Synthesis and DNA Cleavage Activity of Triazacrown-anthraquinone Conjugates

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1. ESI–MS and NMR spectra

ESI–MS and NMR spectra of 1

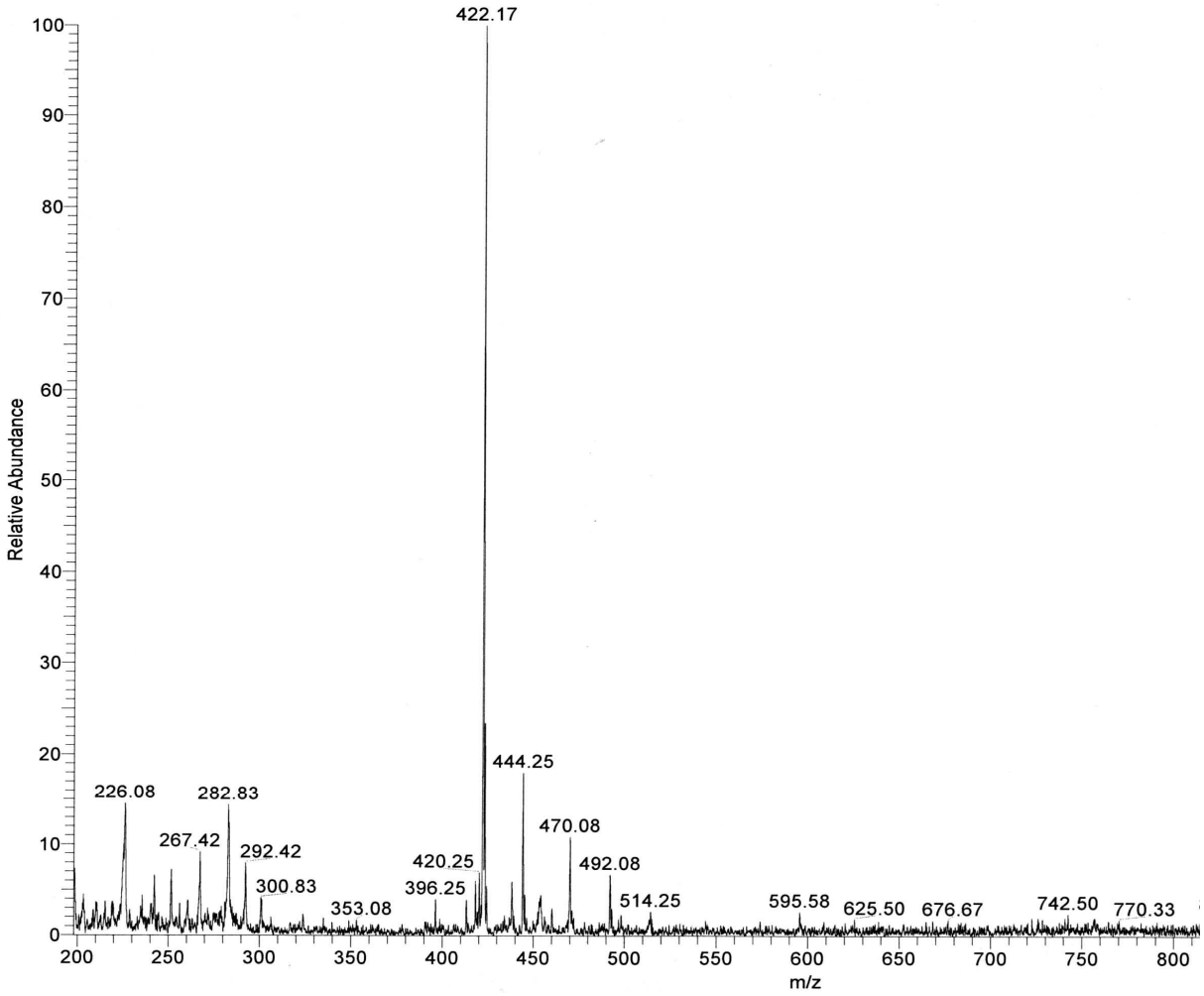


Figure S1. ESI–MS spectra of **1**

