Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary information

Turn-on fluorescence of ruthenium pyrene complexes in response to bovine serum albumin

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Contents

Compounds

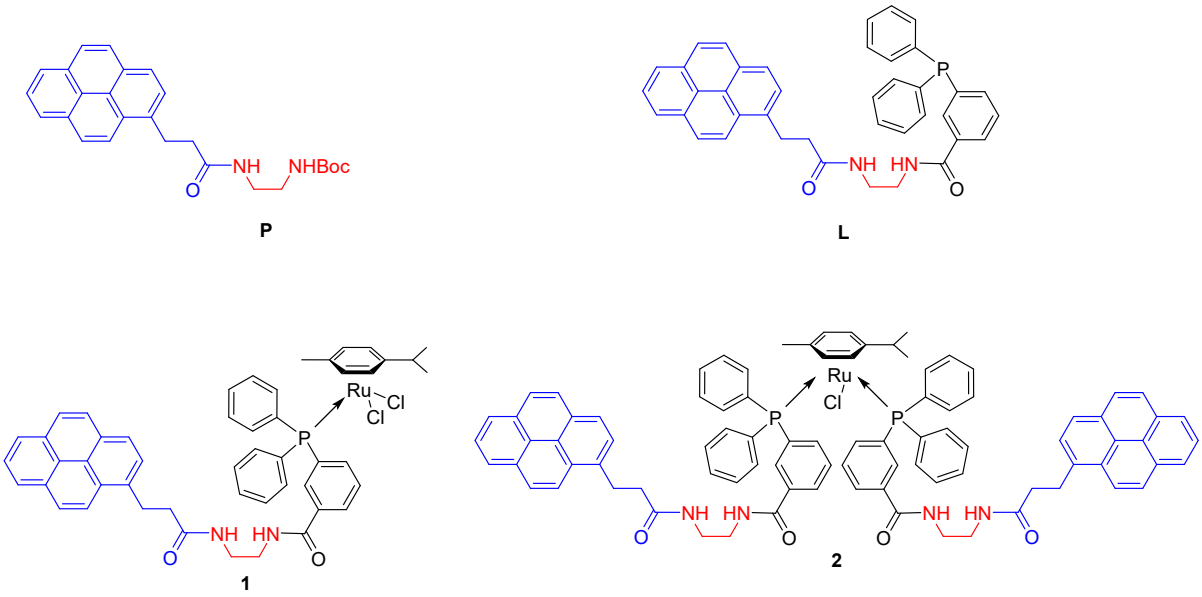
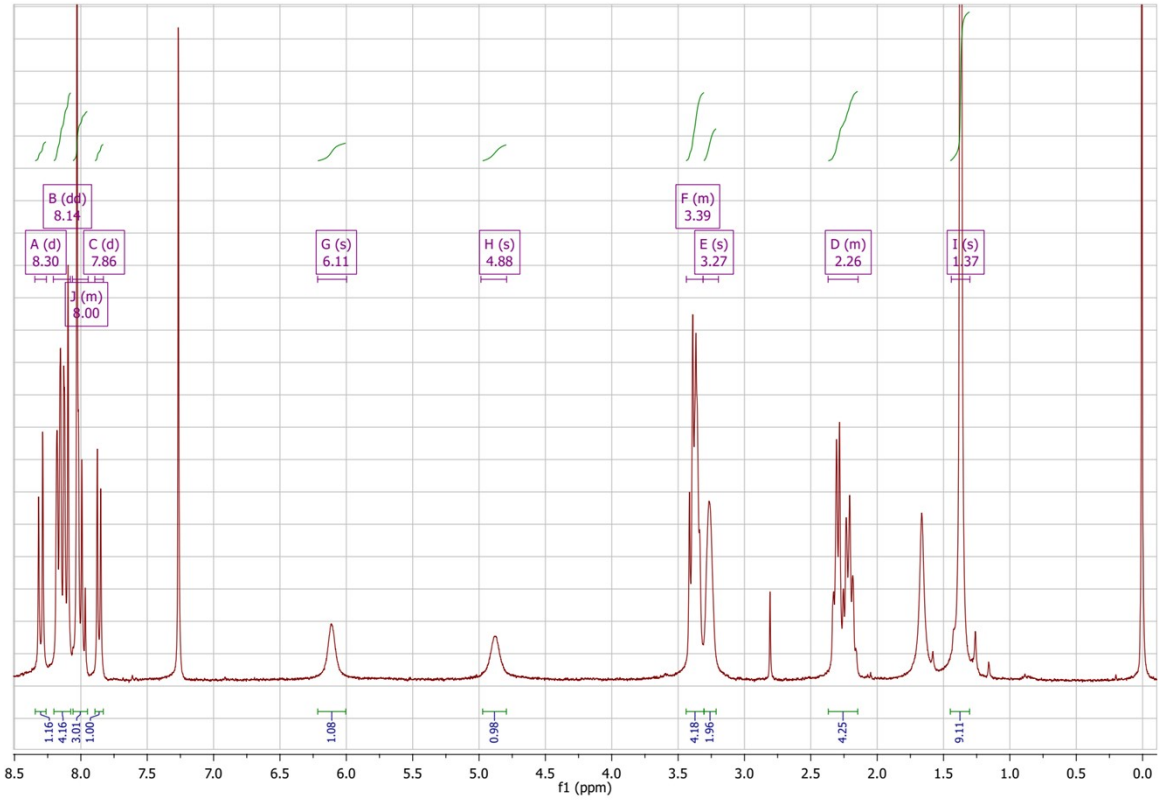


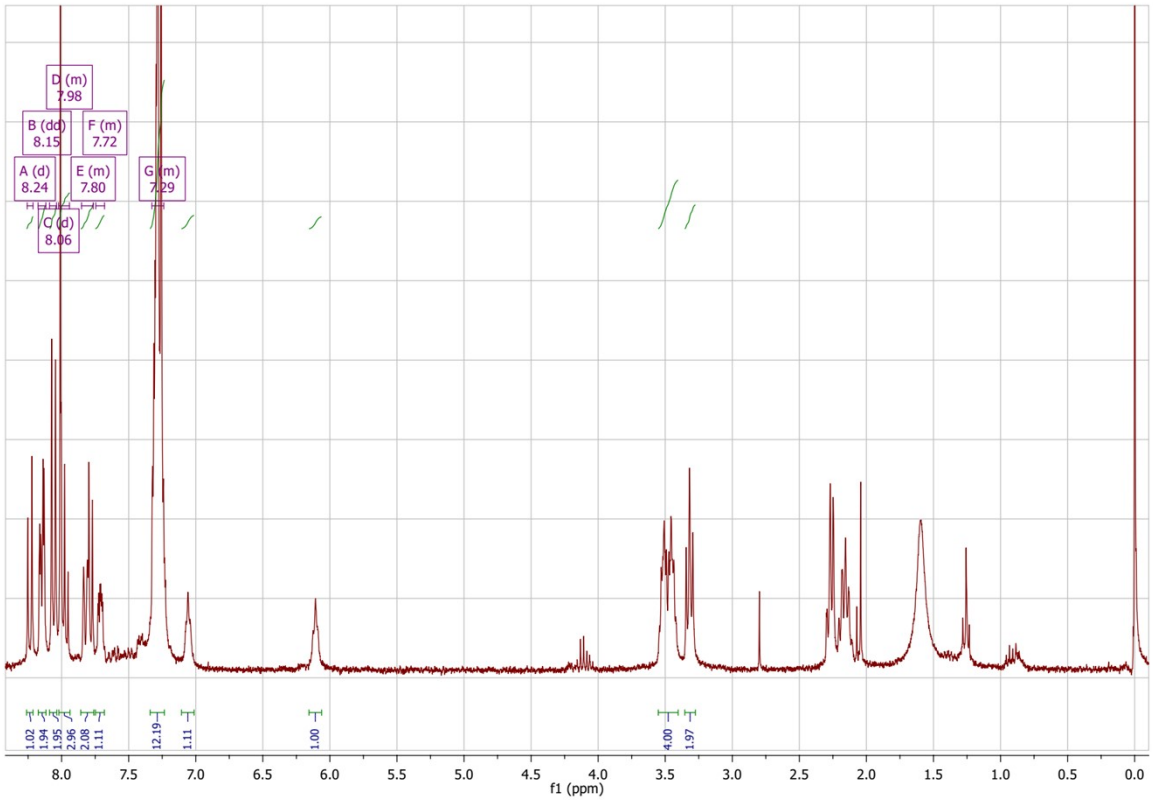
Figure s1 Compounds presented in this paper

