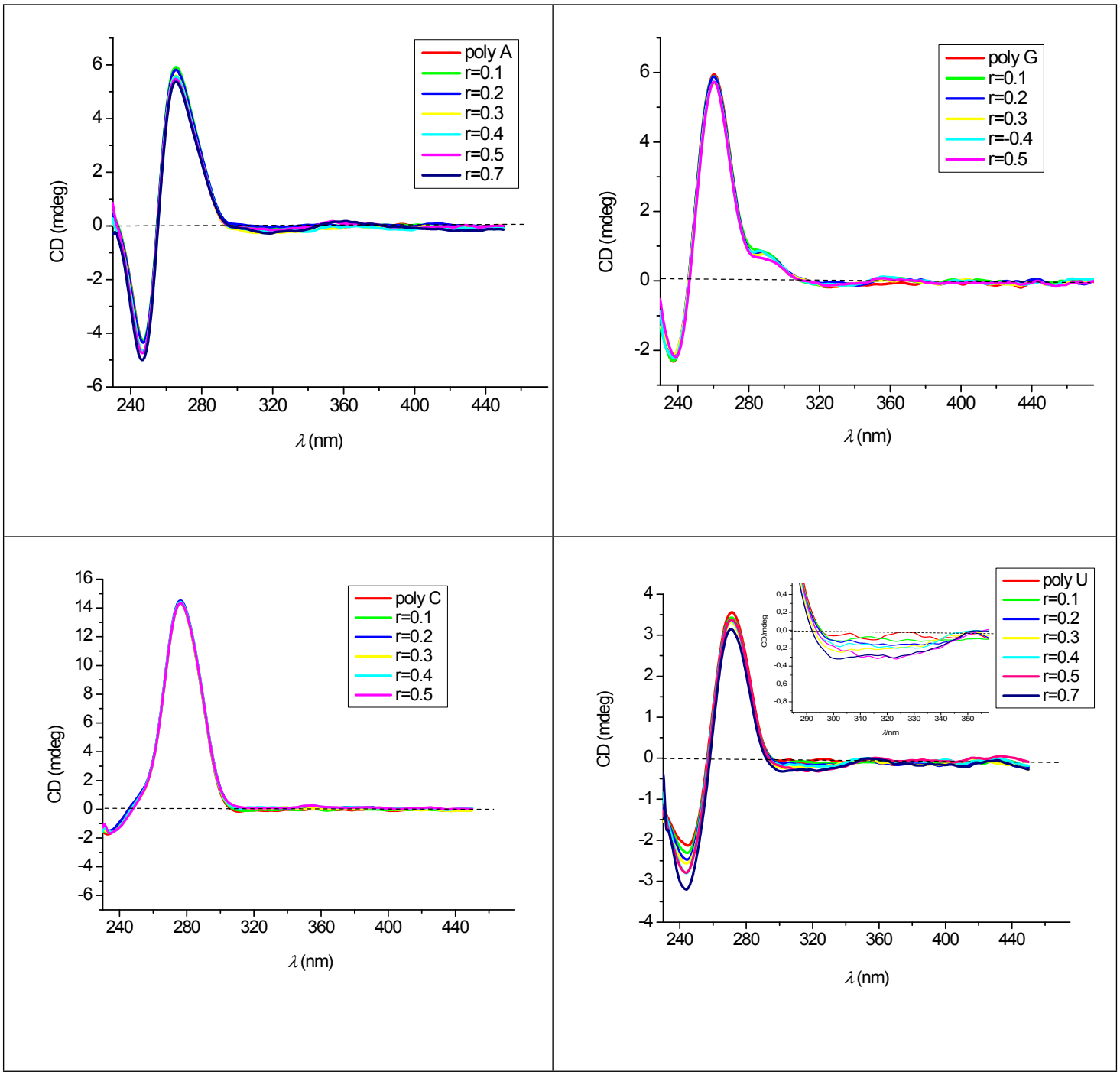
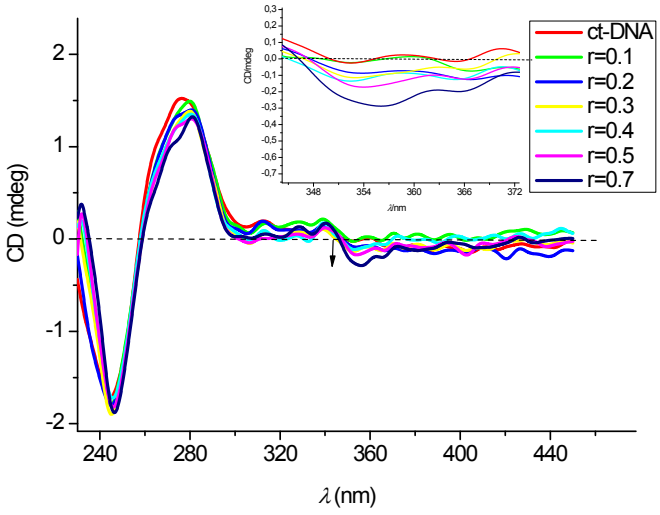
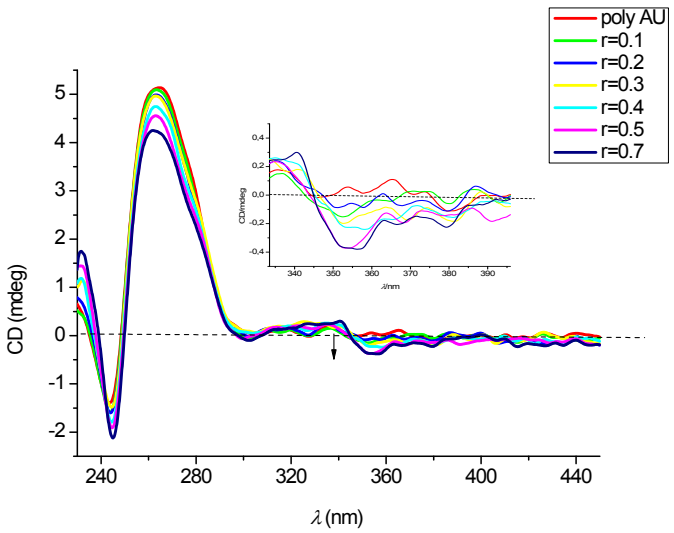
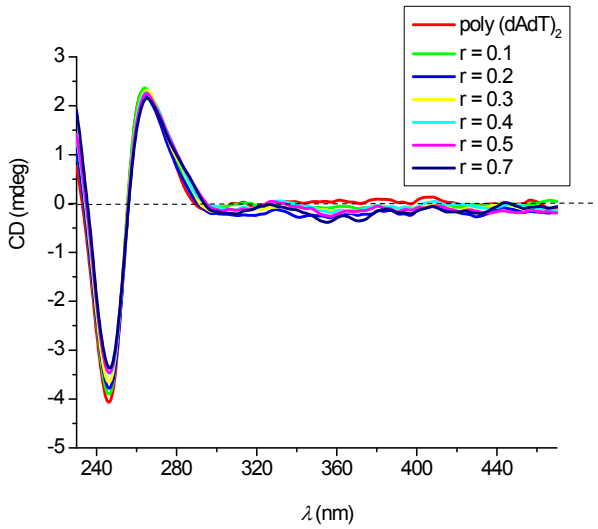
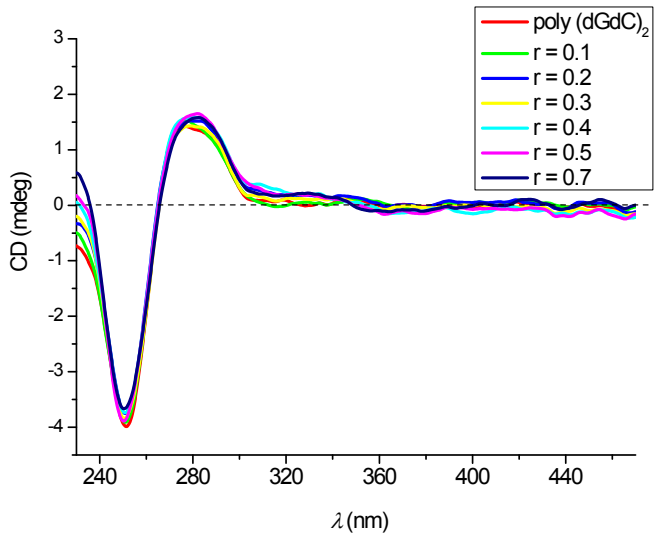


Figure S15. CD titration of **ct-DNA, poly A – poly U, poly (dAdT)$_2$**, **poly (dGdC)$_2$**, **poly A**, **poly G**, **poly C** and **poly U** (all DNA and RNA $c = 2\times10^{-5}$ mol/L) with **2** at molar ratios $\boldsymbol{r}$ [**2**] / [polynucleotide] = 0.1 - 0.5 (0.7). Done at pH 7, sodium cacodylate buffer, $I = 0.05$ mol/L.


ct-DNA
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
r=0.7
CD (mdeg)
λ (nm)


poly AU
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
r=0.7
CD (mdeg)
λ (nm)


poly (dAdT)2
r = 0.1
r = 0.2
r = 0.3
r = 0.4
r = 0.5
r = 0.7
CD (mdeg)
λ (nm)


poly (dGdC)2
r = 0.1
r = 0.2
r = 0.3
r = 0.4
r = 0.5
r = 0.7
CD (mdeg)
λ (nm)