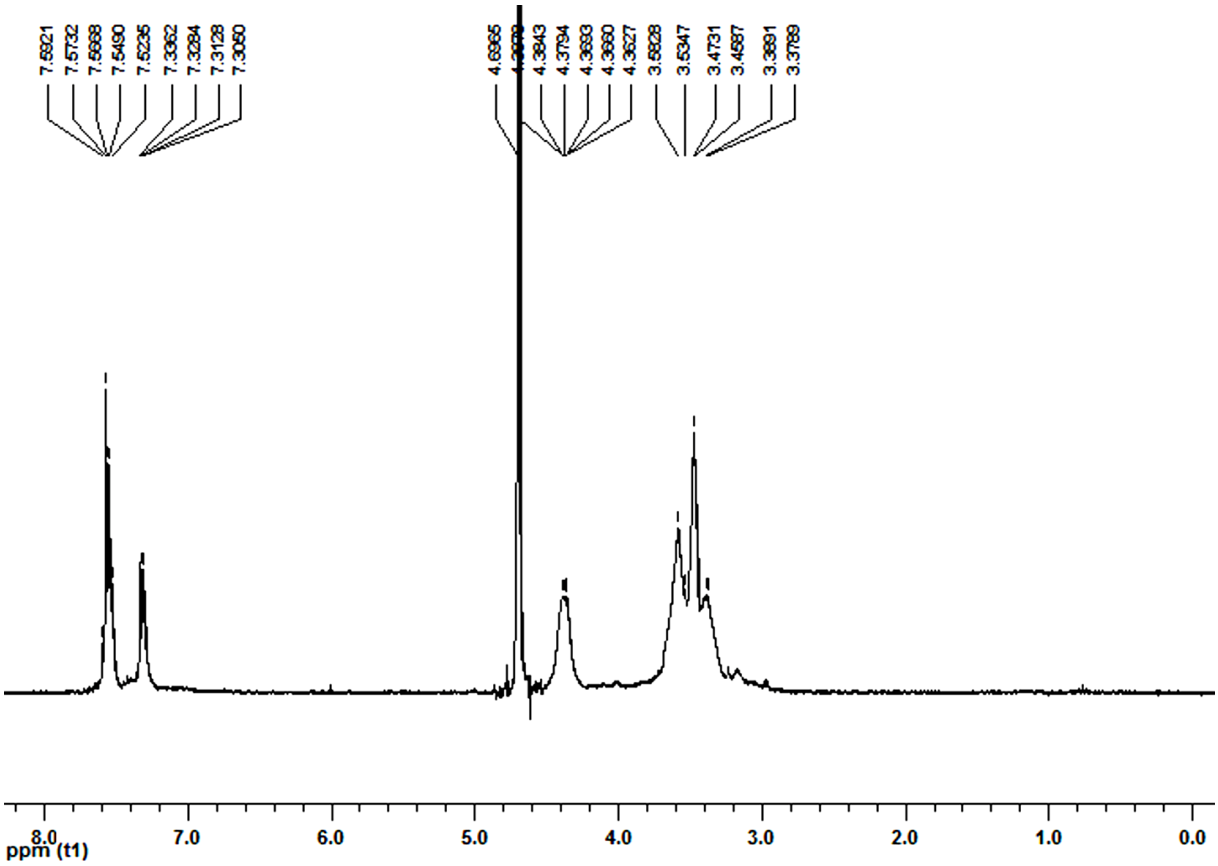


Figure S2. ^{1}H NMR (300 MHz, D_2O) of **1**

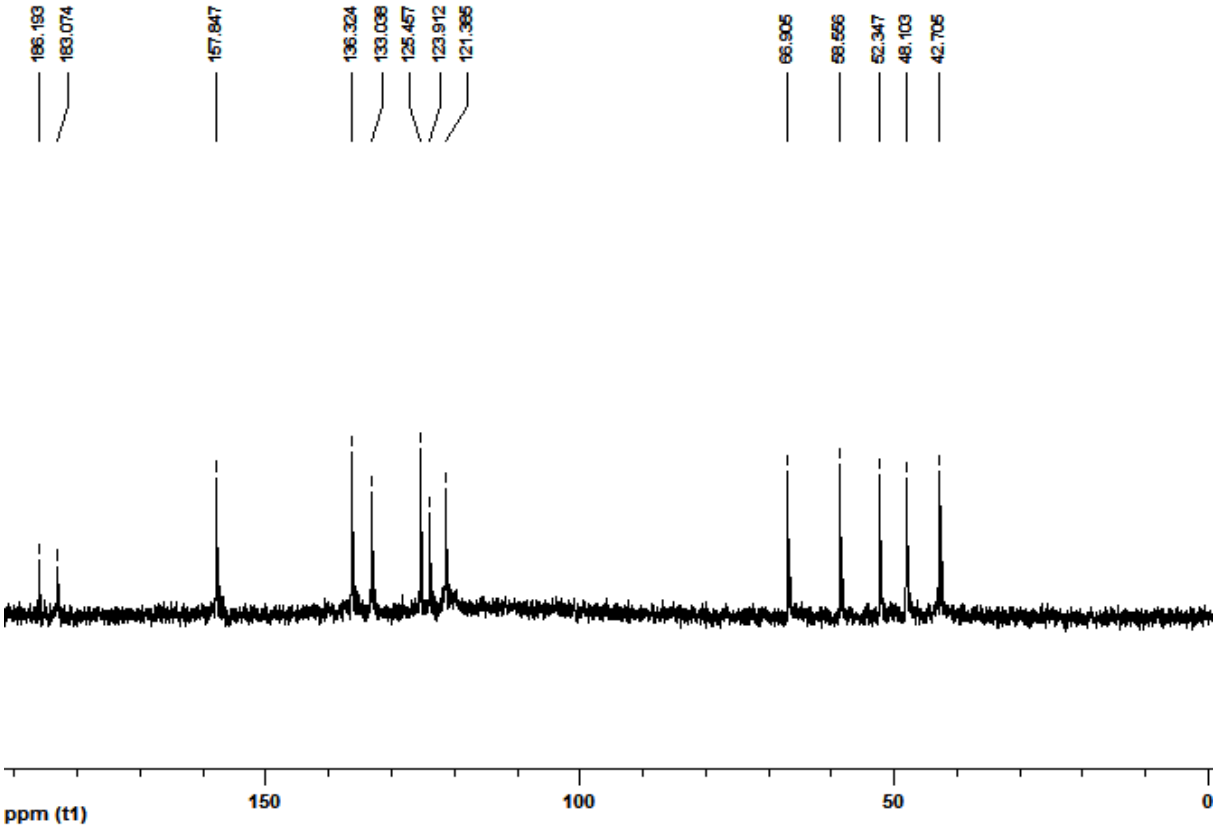