NMR spectra

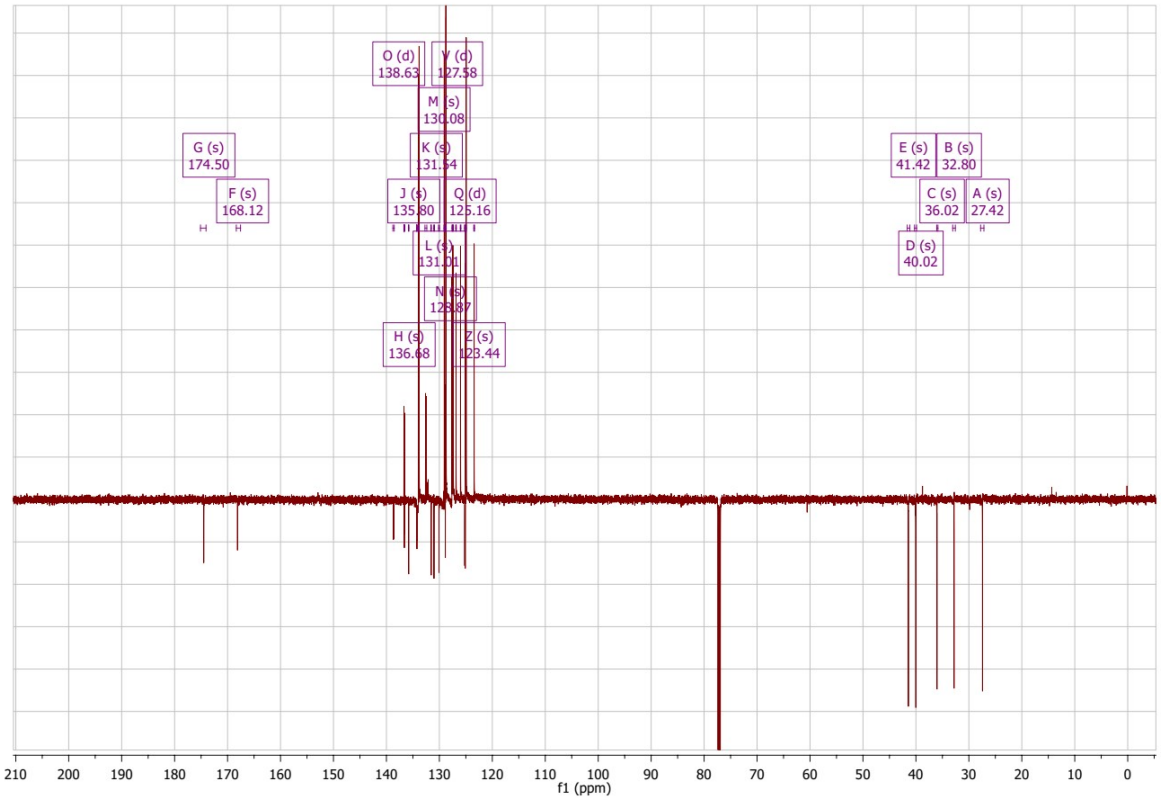
P ^{1}H NMR (300 MHz, $CDCl_3$)

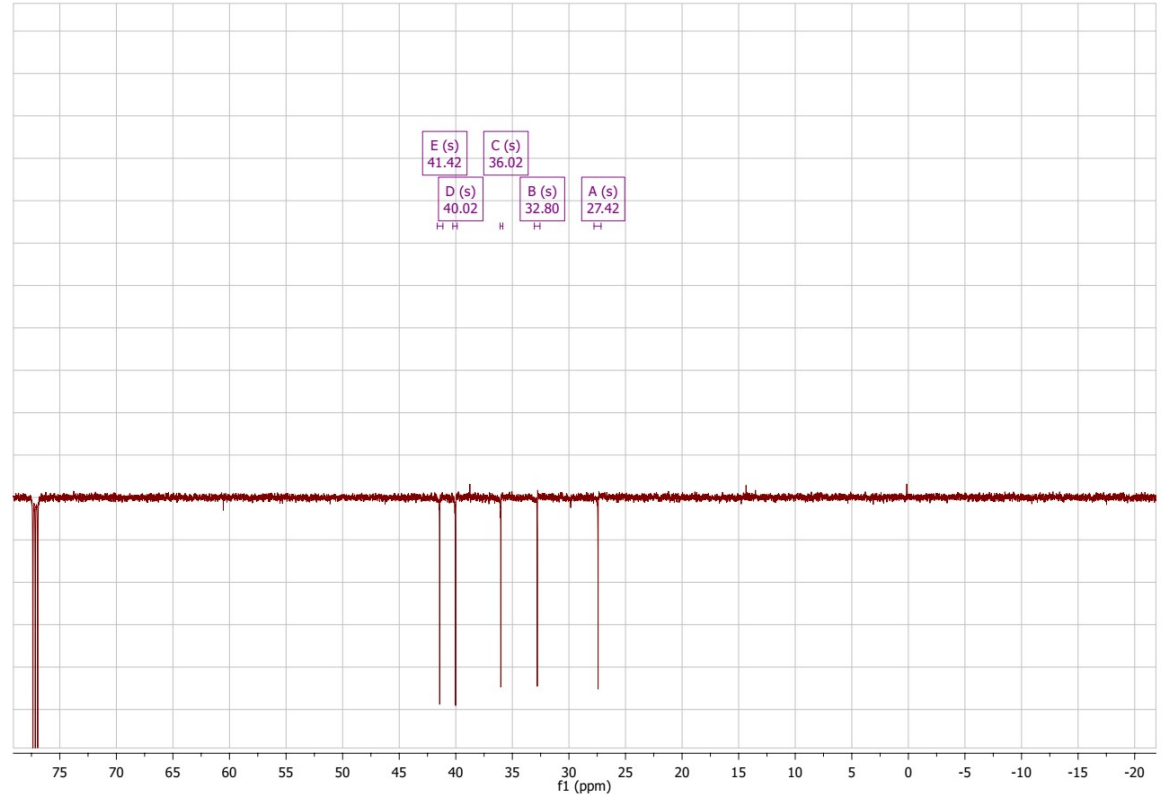


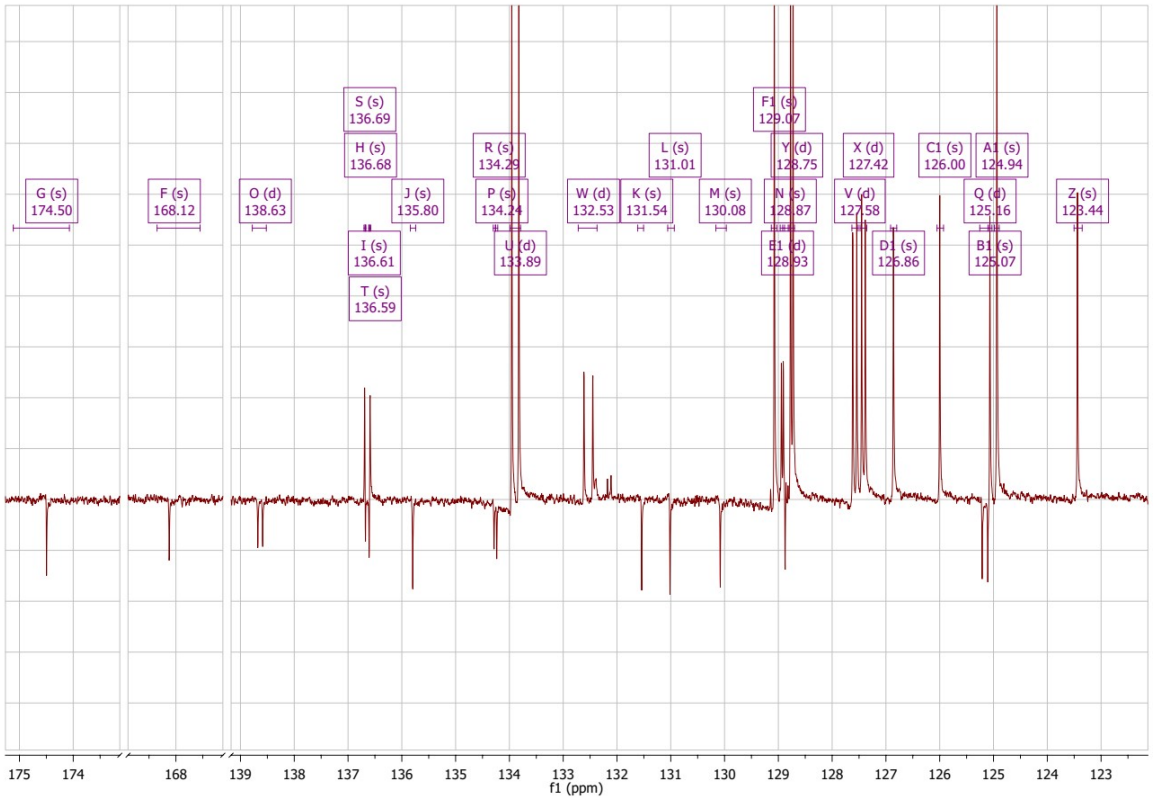
L ^{1}H NMR (300 MHz, $CDCl_3$)



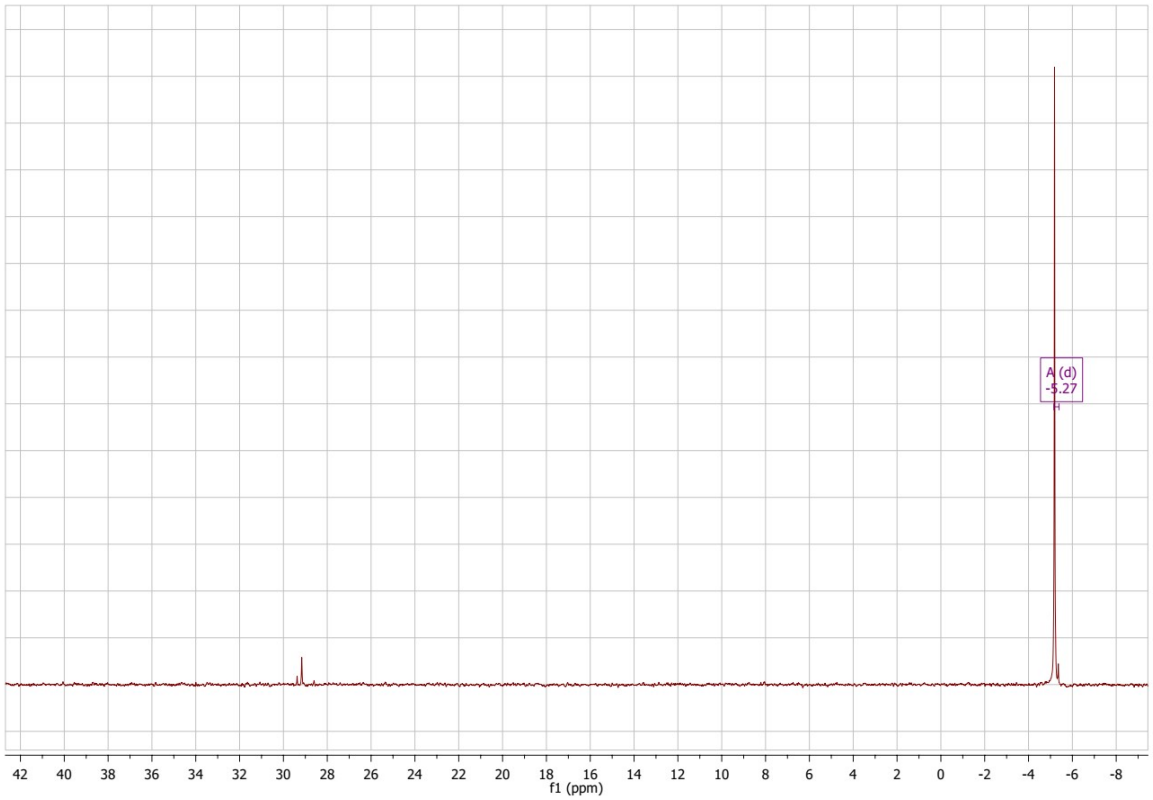
L APT NMR (151 MHz, $CDCl_3$)