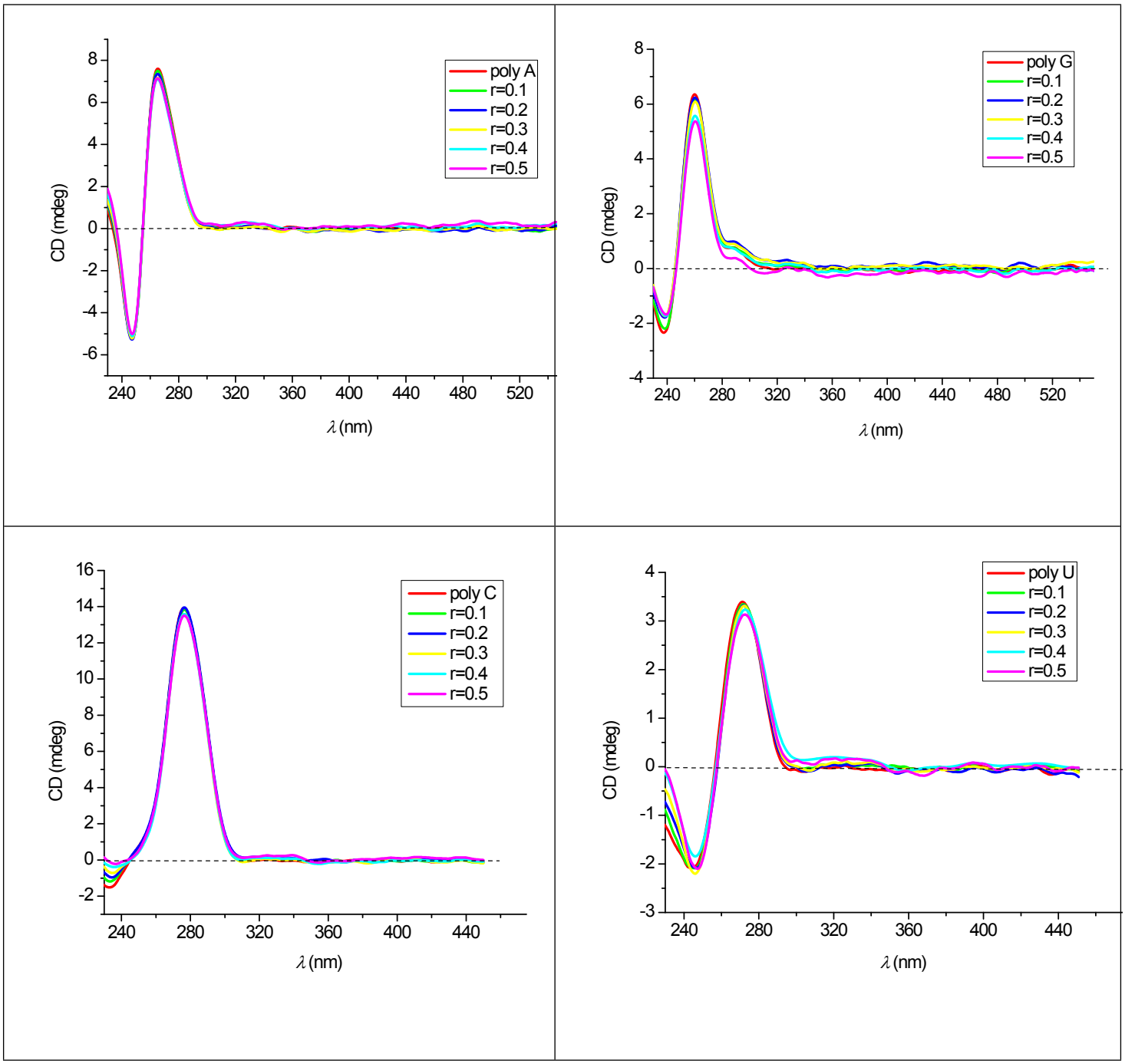
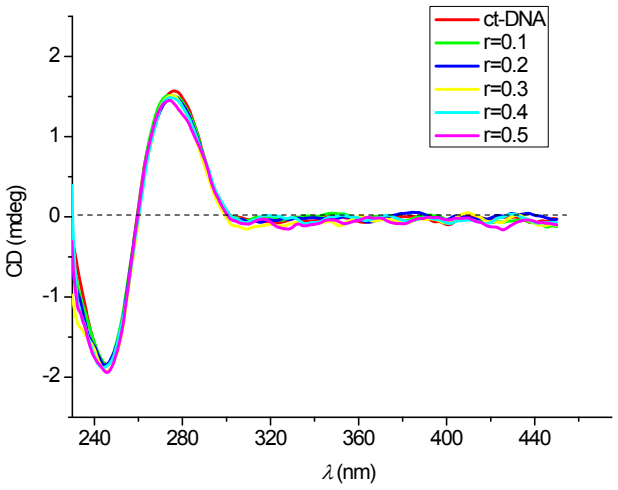
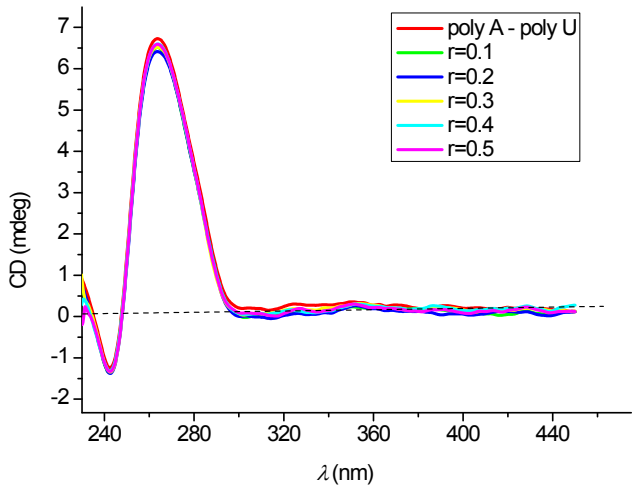
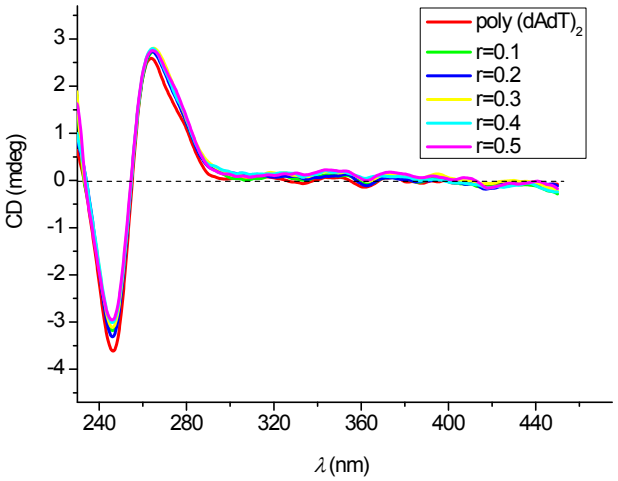
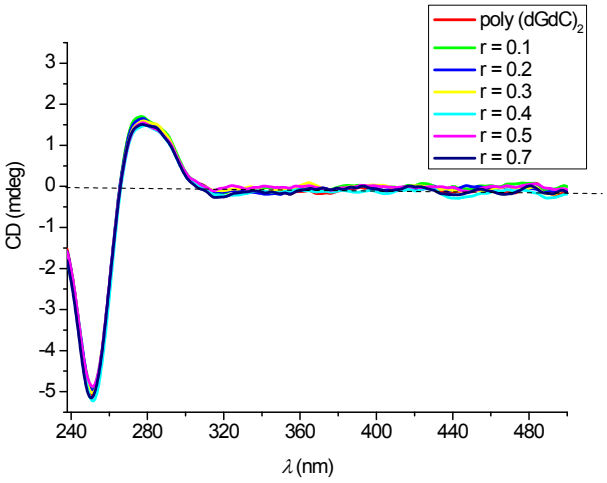


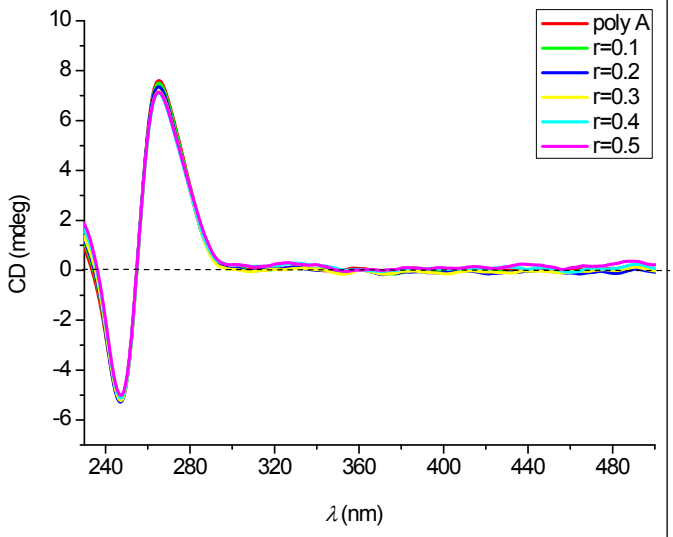
Figure S16. CD titration of **ct-DNA, poly A - poly U, poly (dAdT)$_2$**, **poly (dGdC)$_2$**, **poly A**, **poly G**, **poly C** and **poly U** (all DNA and RNA $c = 2\times10^{-5}$ mol/L) with **3** at molar ratios $\boldsymbol{r}$ [**3**] / [polynucleotide] = 0.1 - 0.5 (0.7). Done at pH 7, sodium cacodylate buffer, $I = 0.05$ mol/L.

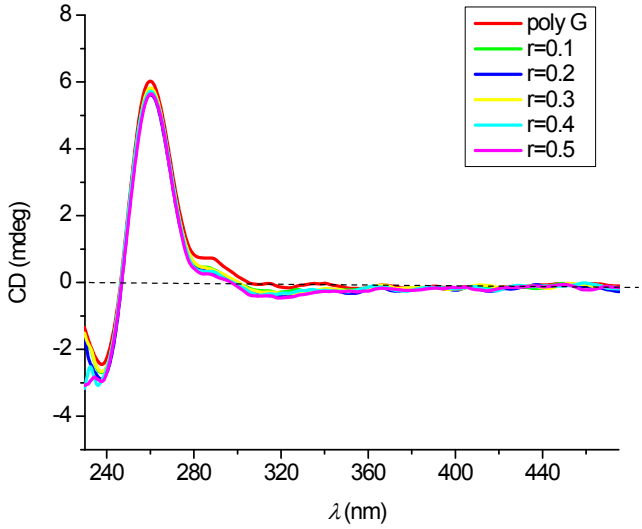

ct-DNA
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
CD (mdeg)
λ (nm)


poly A - poly U
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
CD (mdeg)
λ (nm)


poly (dAdT)2
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
CD (mdeg)
λ (nm)


poly (dGdC)2
r = 0.1
r = 0.2
r = 0.3
r = 0.4
r = 0.5
r = 0.7
CD (mdeg)
λ (nm)


poly A
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
CD (mdeg)
λ (nm)


poly G
r=0.1
r=0.2
r=0.3
r=0.4
r=0.5
CD (mdeg)
λ (nm)

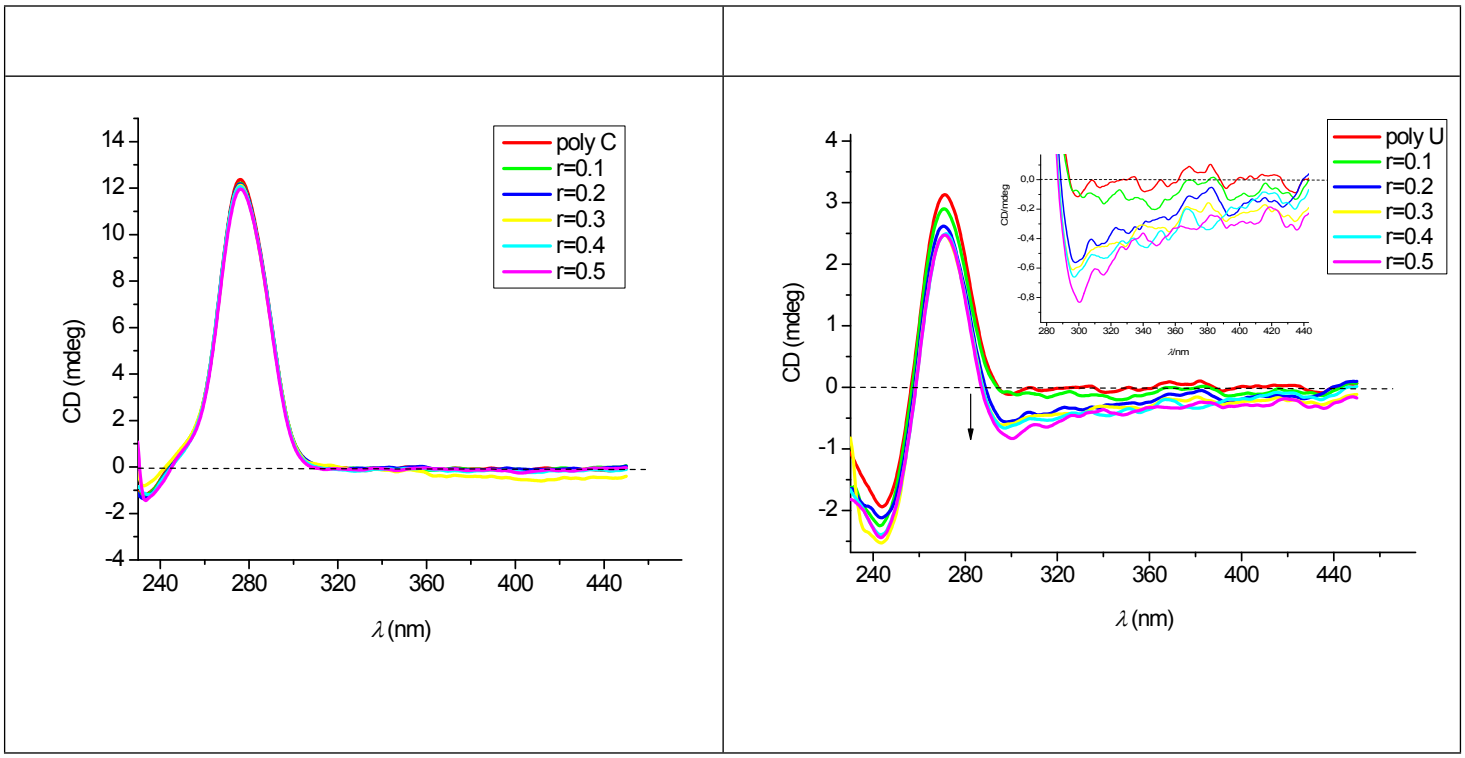


Figure S17. CD titration of **ct-DNA, poly A - poly U, poly (dAdT)$_2$, poly (dGdC)$_2$, poly A**, **poly G**, **poly C** and **poly U** (all DNA and RNA $c = 2\times10^{-5}$ mol/L) with **4** at molar ratios $\boldsymbol{r}$ [**4**] / [polynucleotide] = 0.1 - 0.5. Done at pH 7, sodium cacodylate buffer, $I = 0.05$ mol/L.