Figure S3. ^{13}C NMR (300 MHz, D_2O) of **1**

ESI–MS and NMR spectra of 2

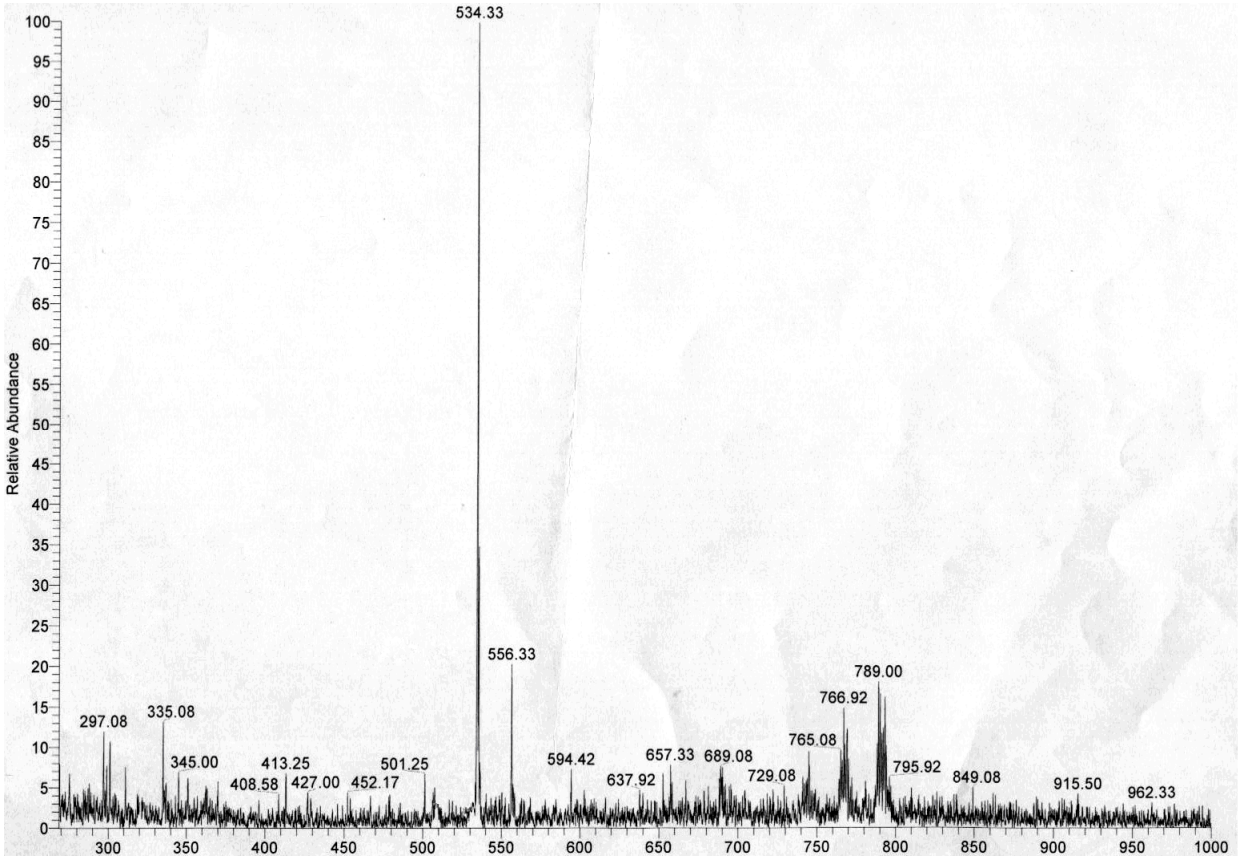