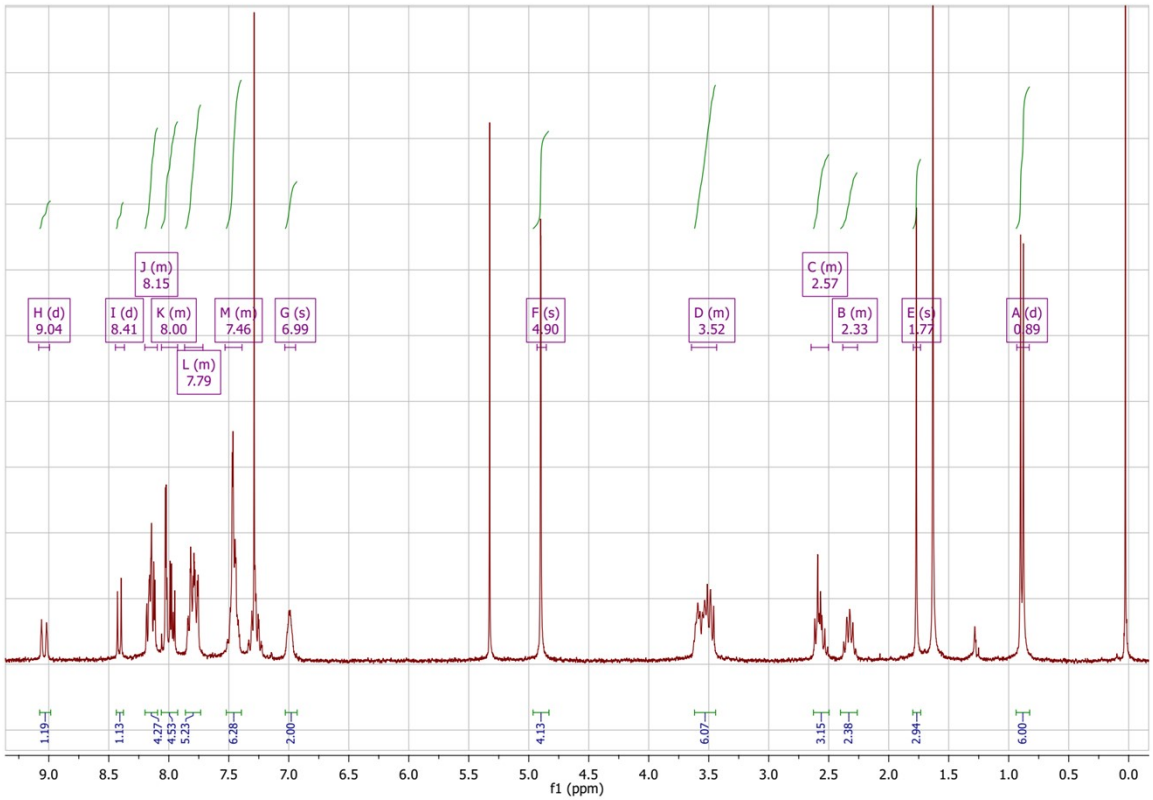




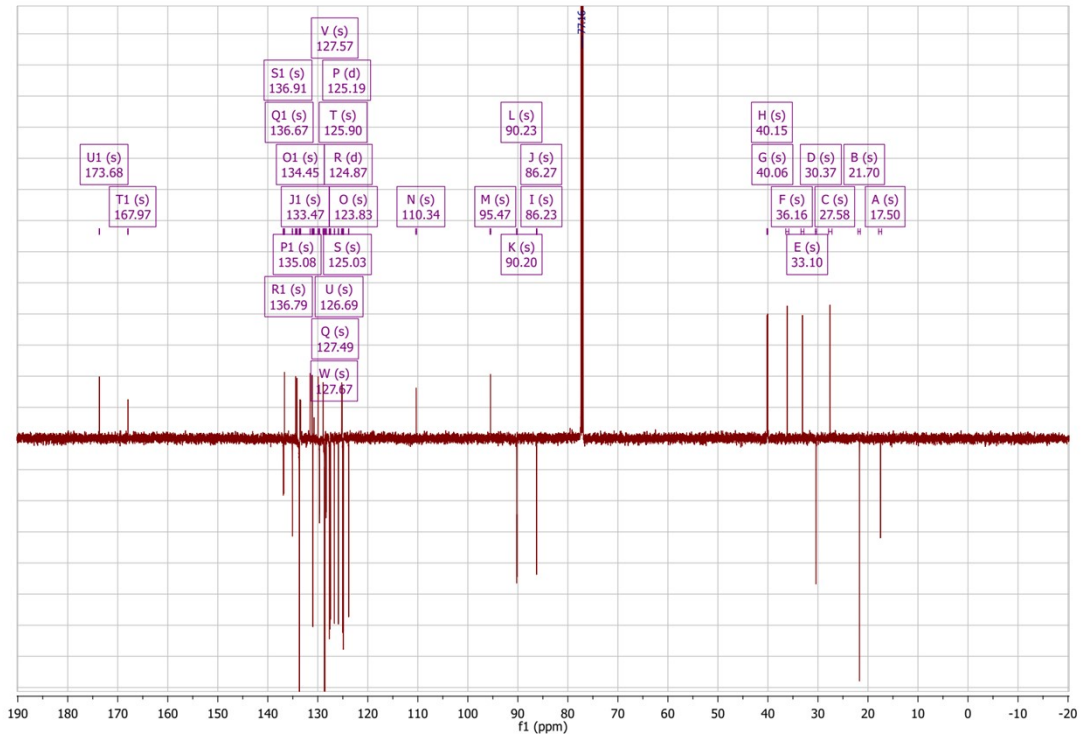
L ^{31}P NMR (243 MHz, $CDCl_3$)

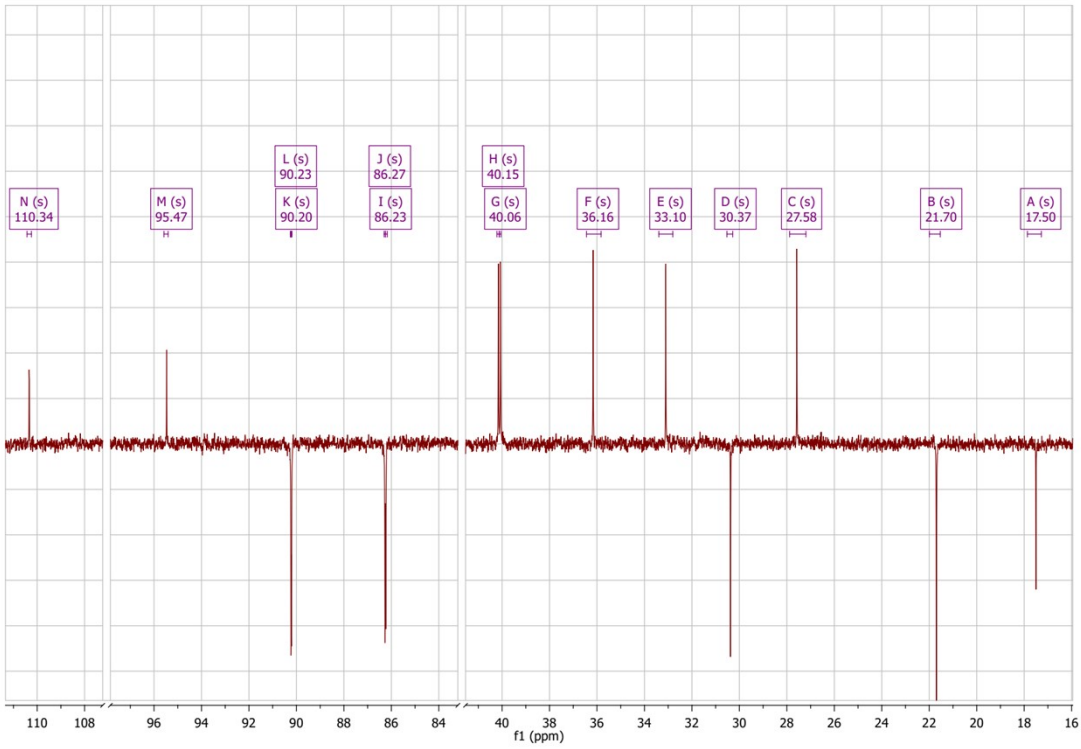


1 ^{1}H NMR (300 MHz, $CDCl_3$)