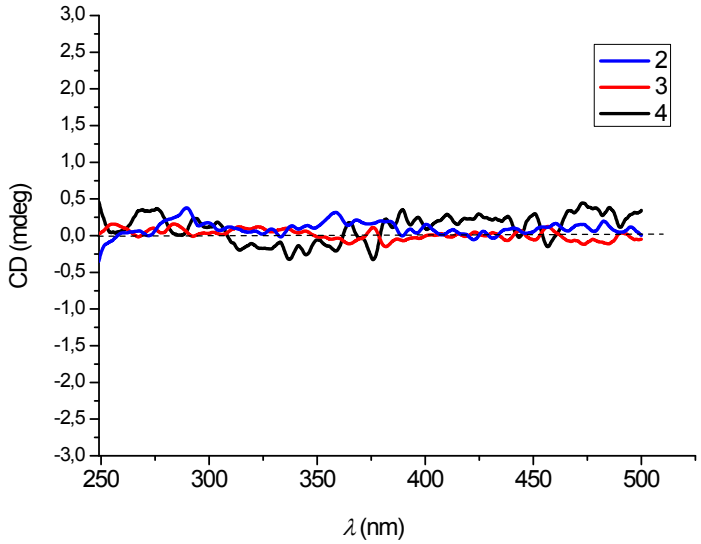


Figure S18. CD spectra of compounds **2, 3** and **4** ($c = 6\times10^{-6}$ mol/L). Done at pH 7, sodium cacodylate buffer, $I = 0.05$ mol/L.

Biological experiments:

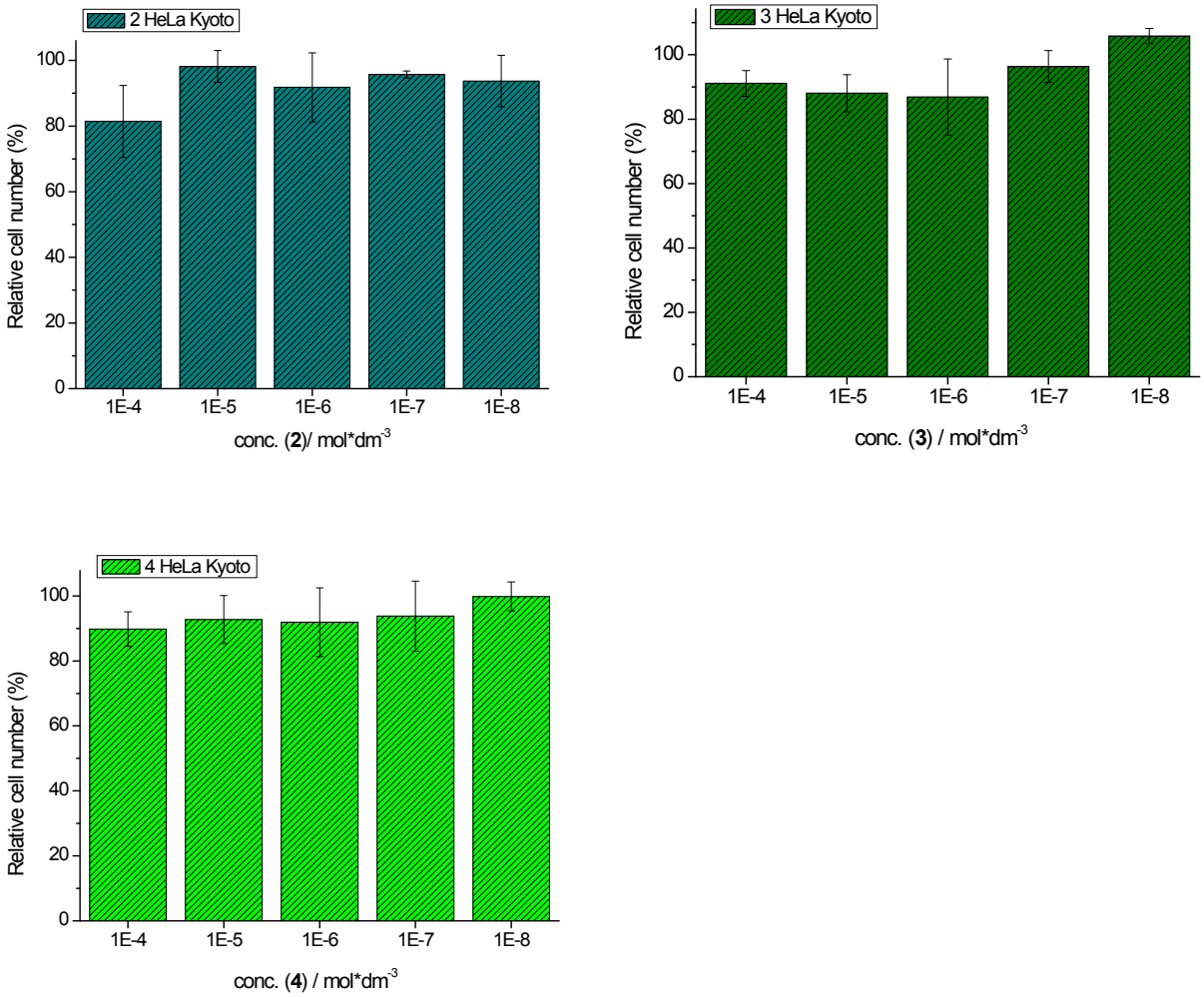


Figure S19. Effect of compounds **2-4** on HeLa Kyoto cell proliferation after 72 h treatment

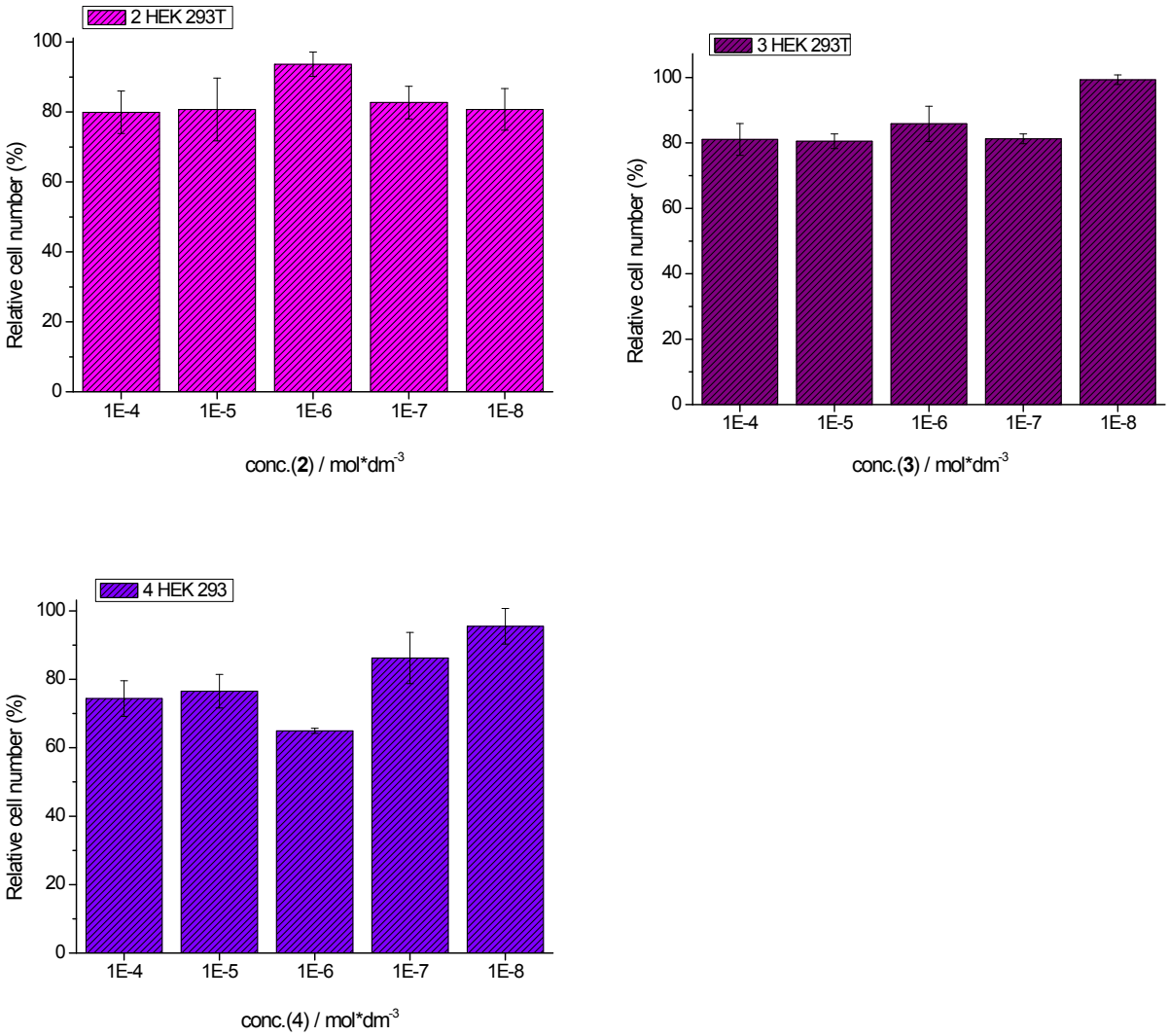


Figure S20. Effect of compounds **2-4** on HEK 293T cell proliferation after 72 h treatment