Figure S4. ESI–MS spectra of **2**

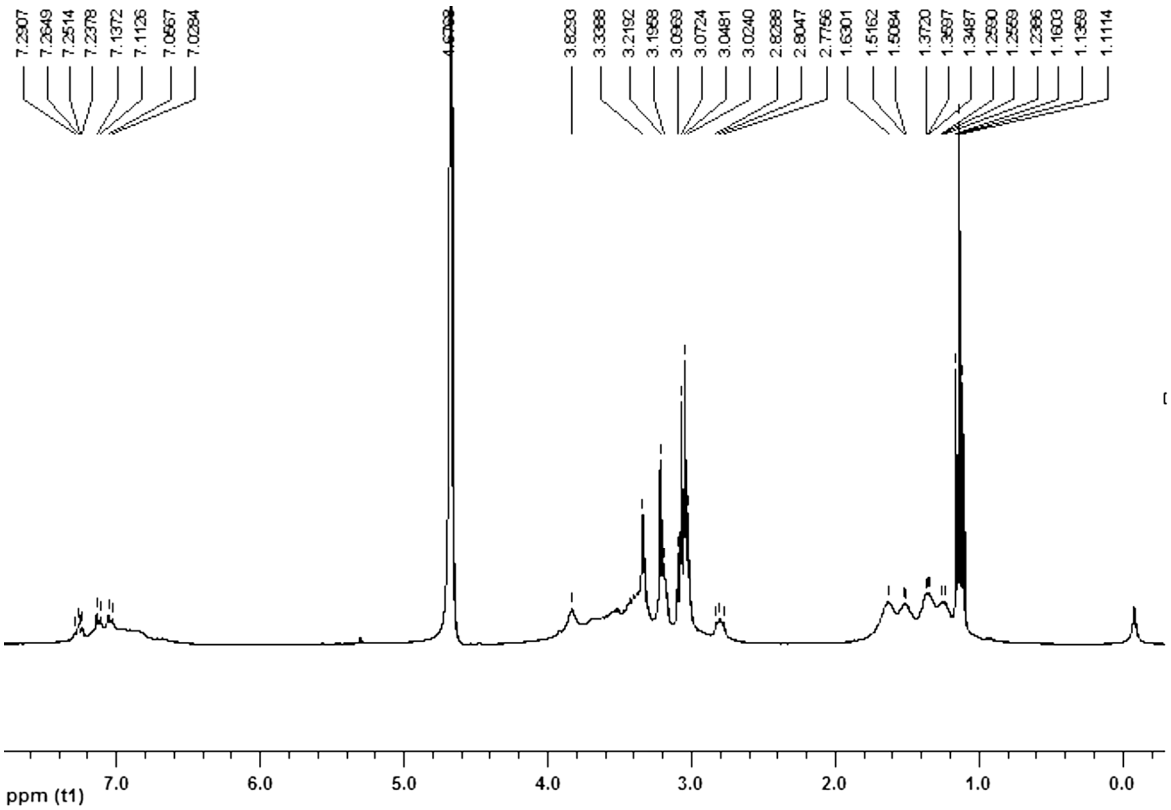


Figure S5. ^{1}H NMR (300 MHz, D_2O) of **2**