1 APT NMR (151 MHz, $CDCl_3$)

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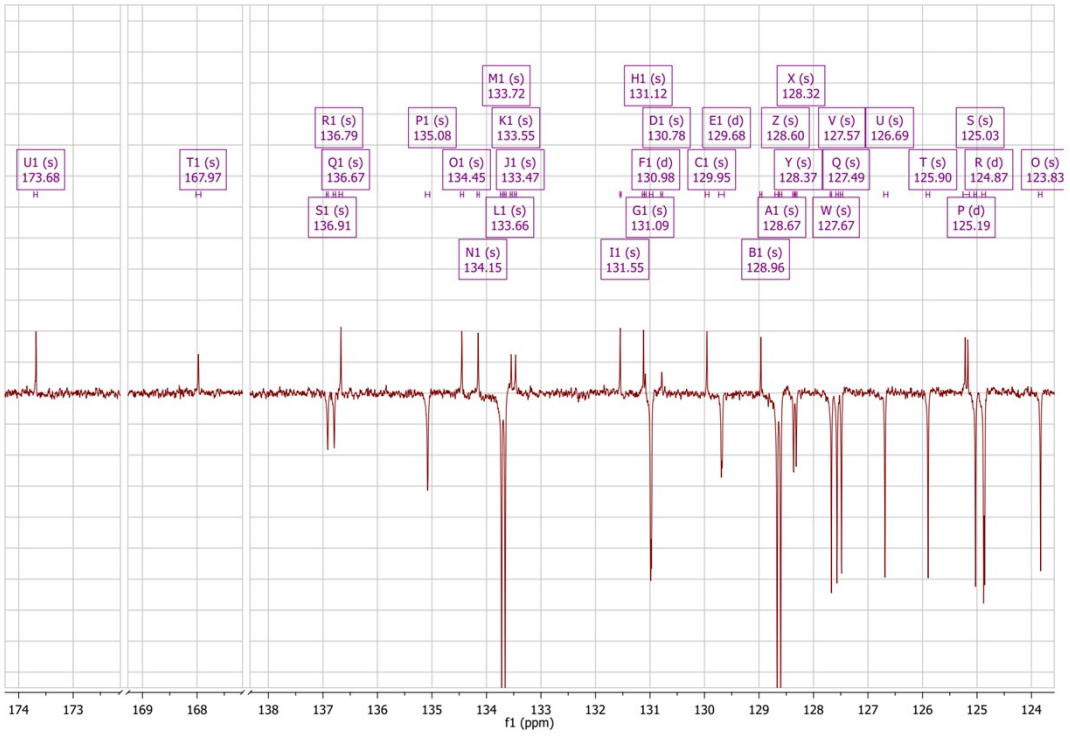
110.34
M (s)
95.47
L (s)
90.23
K (s)
90.20
J (s)
86.27
I (s)
86.23
H (s)
40.15
G (s)
40.06
F (s)
36.16
E (s)
33.10
D (s)
30.37
C (s)
27.58
B (s)
21.70
A (s)
17.50
f1 (ppm)


U1 (s)
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T1 (s)
167.97
R1 (s)
136.79
Q1 (s)
136.67
S1 (s)
136.91
P1 (s)
135.08
O1 (s)
134.45
M1 (s)
133.72
K1 (s)
133.55
J1 (s)
133.47
L1 (s)
133.66
N1 (s)
134.15
H1 (s)
131.12
D1 (s)
130.78
F1 (d)
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G1 (s)
131.09
I1 (s)
131.55
E1 (d)
129.68
C1 (s)
129.95
X (s)
128.32
Z (s)
128.60
Y (s)
128.37
A1 (s)
128.67
B1 (s)
128.96
V (s)
127.57
Q (s)
127.49
W (s)
127.67
U (s)
126.69
T (s)
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S (s)
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R (d)
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P (d)
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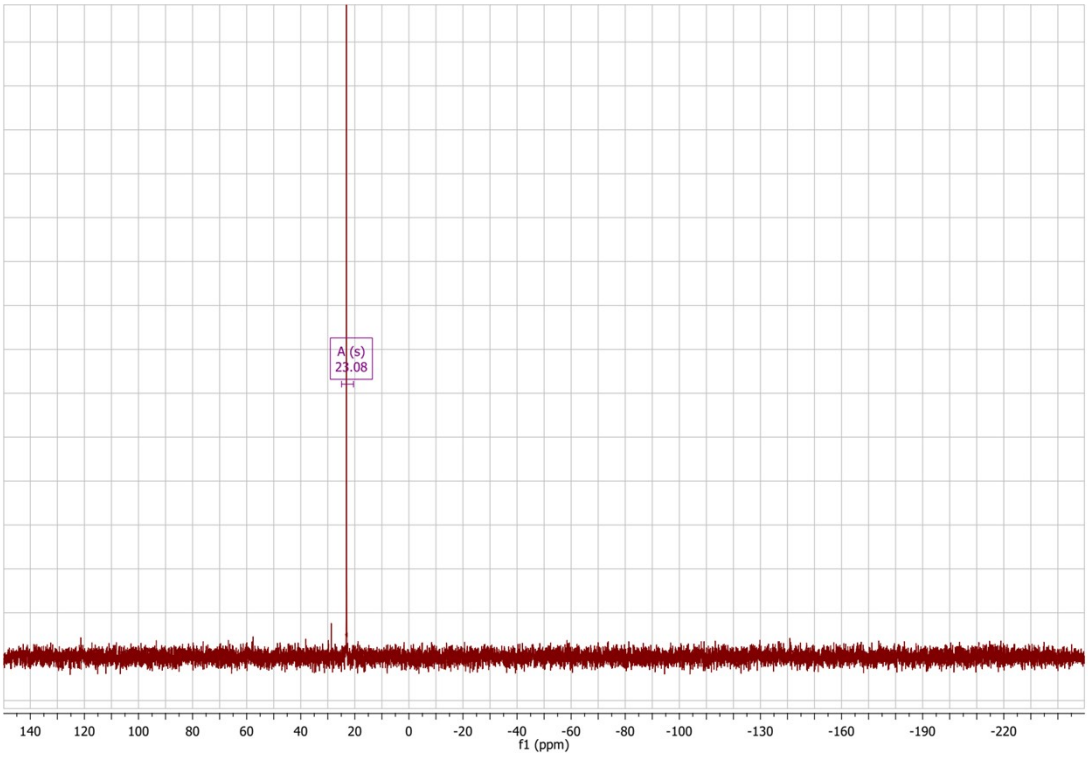
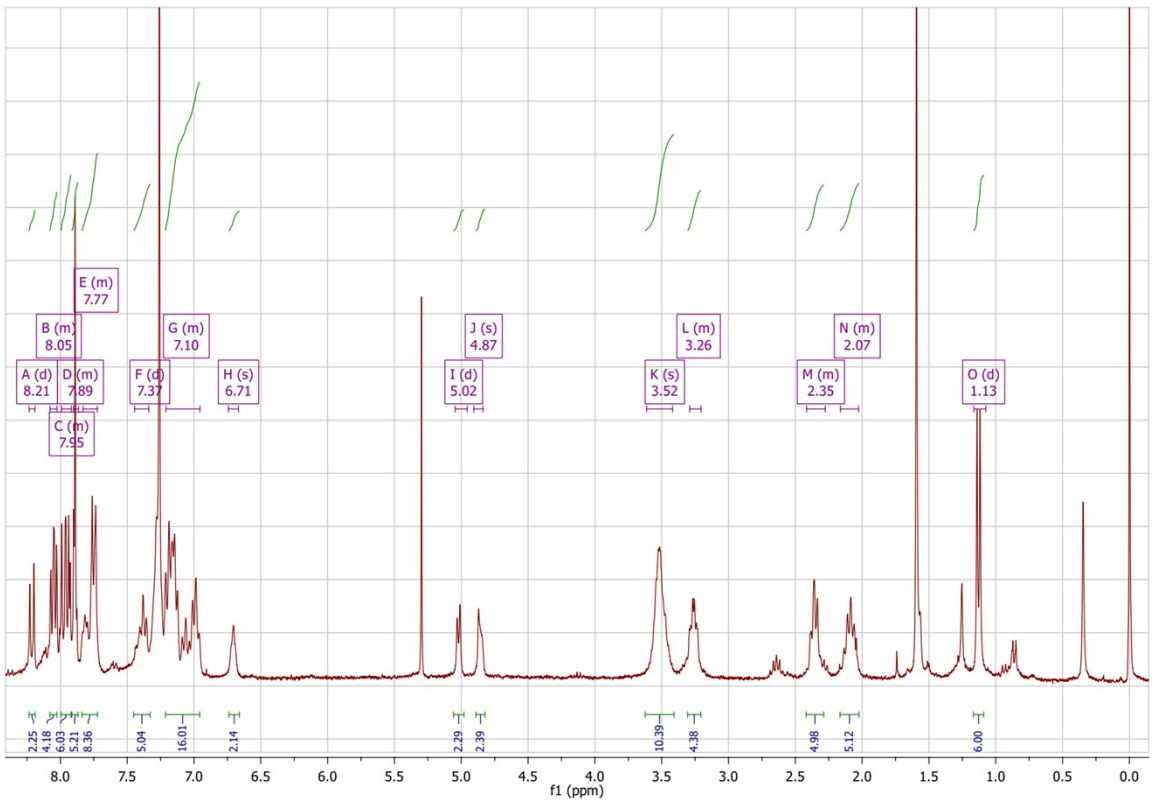
O (s)
123.83
f1 (ppm)

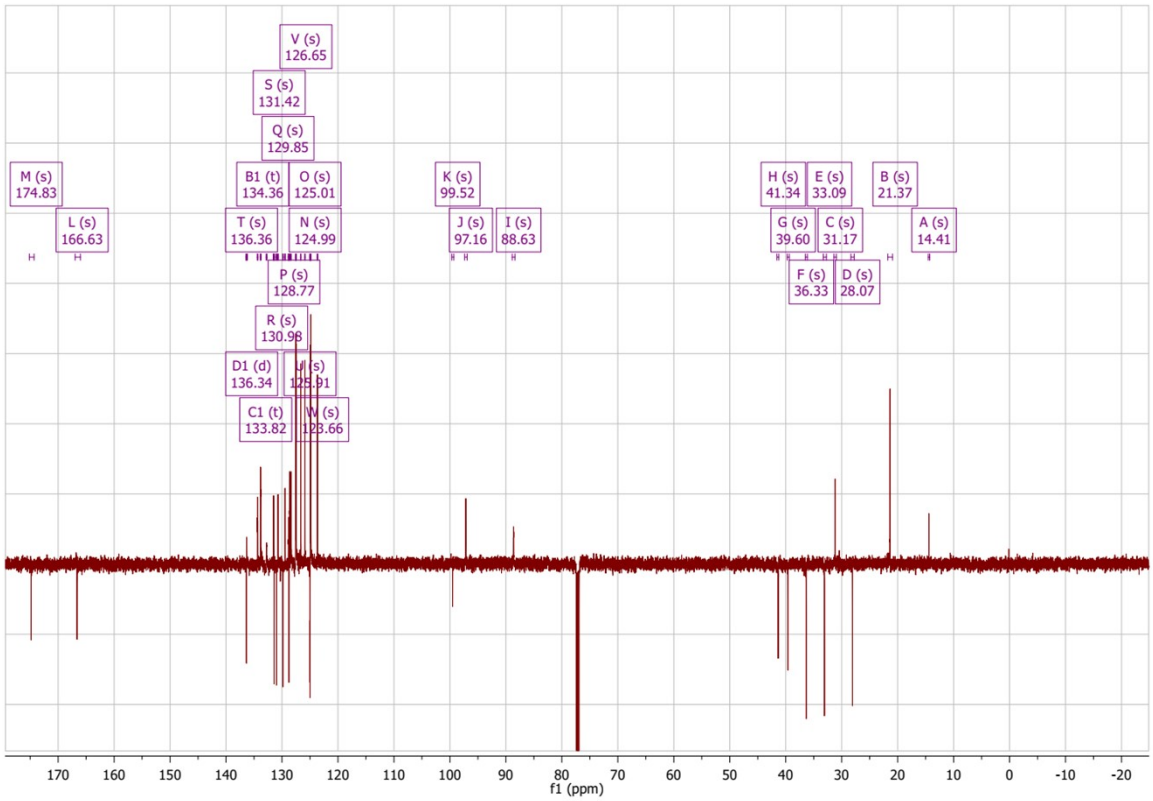
2 ^{31}P NMR (243 MHz, $CDCl_3$)