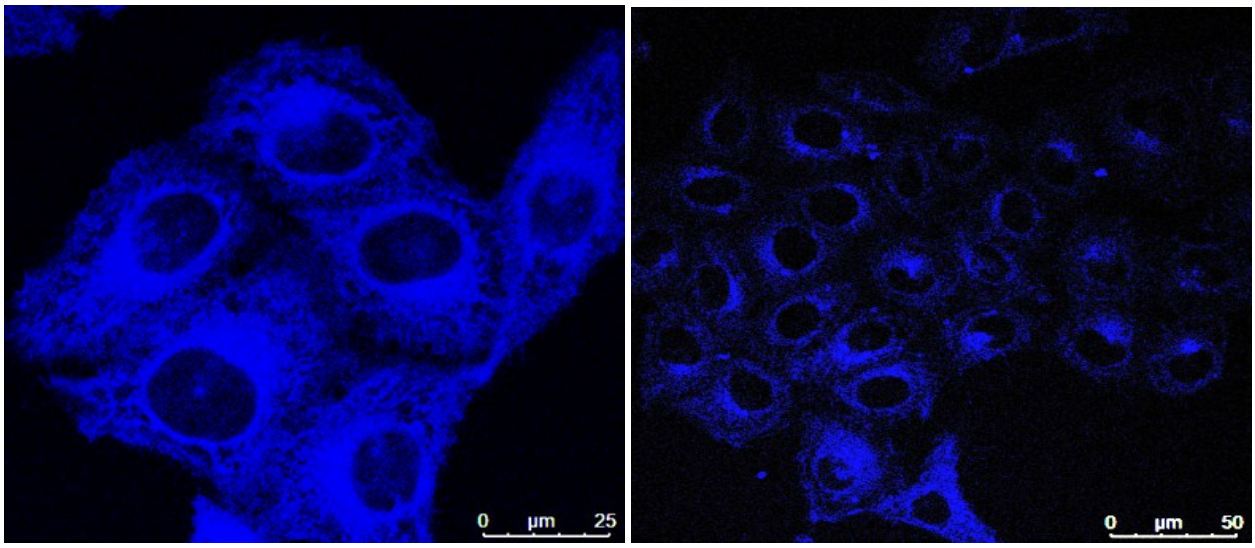
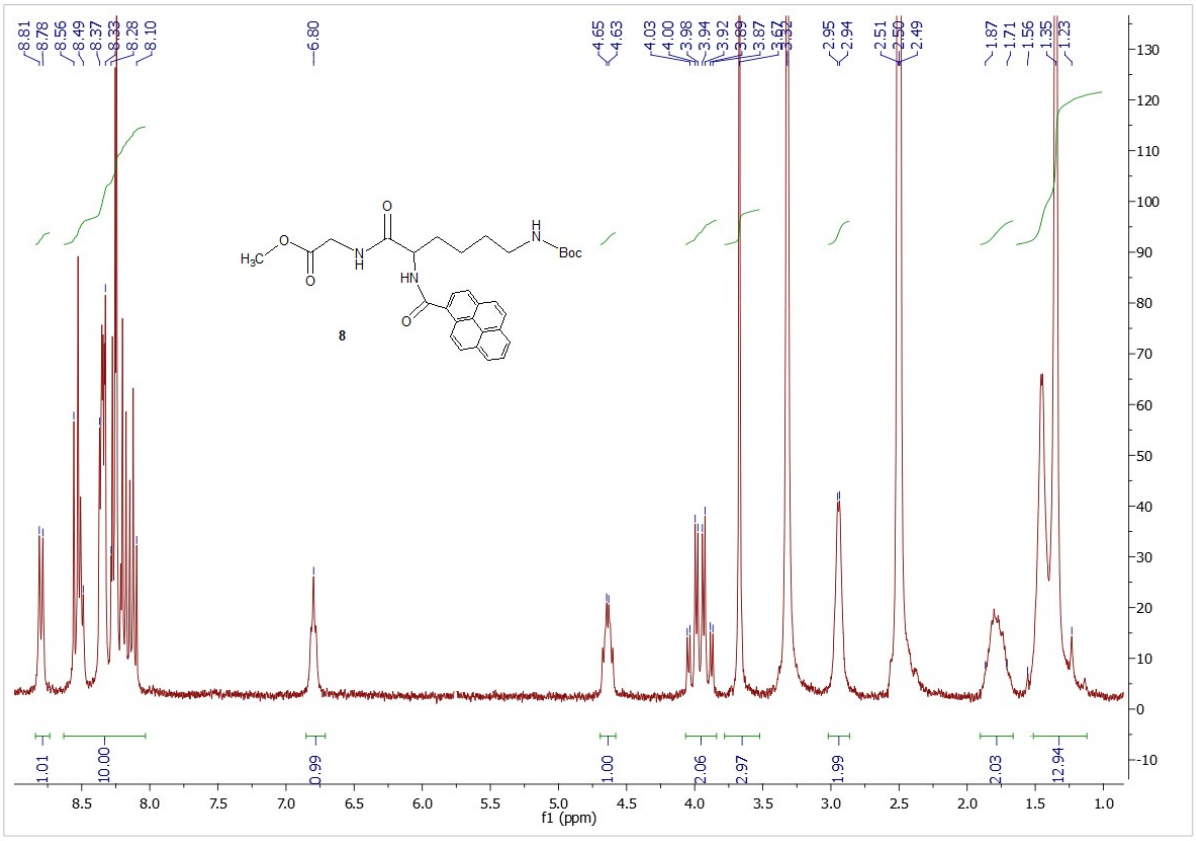


Figure S21. Confocal microscopy (Leica SP8X FLIM) of HeLa Kyoto cells after 2h of incubation with **2** (left) and **4** (right) hybrids (c=2 x 10^{-5} mol/L). The excitation peak value is 340 nm. Emission was detected at 360-405 nm.

1H and ^{13}C NMR Spectra:



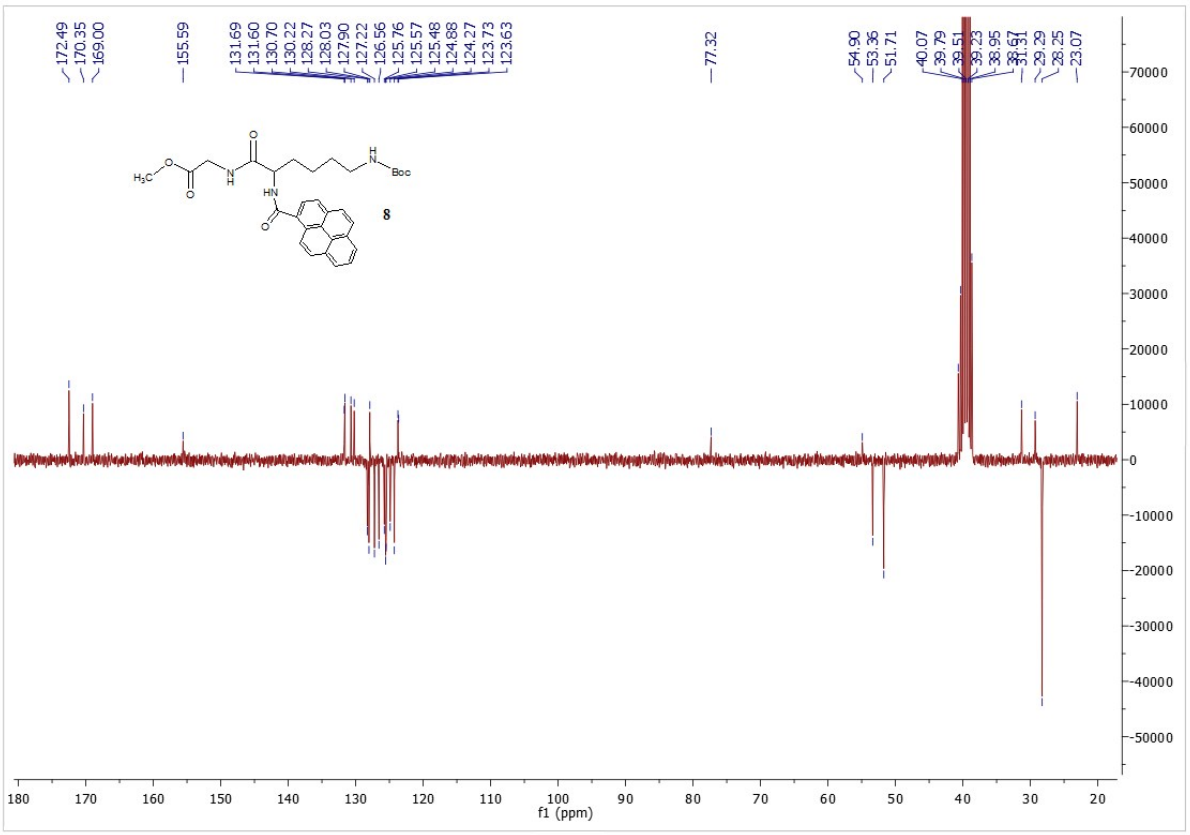
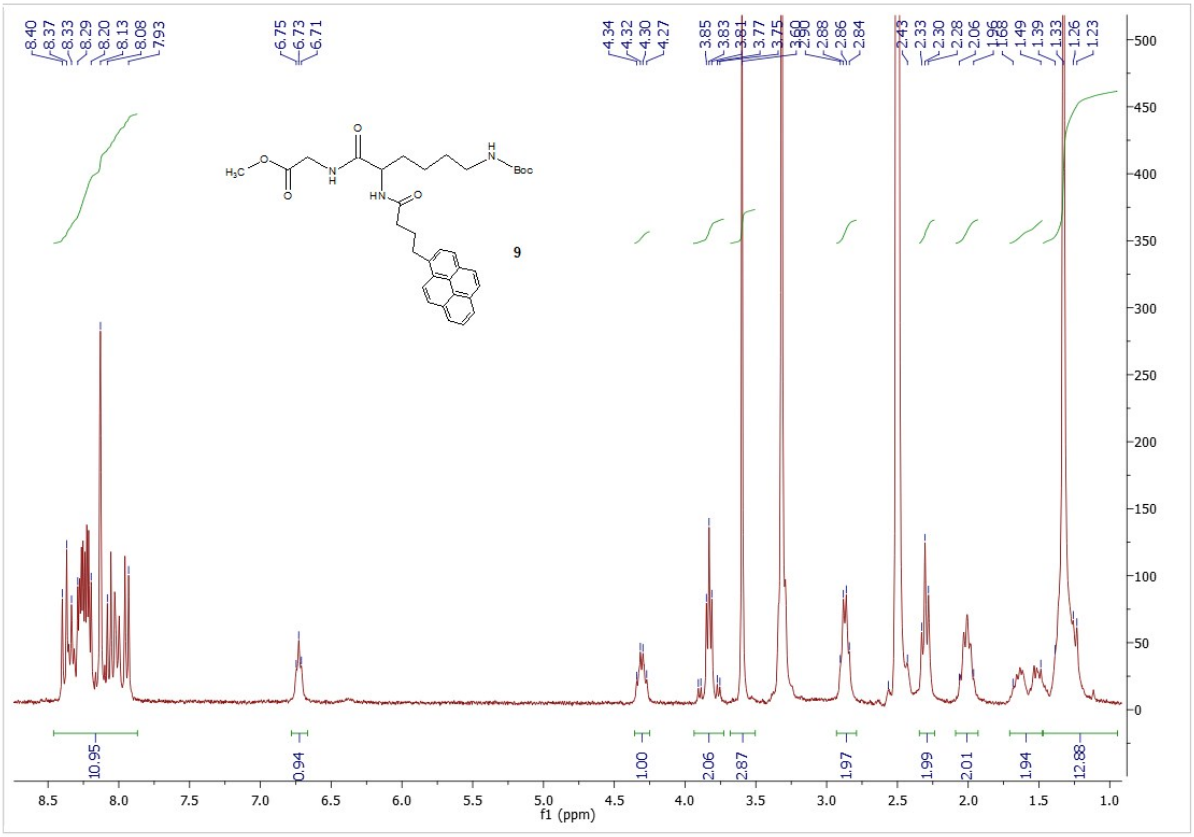


Figure S22. ^{1}H and ^{13}C NMR spectra of compound **8** in DMSO.