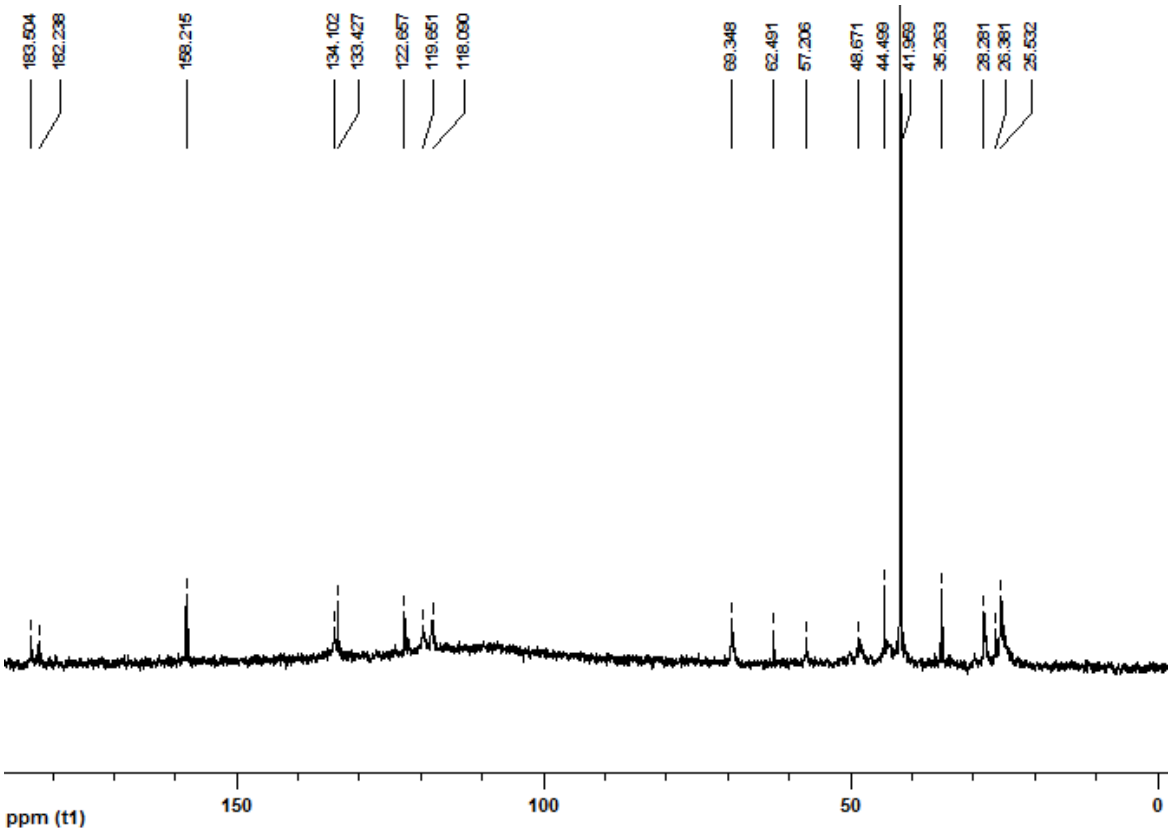


Figure S6. ^{13}C NMR (300 MHz, D_2O) of **2**

NMR spectra of 3

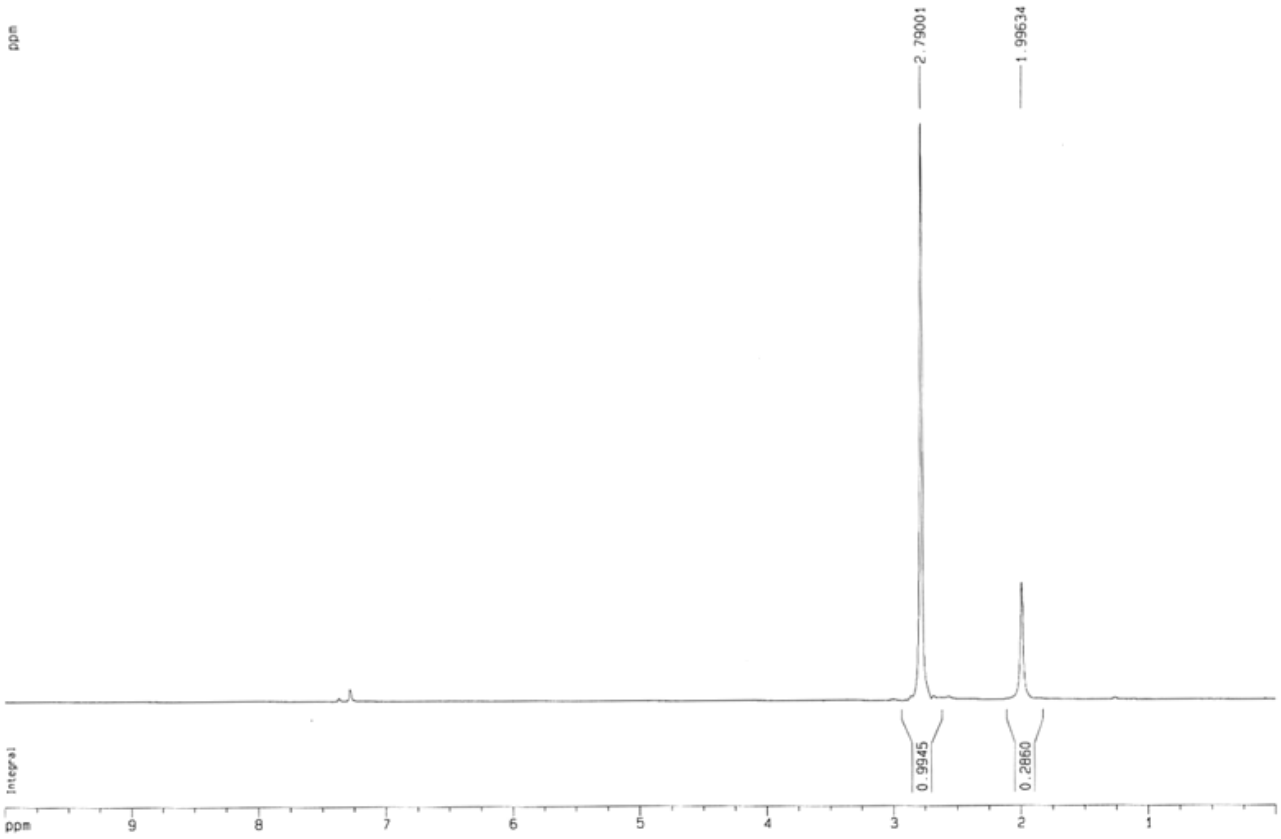