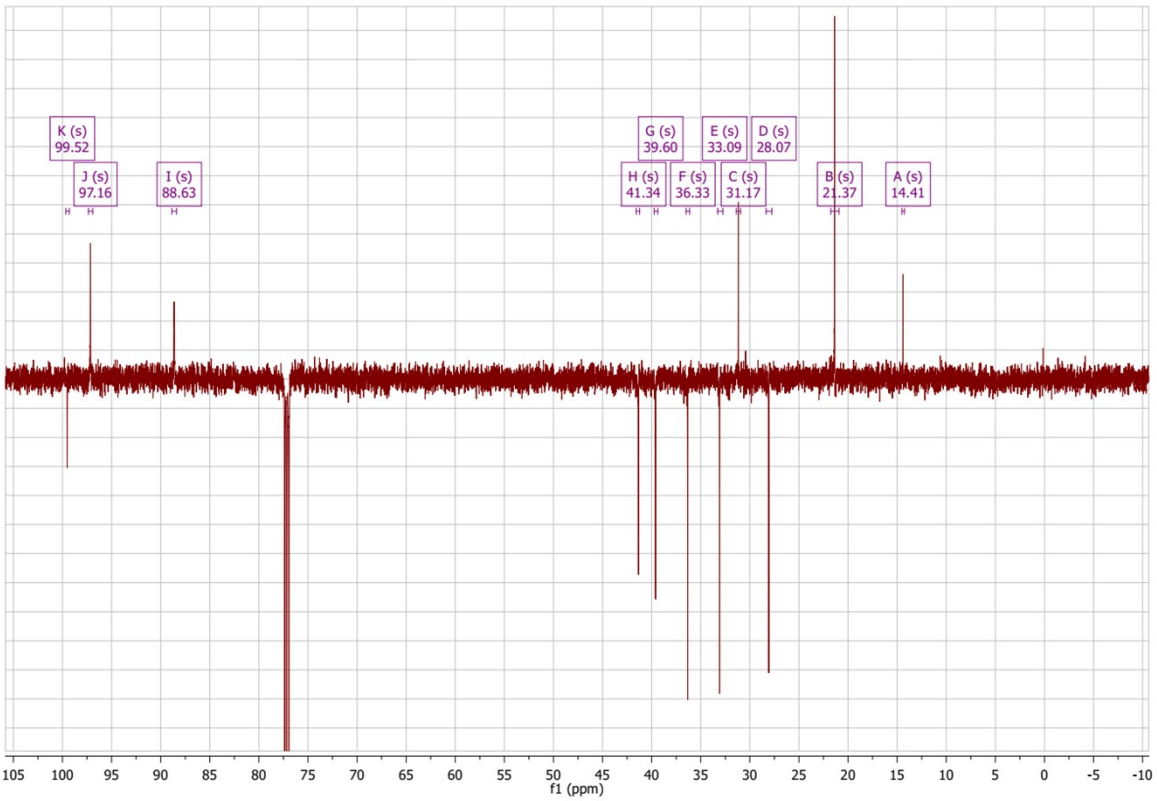


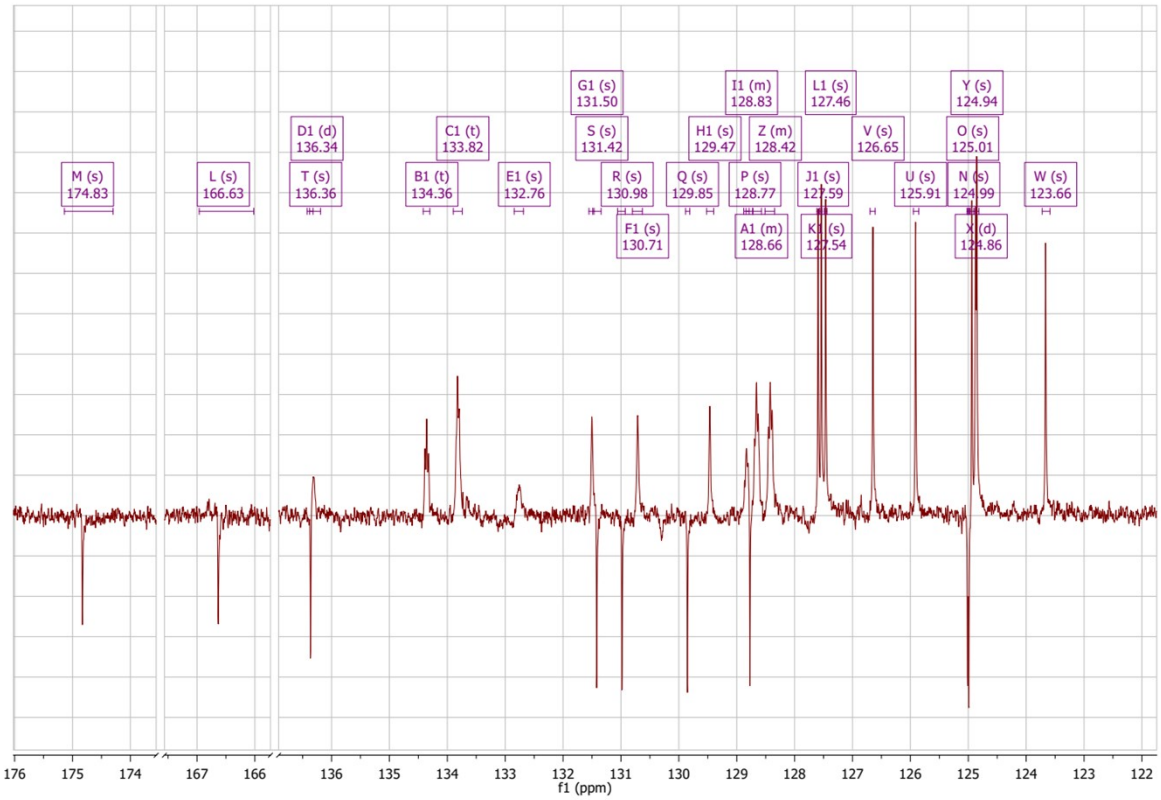
2 ^{1}H NMR (300 MHz, $CDCl_3$)



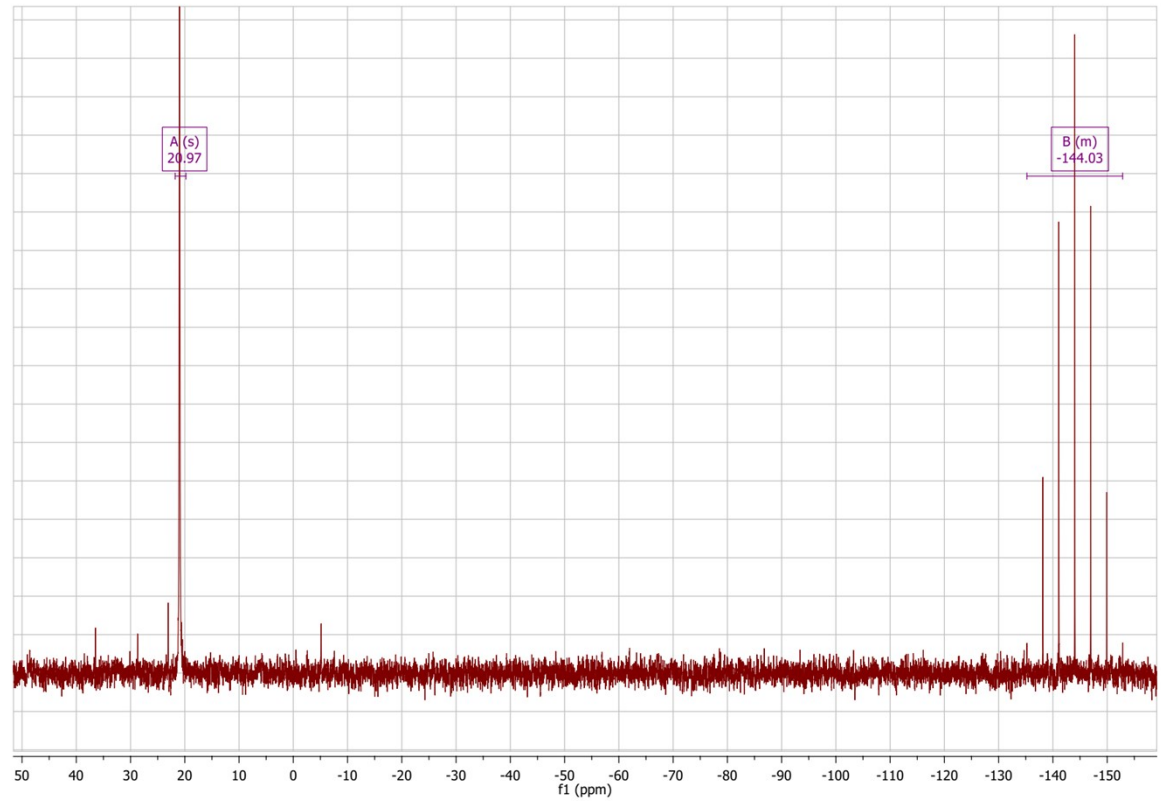
2 ^{13}C NMR (151 MHz, $CDCl_3$)