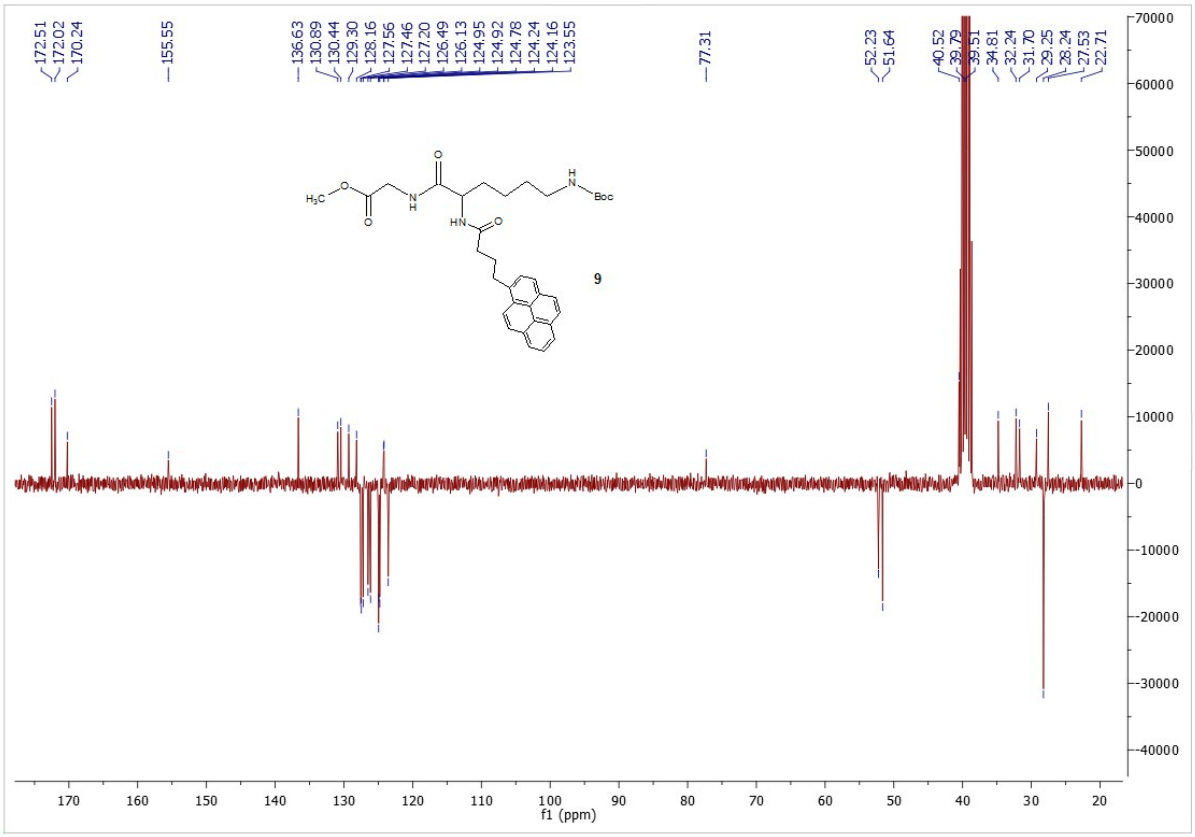
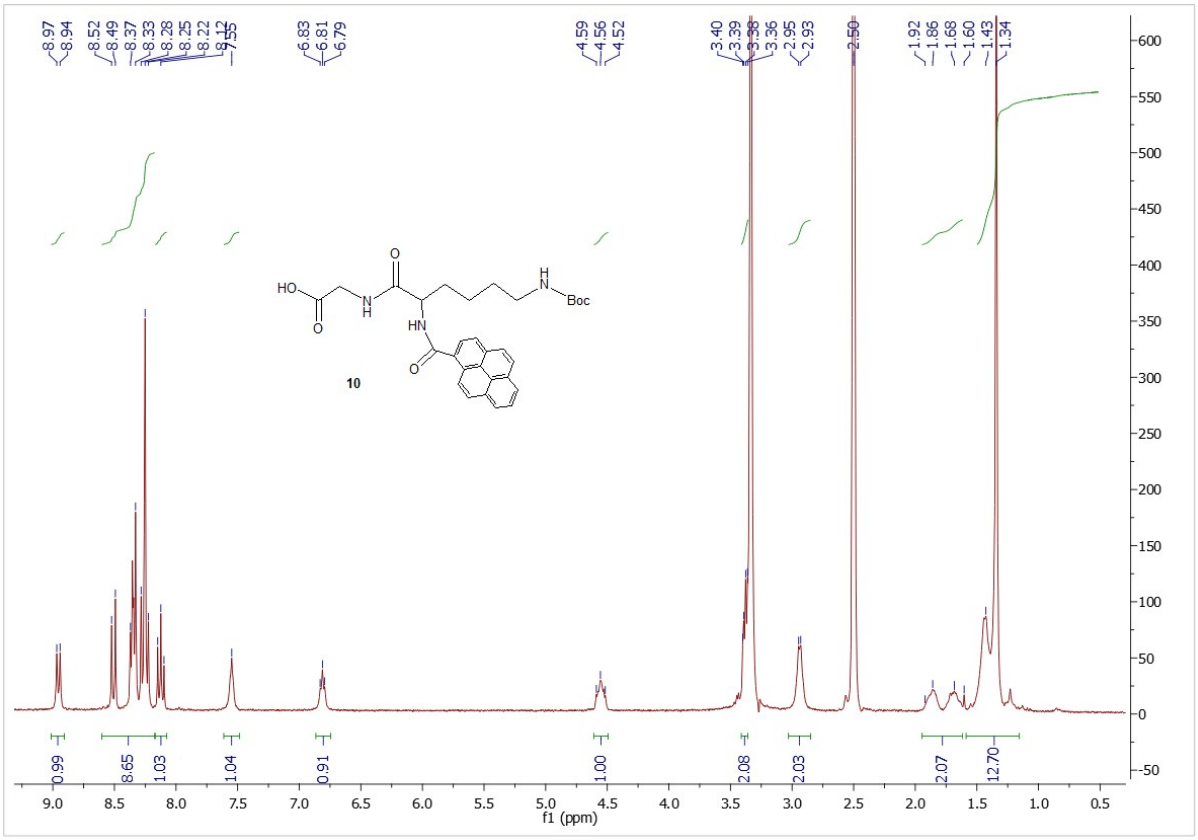


Figure S23. ^{1}H and ^{13}C NMR spectra of compound **9** in DMSO.


8.97
8.94
8.52
8.49
8.37
8.33
8.28
8.25
8.22
8.12
7.55
6.83
6.81
6.79
4.59
4.56
4.52
3.40
3.39
3.38
3.36
2.95
2.93
2.50
1.92
1.86
1.68
1.60
1.43
1.34
HO
O
N
H
O
HN
O
N
H
Boc
10
0.99
8.65
1.03
1.04
0.91
1.00
2.08
2.03
2.07
12.70
f1 (ppm)

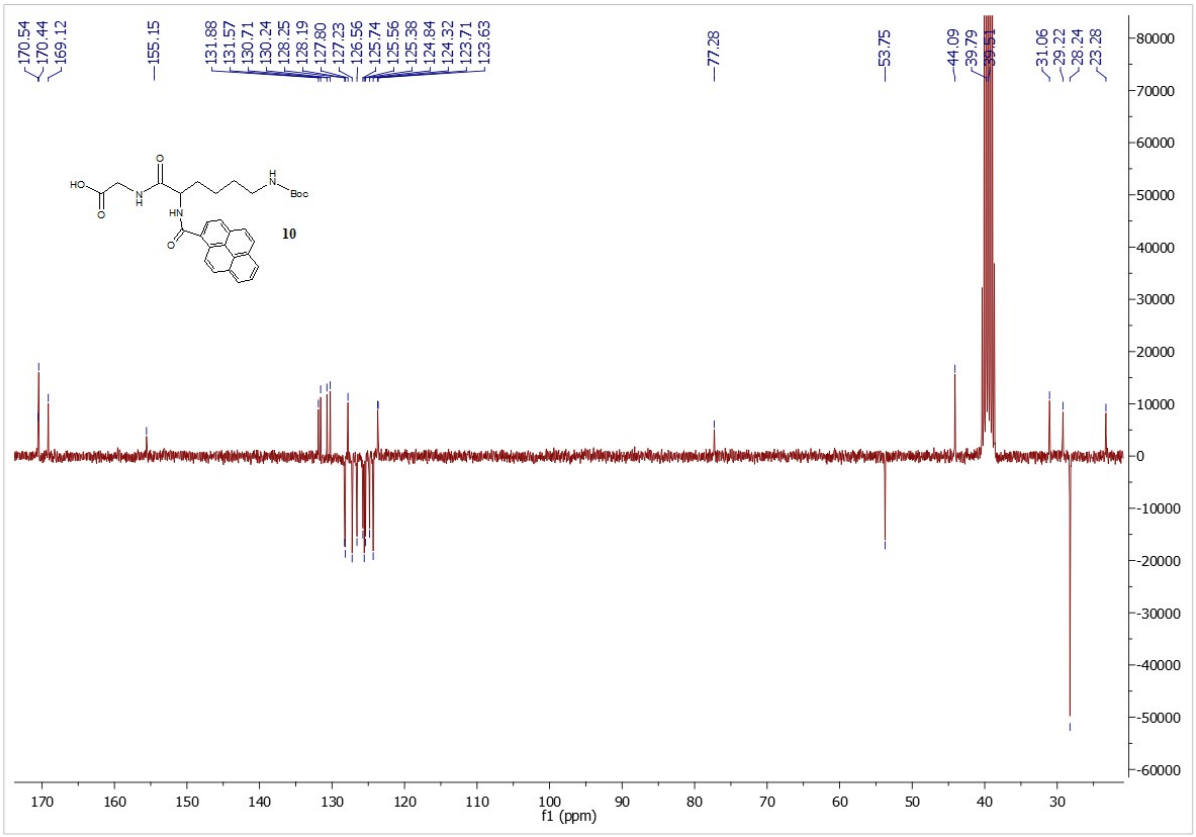
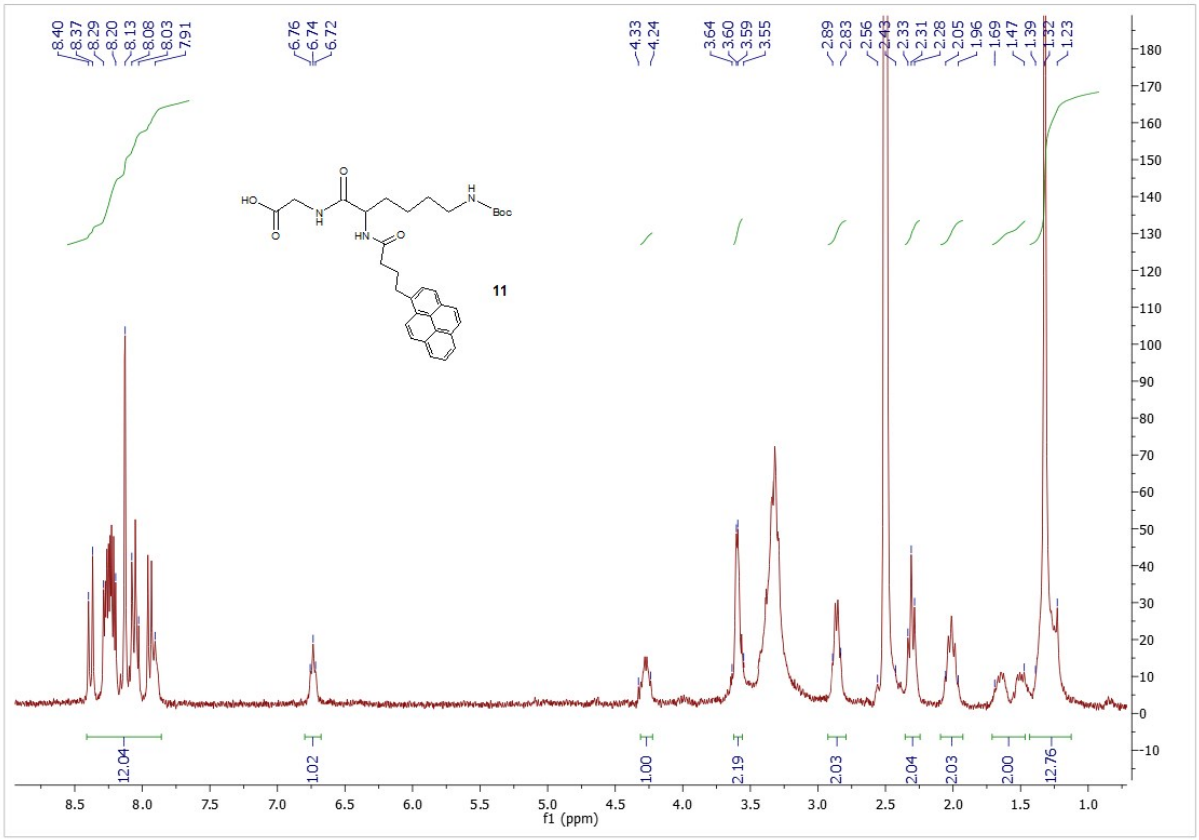

170.54
170.44
169.12
155.15
131.88
131.57
130.71
130.24
128.25
128.19
127.80
127.23
126.56
125.74
125.56
125.38
124.84
124.32
123.71
123.63
77.28
53.75
44.09
39.79
39.51
31.06
29.22
28.24
23.28
HO
O
N
H
O
HN
O
N
H
Boc
10
f1 (ppm)

Figure S24. ^{1}H and ^{13}C NMR spectra of compound **10** in DMSO.