Figure S7. ^{1}H NMR (300 MHz, $CDCl_3$) of **3**

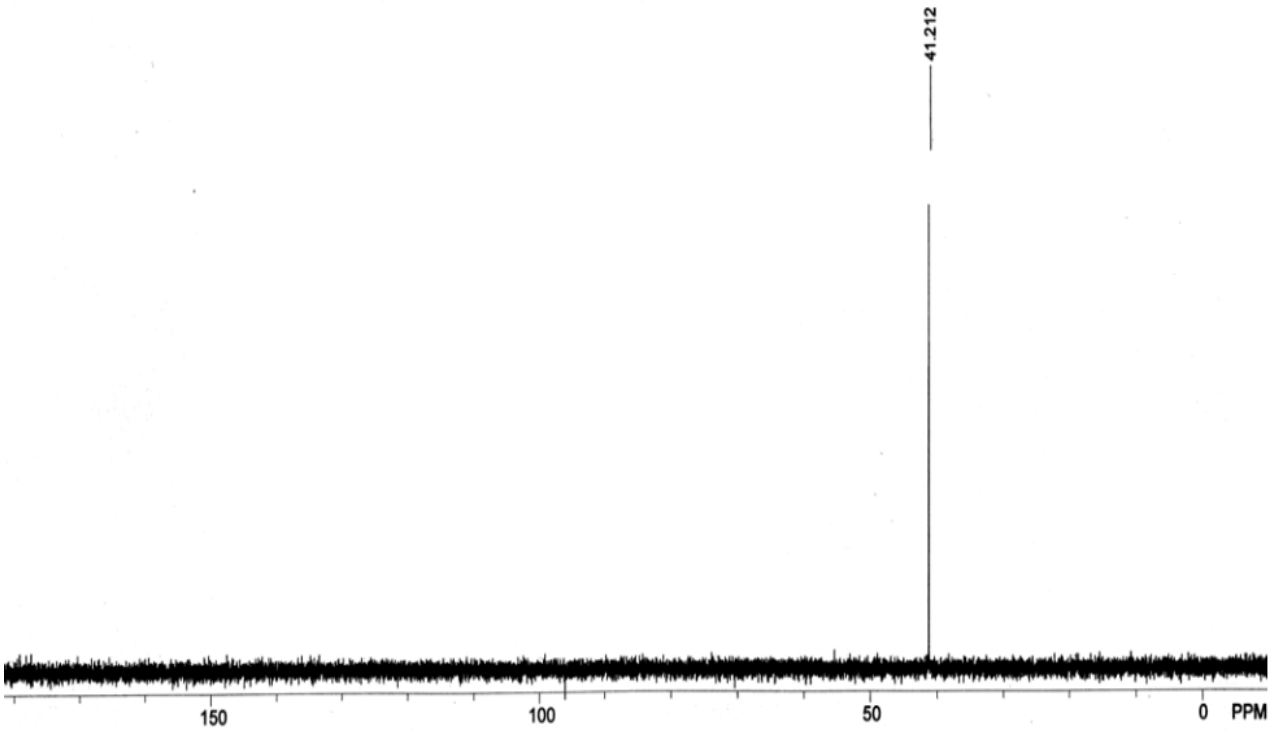


Figure S8. ^{13}C NMR (300 MHz, D_2O) of **3**