2 ^{31}P NMR (243 MHz, $CDCl_3$)



UV/Vis Experiments

Variable concentration (and temperature) spectra of **1** in water

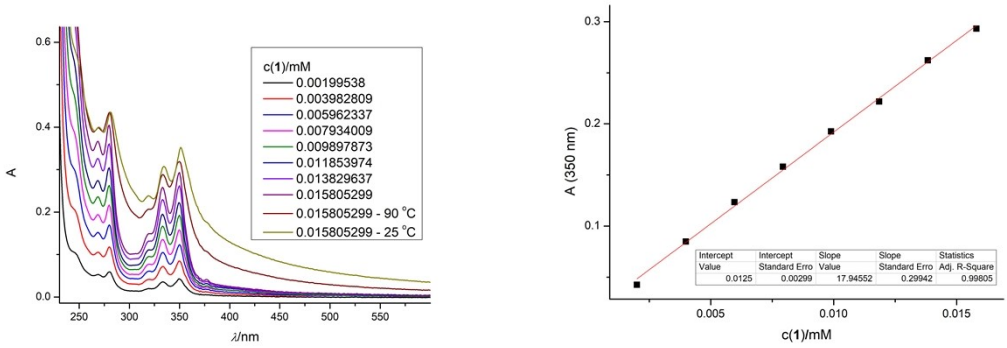


Figure s2: Left: A DMSO solution of **1** was added (**c(1)** =9.99x10^{-4}) to 1 ml of water, heating and cooling was done at the highest concentration measured, 1 cm path length, T = 25, additions of 2 µl °C. **Right:** Linear fit at 350 nm.

Variable concentration spectra od **1** in DMSO

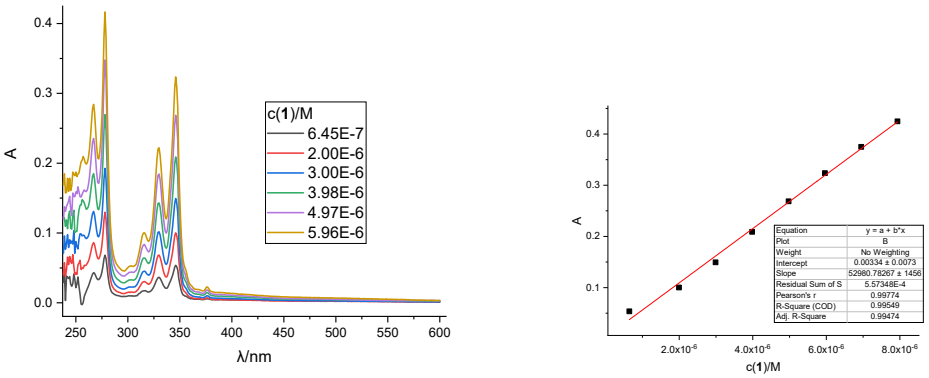


Figure s3: Left: A DMSO solution of **1** was added (**c(1)** =6.46x10^{-4}) to a 2 ml solution of DMSO, 1 cm path length, T = 25 °C. **Right:** Linear fit at 346 nm.

Variable concentration (and temperature) spectra of **2** in DMSO

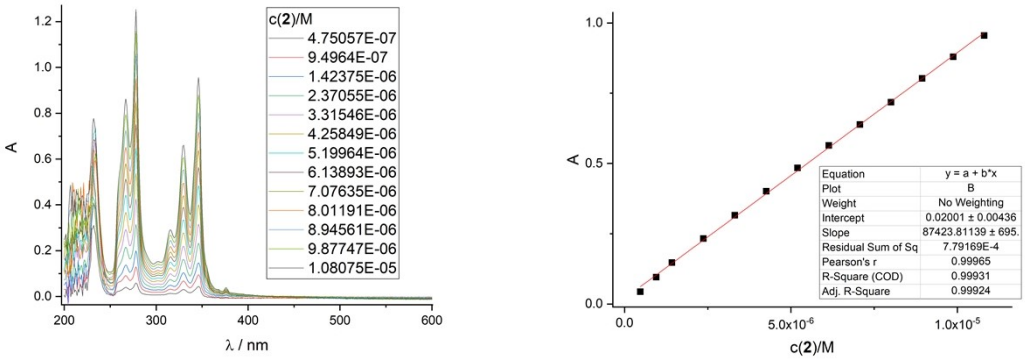


Figure s4: Left: A DMSO solution of **2** was added (**c(2)** =9.51x10^{-4}) to a 2 ml solution of DMSO, 1 cm path length, T = 25 °C. **Right:** Linear fit at 346 nm.

Variable concentration (and temperature) spectra of **2** in Na-cacodylate buffer

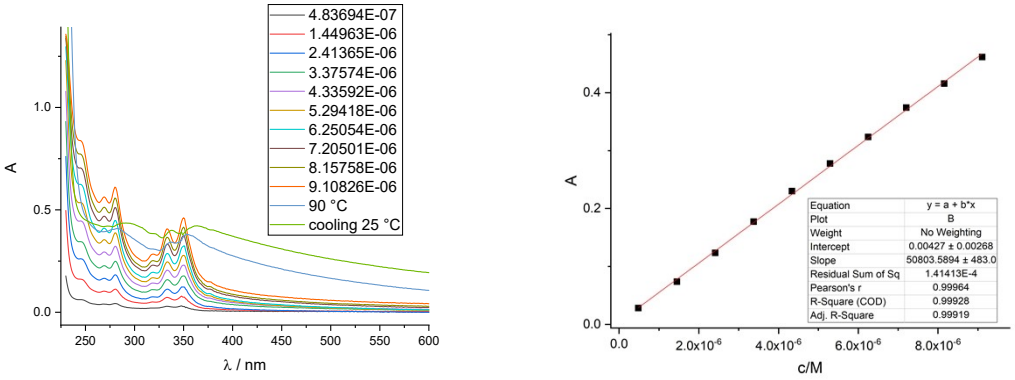


Figure s5: Left: A DMSO solution of **2** was added (**c(2)** =9.68x10^{-4}) to a 2 ml solution of Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), heating and cooling was done at the highest concentration measured, 1 cm path length, T = 25 °C. **Right:** Linear fit at 350 nm.

dTM measurements

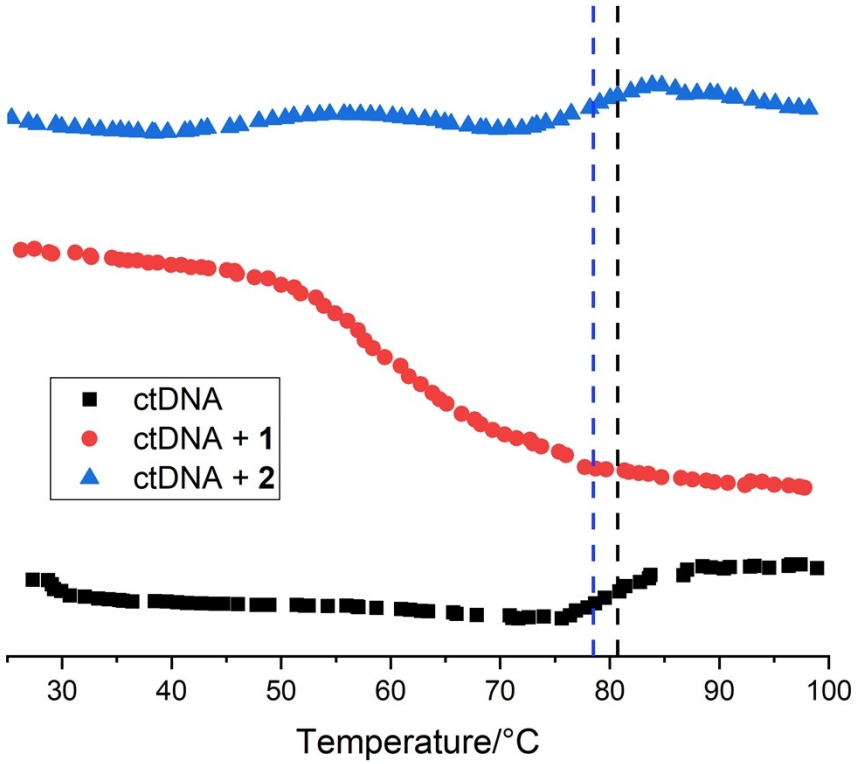


Figure s6 dTM measurements of ctDNA and mixtures of ctDNA with **1** and **2**. r(compound/ctDNA) = 0.3. c(ctDNA) = 2 x 10^{-5} M. Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), 1 cm path length. T_m(ctDNA) = 80.7 °C, T_m(ctDNA + **1**) could not be determined, T_m(ctDNA + **2**) = 78.5 °C.