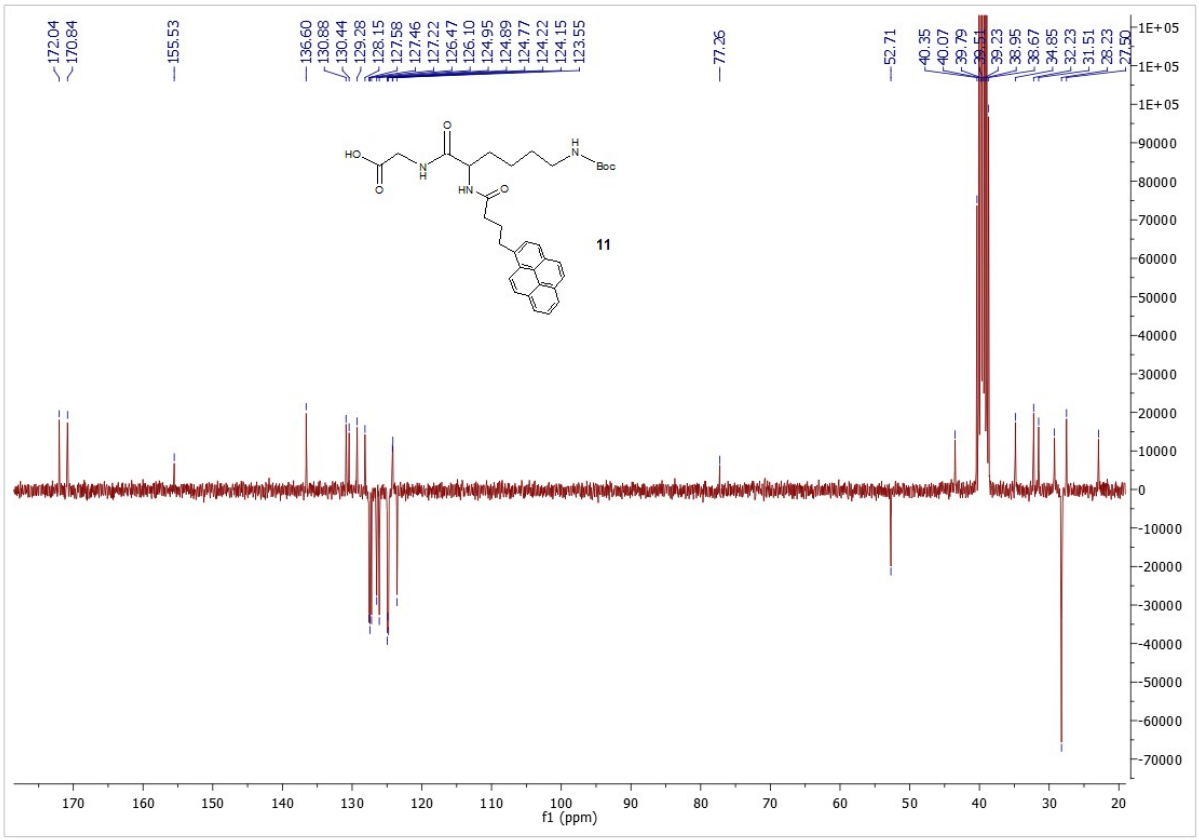


Figure S25. ^{1}H and ^{13}C NMR spectra of compound **11** in DMSO.

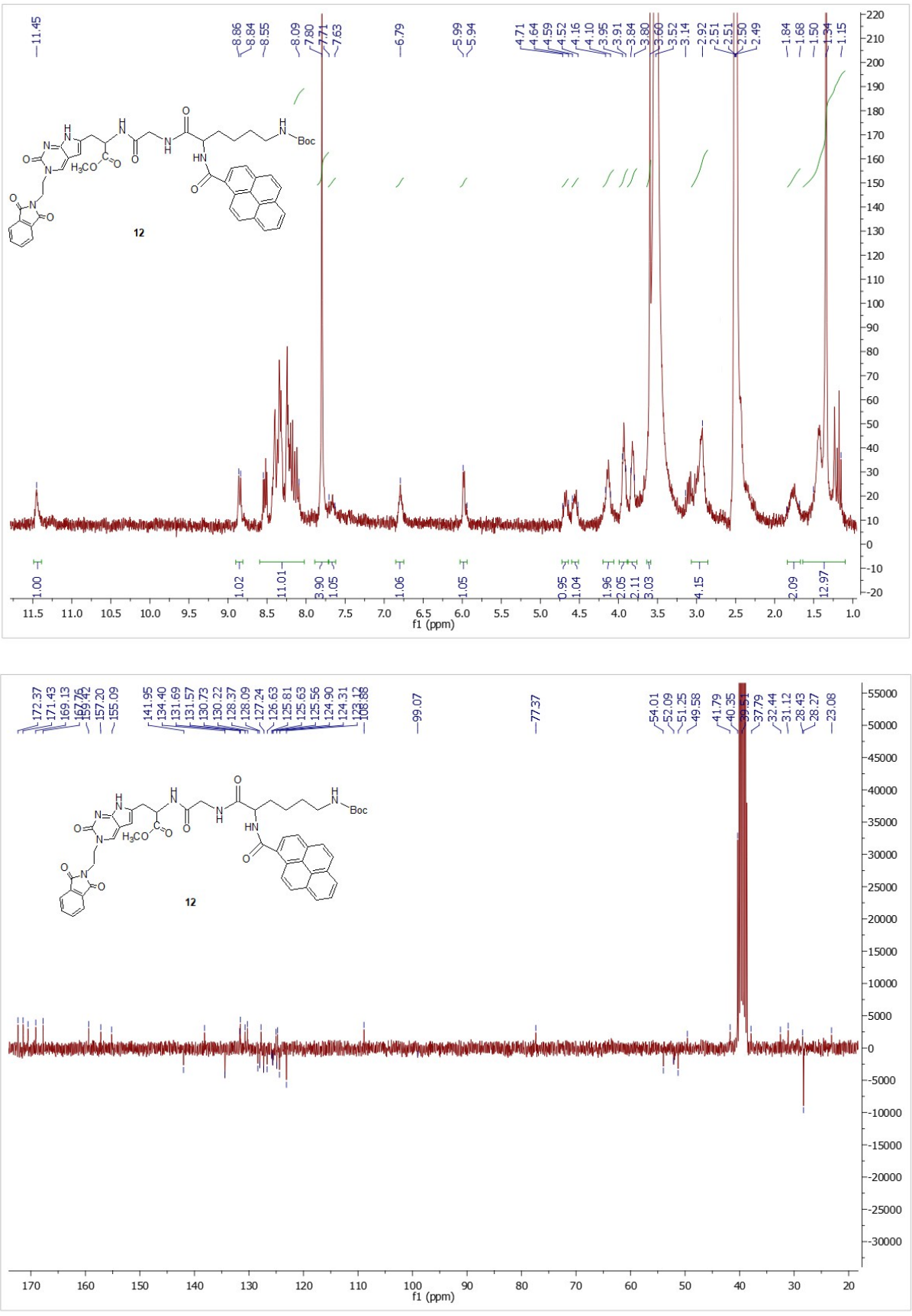


Figure S26. ^{1}H and ^{13}C NMR spectra of compound **12** in DMSO.

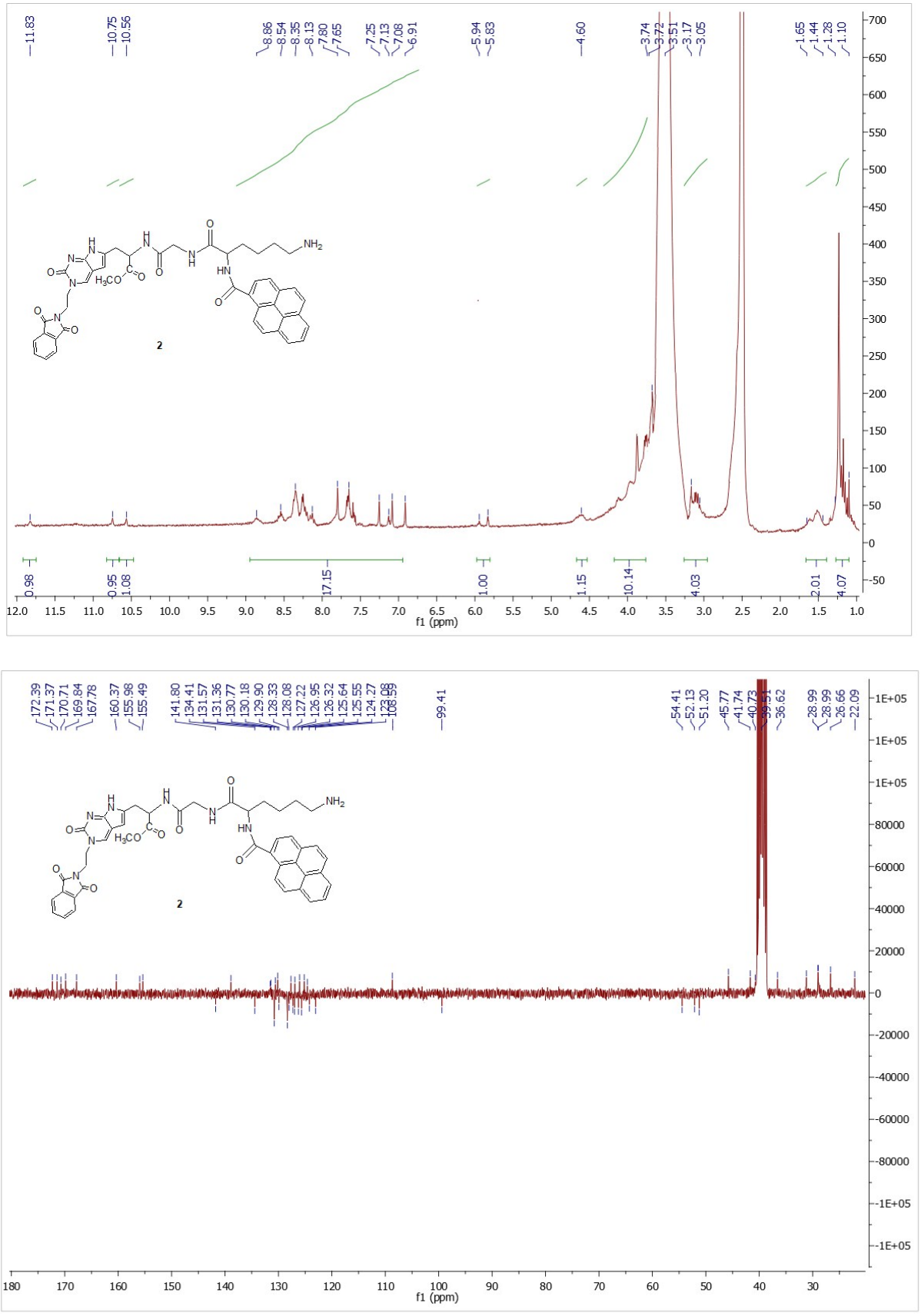


Figure S27. ^{1}H and ^{13}C NMR spectra of compound **2** in DMSO.

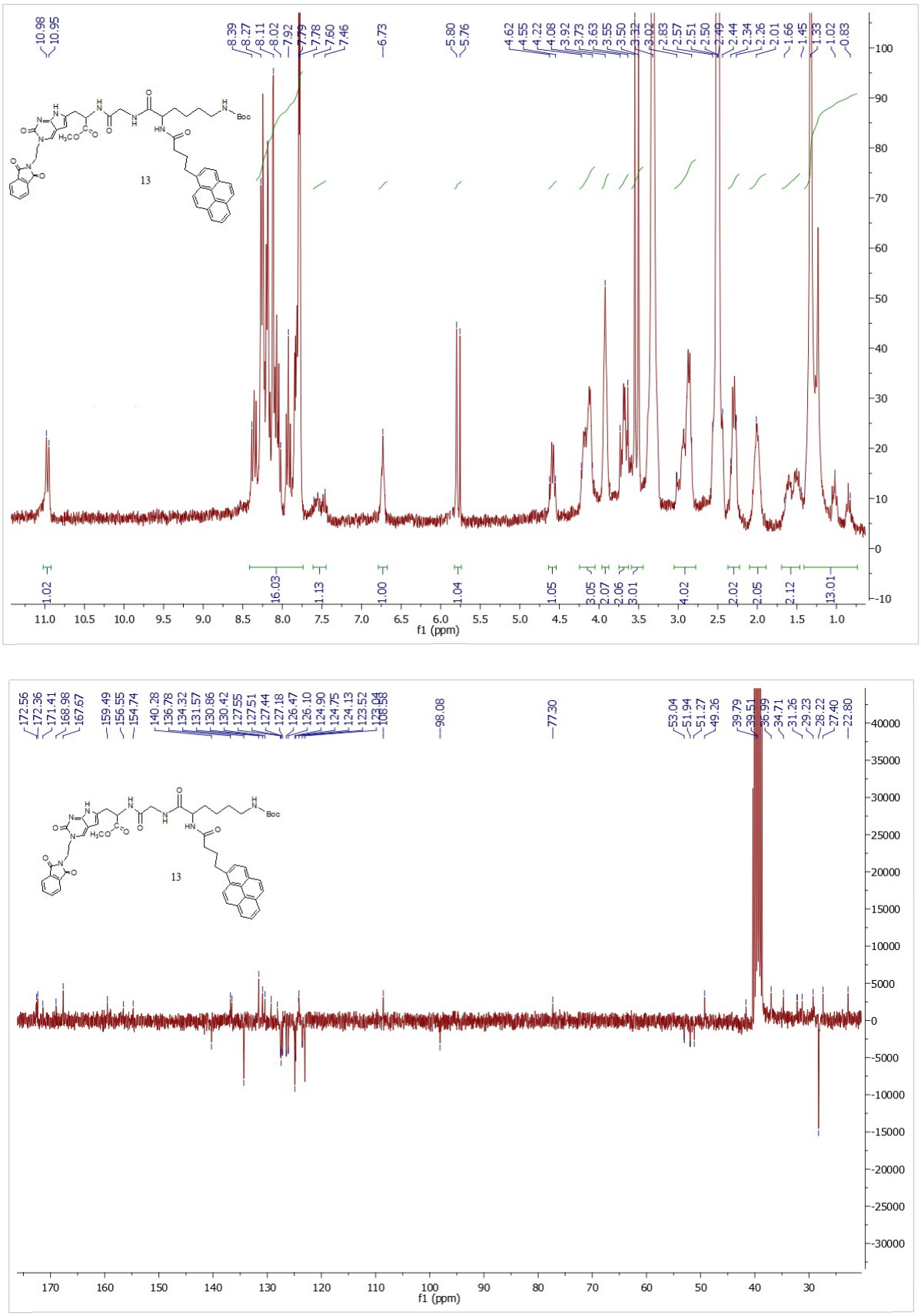


Figure S28. ^{1}H and ^{13}C NMR spectra of compound **13** in DMSO.