Fluorescence experiments

Variable concentration spectra of **1** in DMSO

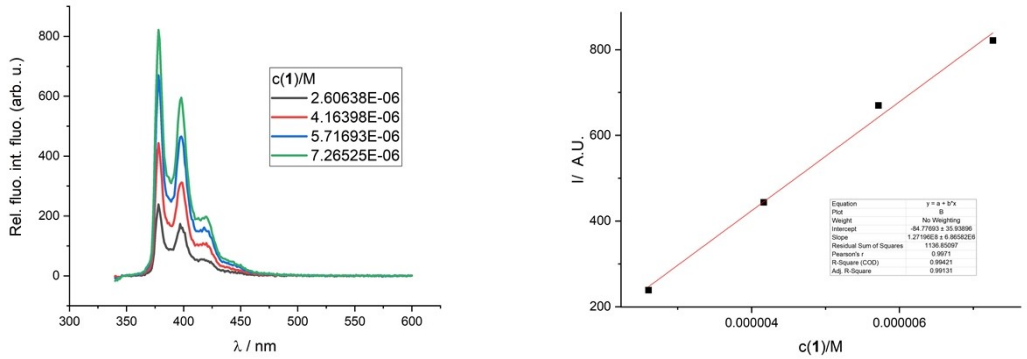


Figure s7: Left: A DMSO solution of **1** was added (**c(2)**=1.045x10^{-3}) to a 2 ml solution of DMSO, 1 cm path length, T = 25 °C, λ_{exc} = 340 nm, additions of 3-5 µl. **Right:** Linear fit at 378 nm.

Excitation spectra of **1**

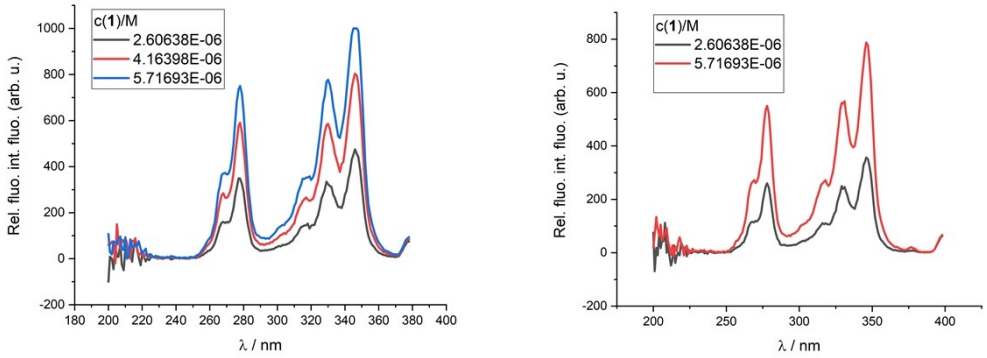


Figure s8: Excitation spectra of **1**. A DMSO solution of **1** was added (**c(1)**=1.045x10^{-3}) to a 2 ml solution of DMSO, 1 cm path length, T = 25 °C, , additions of 3-5 µl.**Left:** λ_{em} = 378 nm **Right:** λ_{em} = 398 nm.

Variable concentration (and temperature) spectra of **2** in DMSO

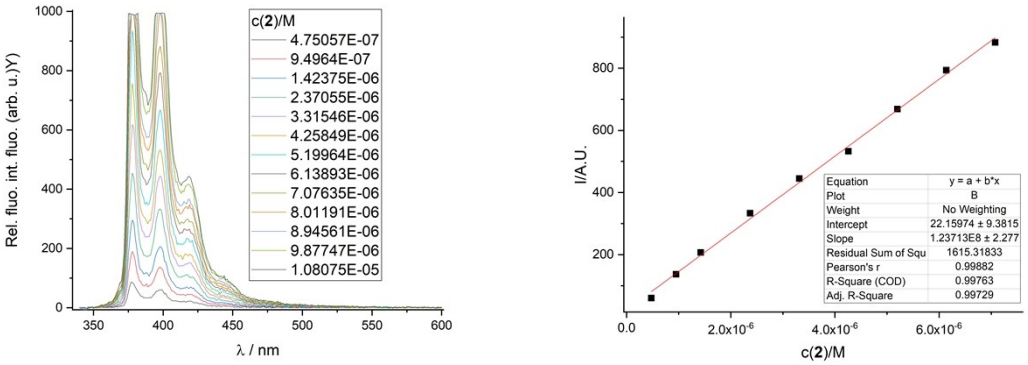


Figure s9: Left: A DMSO solution of **2** was added (**c(2)**=9.51x10^{-4}) to a 2 ml solution of DMSO, 1 cm path length, T = 25 °C, λ_{exc} = 340 nm. **Right:** Linear fit at 398 nm.

Variable concentration (and temperature) spectra of **2** in Na-cacodylate buffer

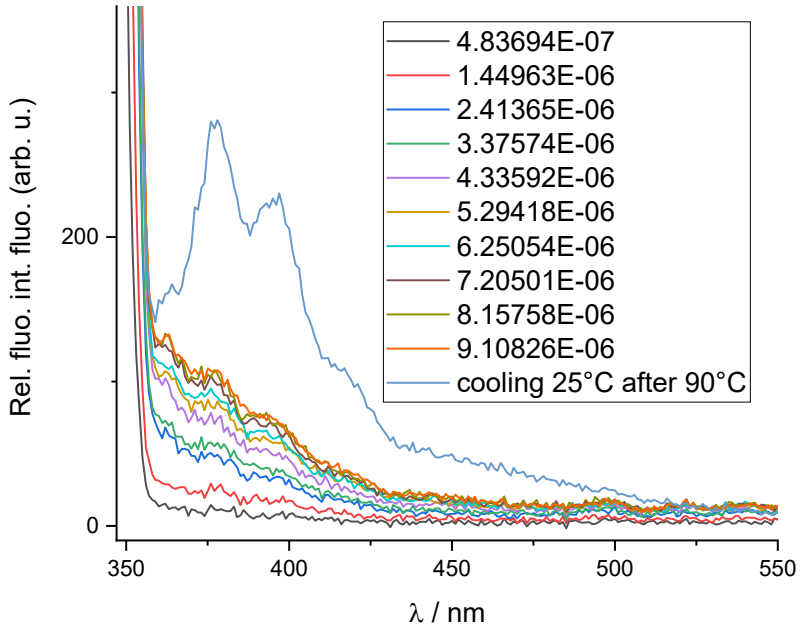


Figure s10: 2 was added (**c(2)** =9.68x10^{-4}) to a 2 ml solution of Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), λ_{exc} = 340 nm, 1 cm path length, T = 25 °C.

BSA titration of **1**

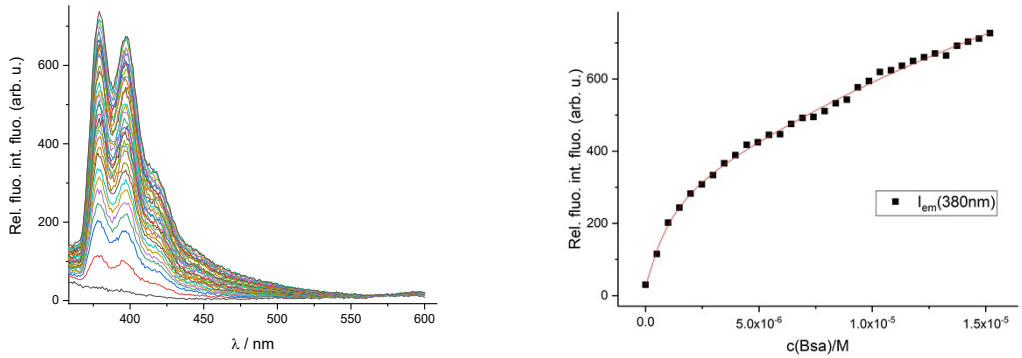


Figure s11 Left: Fluorimetric titration of **1** with BSA (c(BSA) = 1 mM)**,** M, $\mathbf{c_0(1)}$ = 5.23x10^{-6}, λ_{exc} = 340 nm, Na-cacodylate buffer (pH = 7.05, I = 0.05 mol dm^{-3}), 1 cm path length, T = 25 °C, BSA additions of 1 µl, spectra are corrected for dillution. **Right:** Changes in fluorescence of **1** at 380 nm during the titration.

BSA titration of **2**

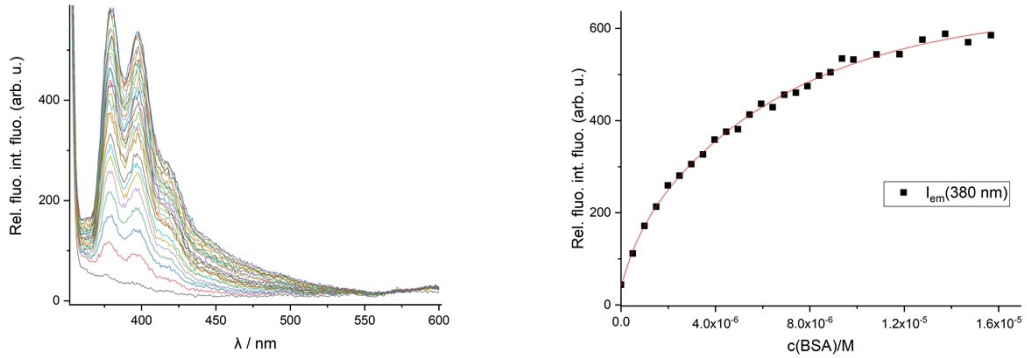


Figure s12 Left: Fluorimetric titration of **2** with BSA (c(BSA) = 1 mM)**,** M, $\mathbf{c_0(2)}$ =4.64x10^{-6}, λ_{exc} = 340 nm, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), 1 cm path length, T = 25 °C, BSA additions of 1-2 µl, spectra are corrected for dillution. **Right:** Changes in fluorescence of **2** at 380 nm during the titration.

Time resolved fluorescence measurements

For **1** and **2** in NaCaco, only samples with BSA added were successfully measured, samples without BSA had very low fluorescence.

Table s1. Measured relaxation times τ and quantum yields for **1**, **2** and **L**.