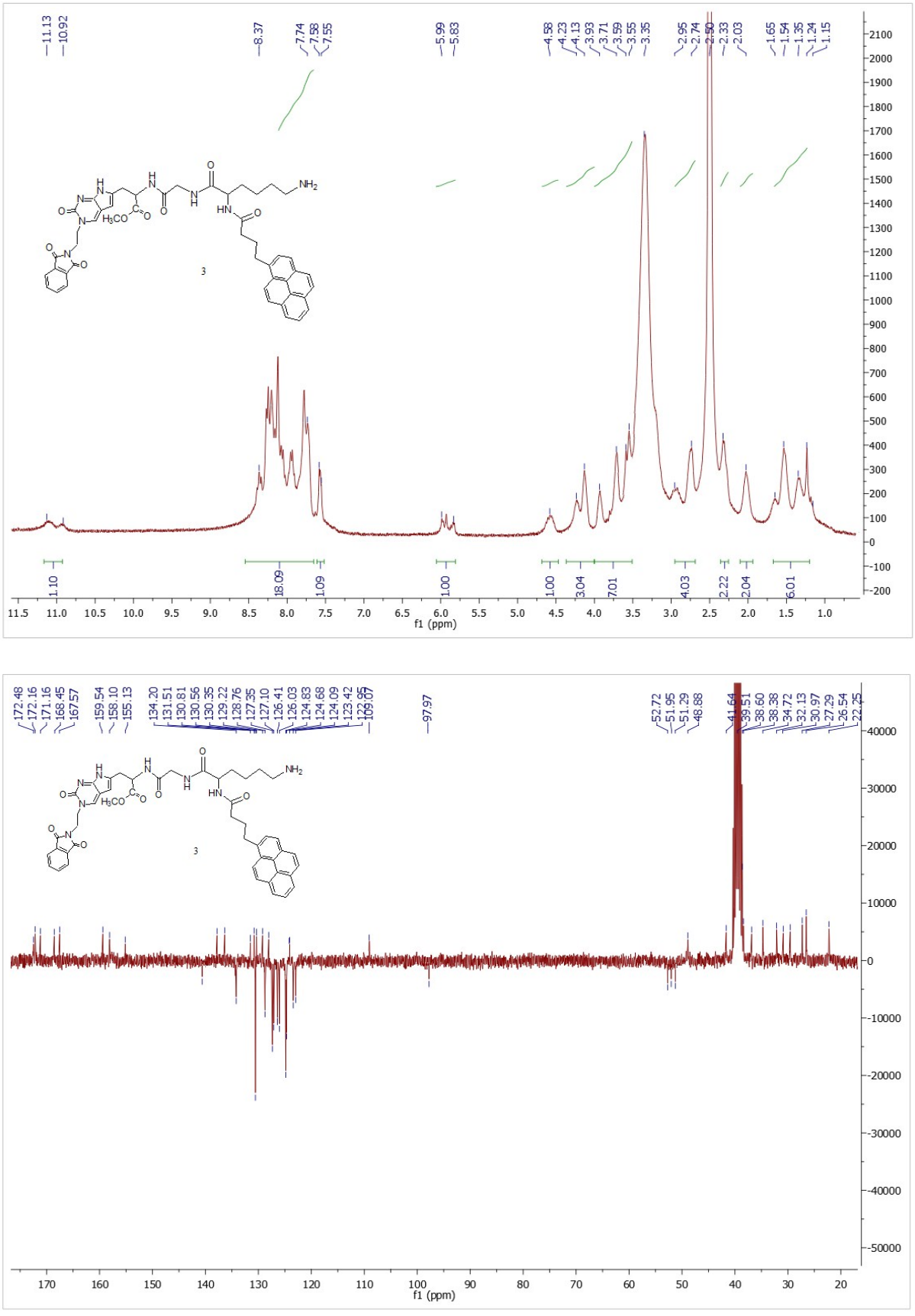
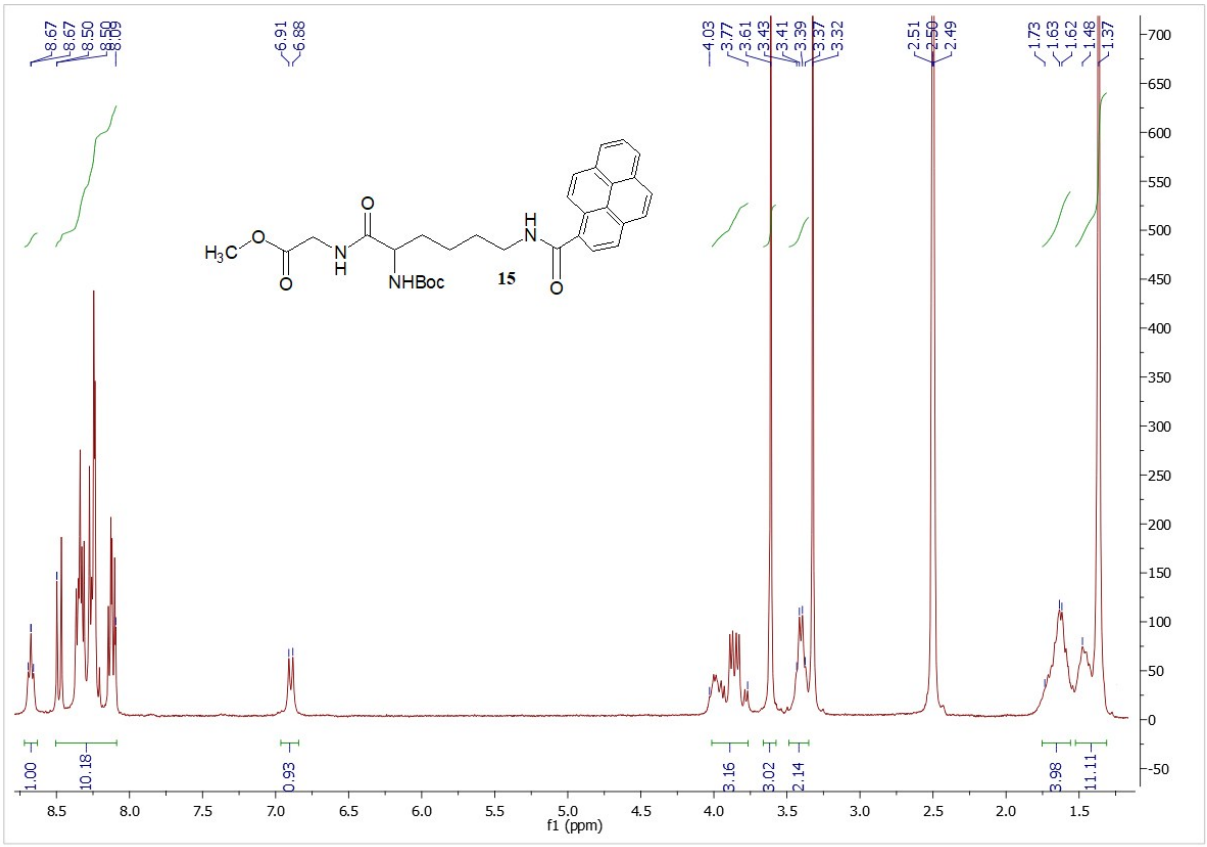


Figure S29. ^{1}H and ^{13}C NMR spectra of compound **3** in DMSO.



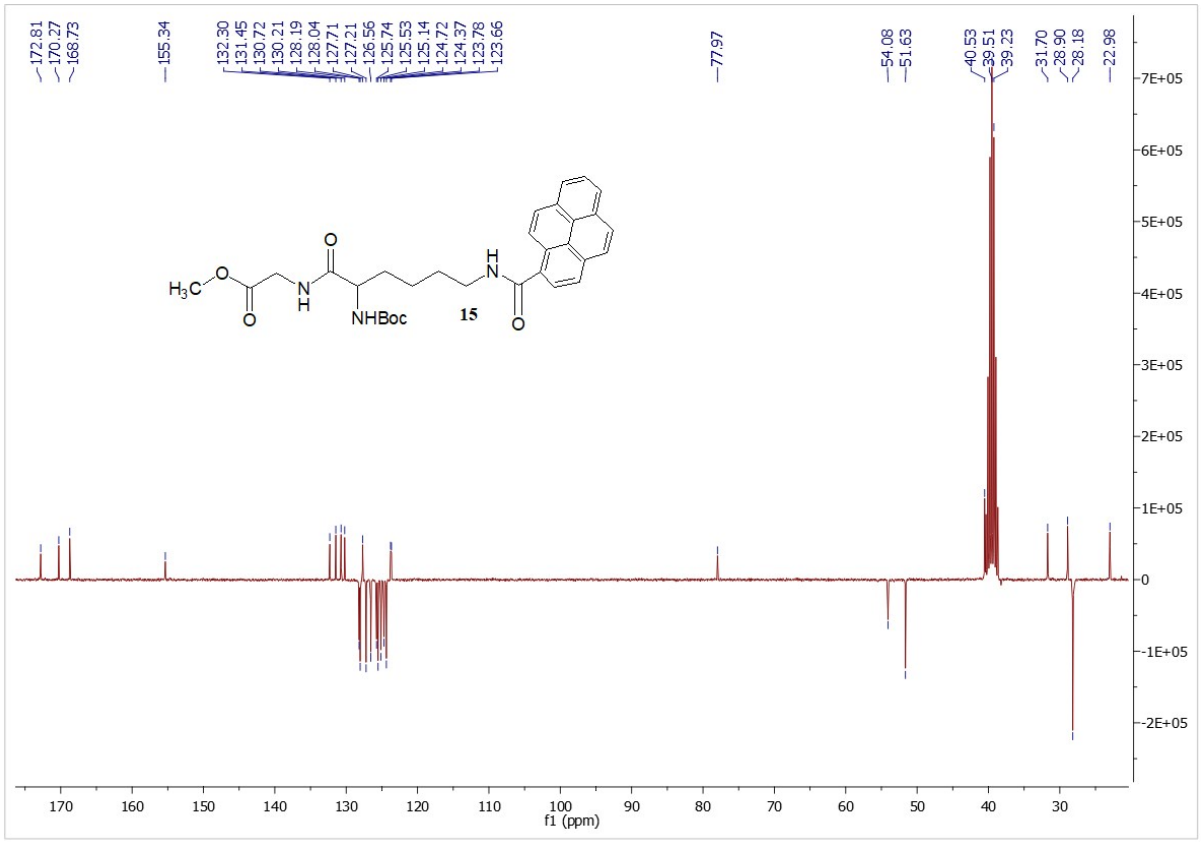


Figure S30. ^{1}H and ^{13}C NMR spectra of compound **15** in DMSO.