Compound (solvent)	λ_{max}/ nm	ε / $M^{-1}cm^{-1}$	Φ_f[a]	λ_{em}/nm[b]	τ / ns (non-degassed)	χ2	τ / ns (degassed)[c]	χ2
1 (DMSO)	346	52981	0.17	-	-	-	-	-
1 (H_2O)	350	17945	-	-	-	-	-	-
1+BSA (NaCaco)	-	-	-	419	12.9 (17%) 41.6 (31%) 181.5 (53%)	1.084	15.3 (61%)[d] 123.7(39%)	1.539
2 (DMSO)	346	87723	0.18	-	-	-	-	-
2 (H_2O)	350	50766	-	-	-	-	-	-
2+BSA (NaCaco)	-	-	-	419	27.2(36 %) 169.1(64%)	1. 194	22.1(51%) 130.8(49%)	1.165
L (NaCaco)	350	28640	0.61	470	-	-	32.2 (43%) 72.6 (57%)	1.003
A[1] **(NaCaco)**	342	62596	0.15	377, 398, 418	94.0 (100%)	1.068	2.5 (1%) 100.3(99.2%)	1.060

[a] Absolute fluorescence quantum yield was determined by integrating sphere SC-30, Edinburgh Inst., for Argon purged solutions, by λ_{exc} = 340 nm [b] Pulsing diode excitation at 340 nm. [c] Degassed by ultrasonic bath for 30 min,[d] the values could not be determined reliably.

1 R. J. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New york, 3rd edn., 2006.

ctDNA titration of **1**

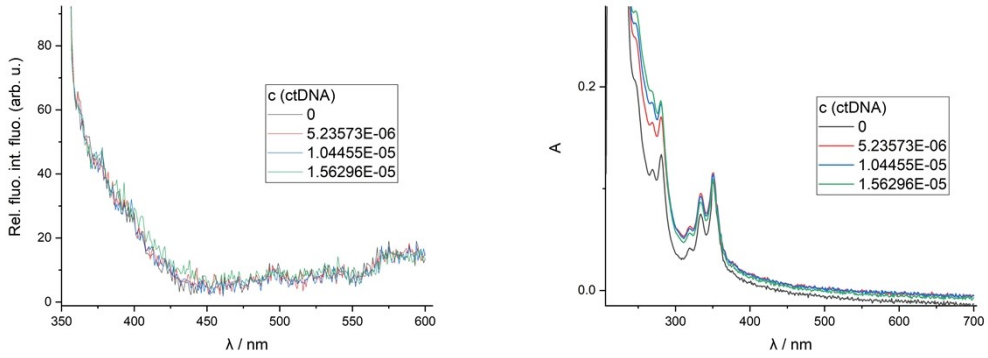


Figure s15 Left: Fluorimetric titration of **1** with ctDNA**,** c(ctDNA) = 2.11x10^{-3} M, $\mathbf{c_0(1)}$ =4.89x10^{-6}, λ_{exc} = 340 nm, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), 1 cm path length, T = 25 °C, additions of 5 µl, spectra are not corrected for dillution. **Right:** Corresponding UV spectra.

ctDNA titration of **2**

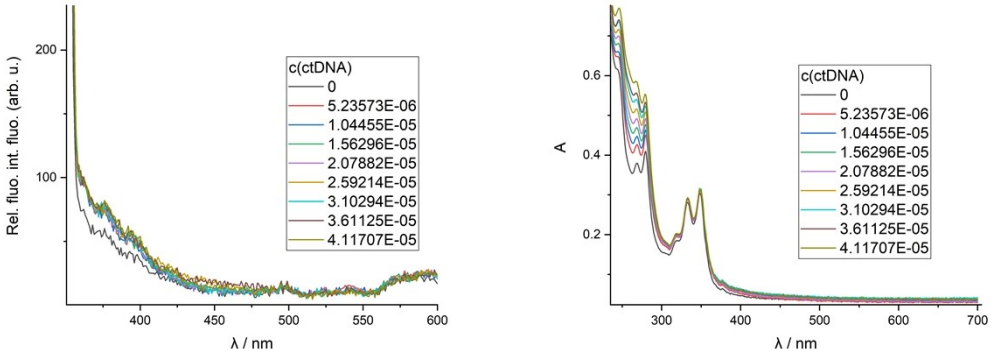


Figure s16 Left: Fluorimetric titration of **2** with ctDNA**,** c(ctDNA) = 2.11x10^{-3} M, $\mathbf{c_0(1)}$ =4.92x10^{-6}, λ_{exc} = 340 nm, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), 1 cm path length, T = 25 °C, additions of 5 µl, spectra are not corrected for dillution. **Right:** Corresponding UV spectra.

Competition experiments ES283 titration of ctDNA + ethidium bromide

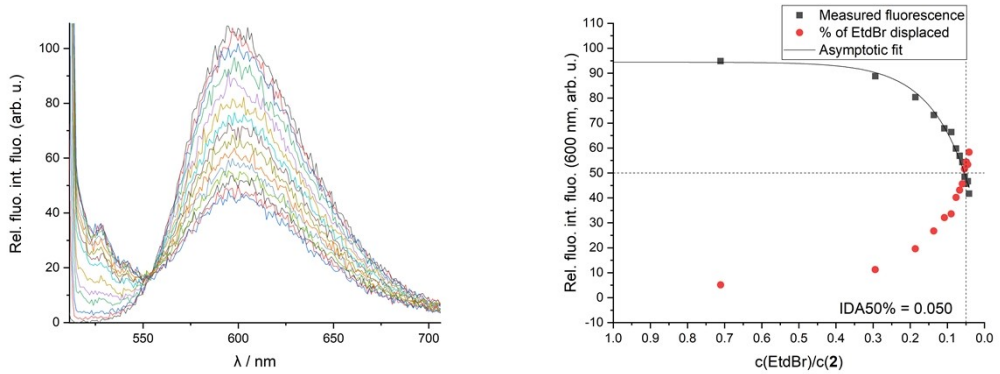


Figure S17 Left: Ethidium Bromide displacement assay, λ_{exc} = 505 nm, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), c_0(ctDNA) = 4.99 x10^{-5} M, c_0(EtdBr) = 4.96x10^{-6} M, 1 cm path length, T = 25 °C, c (**2**) = 2.02 x 10^{-3} M – additions of 2-10 µl, baseline was subtracted and the spectra were corrected for dilution. **Right:** Changes in fluorescence of EtdBr at 600 nm during the titration, IDA_{50}(**2**) = 0.050.

Competition experiments ES282 titration of ctDNA + ethidium bromide

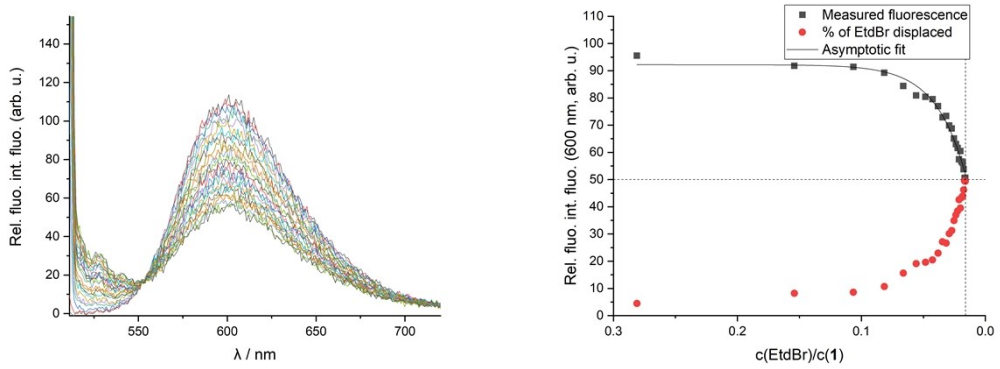


Figure S18 Left: Ethidium Bromide displacement assay, λ_{exc} = 505 nm, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), c_0(ctDNA) = 5.27x10^{-5} M, c_0(EtdBr) = 4.96x10^{-6} M, 1 cm path length, T = 25 °C, c (**1**) = 2.98 x 10^{-3} M – additions of 2-10 µl, baseline was subtracted and the spectra were corrected for dilution. **Right:** Changes in fluorescence of EtdBr at 600 nm during the titration, IDA_{50}(**1**) = 0.016.

CD spectroscopy

Titration of 1 with ctDNA

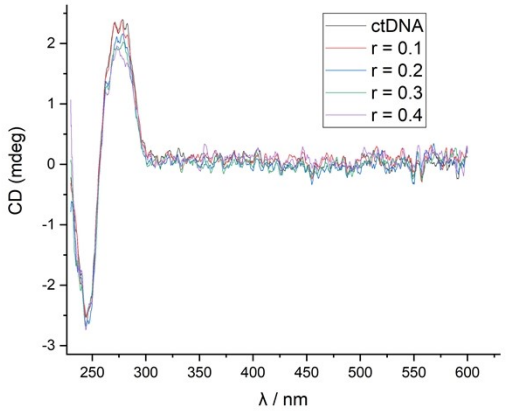


Figure s19 Titration of ctDNA with **1.** c_0(ctDNA)=1.95 x 10^{-5}, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), 1 cm path length, T = 25 °C, baseline was subtracted from each spectrum.

Titration of 2 with ctDNA

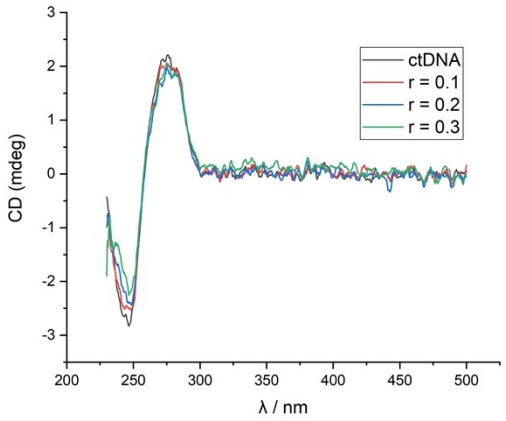


Figure s20 Titration of ctDNA with **2.** c_0(ctDNA)= 1.97 x 10^{-5}, Na-cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}), 1 cm path length, T = 25 °C, baseline was subtracted from each spectra.

Biology

Toxicity on HeLa cells

Compound	HeLa		Fibroblasts
	IC_{50} (µM) ± SD	**IC_{50} (µM) ± SD λ = 300 nm**	**IC_{50} (µM) ± SD**
1	5.13 ± 1.10	3.24±0.4	12 ± 2 .13
2	12.50 ± 0.50	8.75±1.8	11.8 ± 1.1

Table s2. IC_{50} values of compounds **1** and **2** measured on HeLa cells with and without 300 nm irradiation and on fibroblasts.