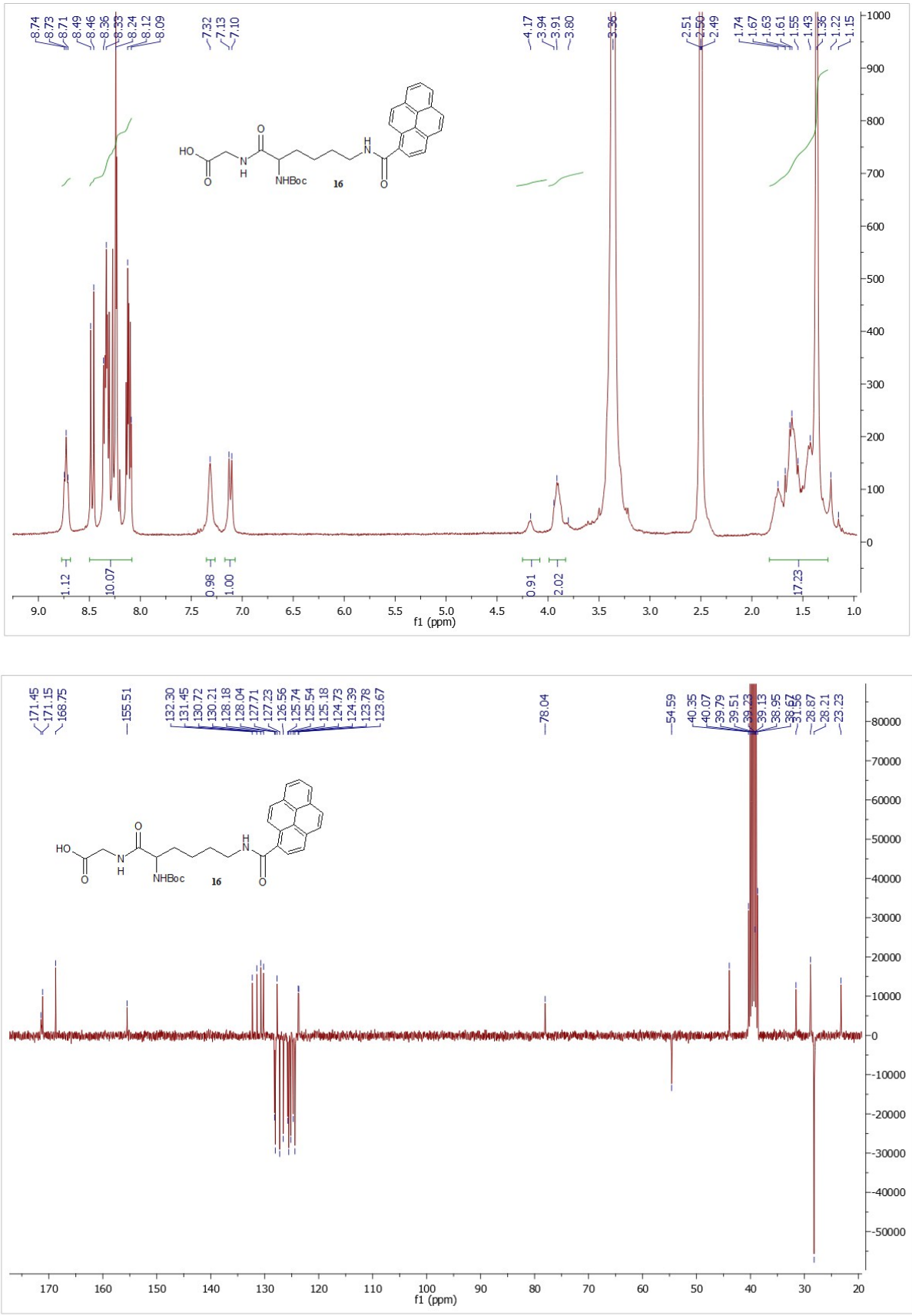


Figure S31. ^{1}H and ^{13}C NMR spectra of compound **16** in DMSO.

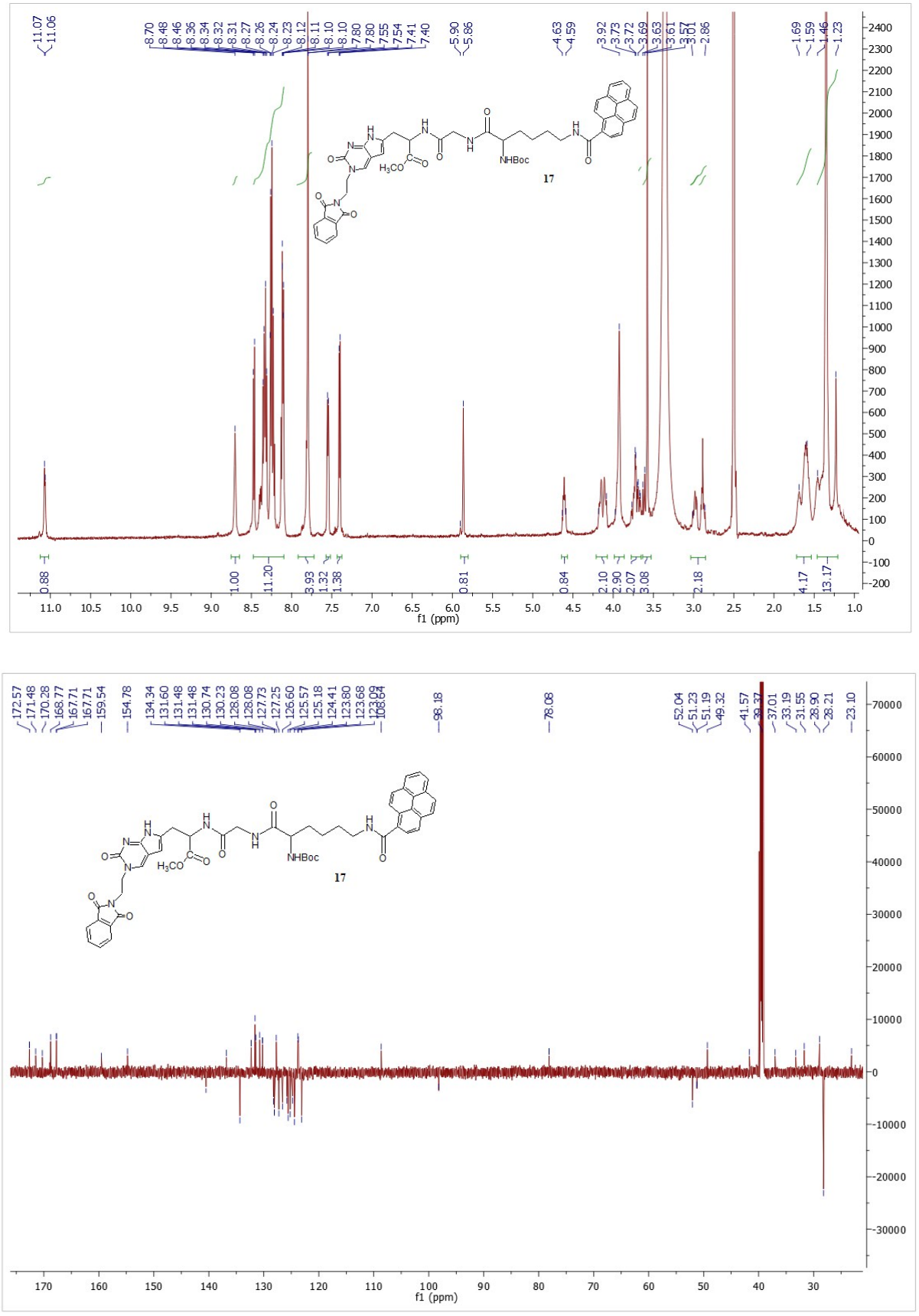


Figure S32. ^{1}H and ^{13}C NMR spectra of compound **17** in DMSO.

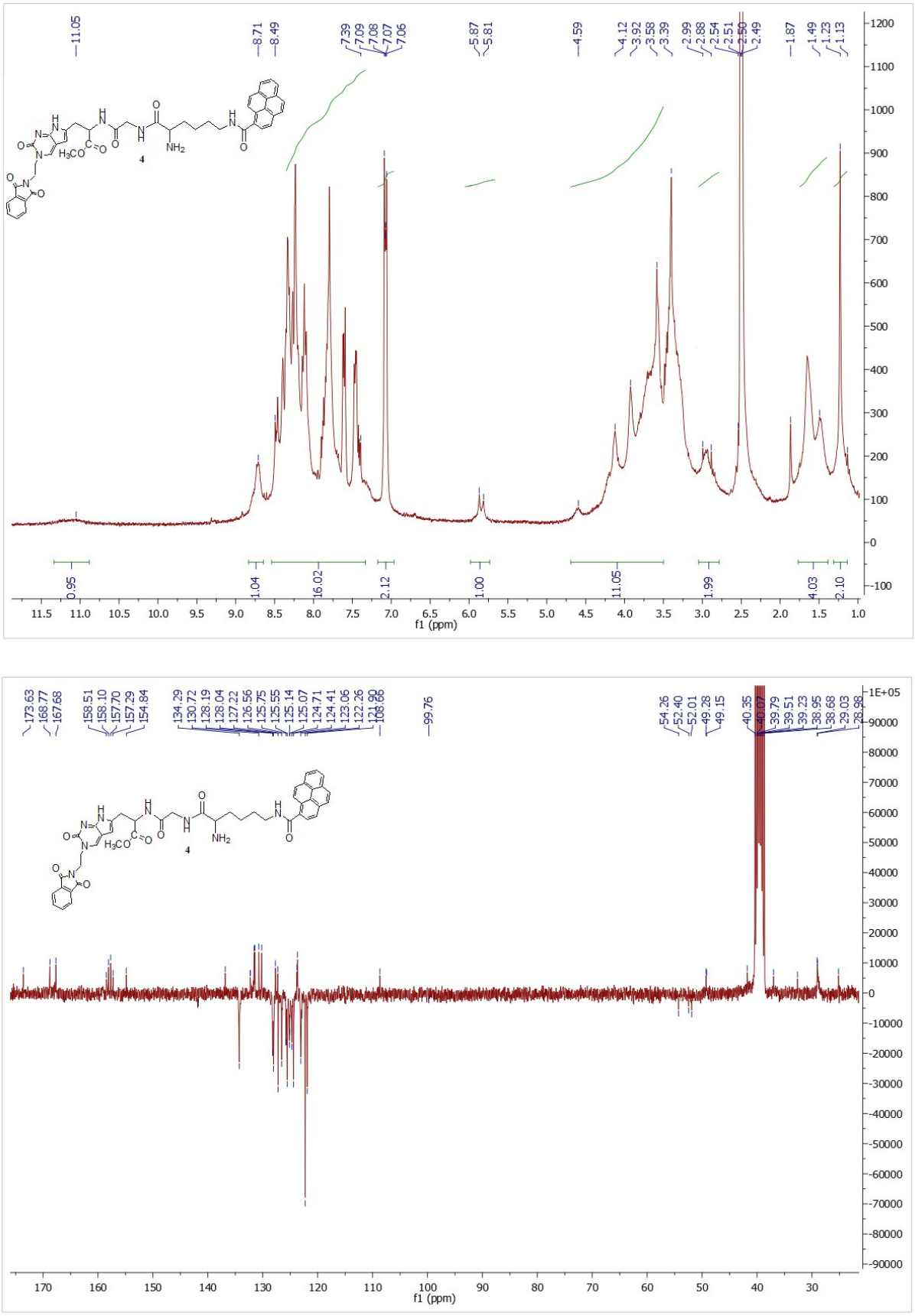


Figure S33. ^{1}H and ^{13}C NMR spectra of compound **4** in DMSO.

REFERENCES

[1] Cantor, C. R., and Schimmel, P. R. (1980) Biophysical Chemistry, Vol. 3, , WH Freeman and Co., San Francisco.; Egli, M., and Saenger, W. (1983) Principles of Nucleic Acid Structure, Springer-Verlag, New York
[2] Rodger A., Norden B., In *Circular Dichroism and Linear Dichroism*; Oxford University Press: New York, 1997, Chapter 2.