NMR spectra of 4a

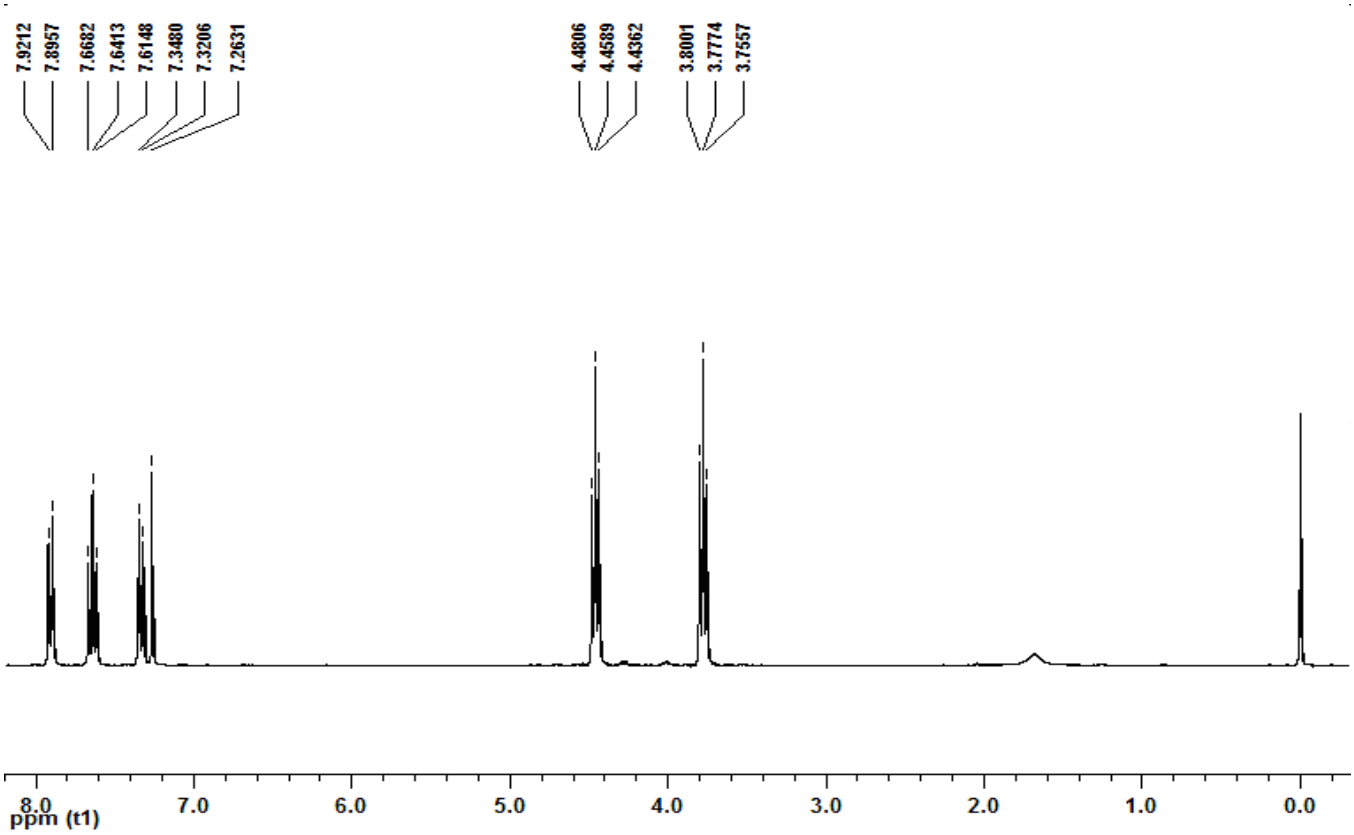


Figure S9. ^{1}H NMR (300 MHz, $CDCl_3$) of **4a**

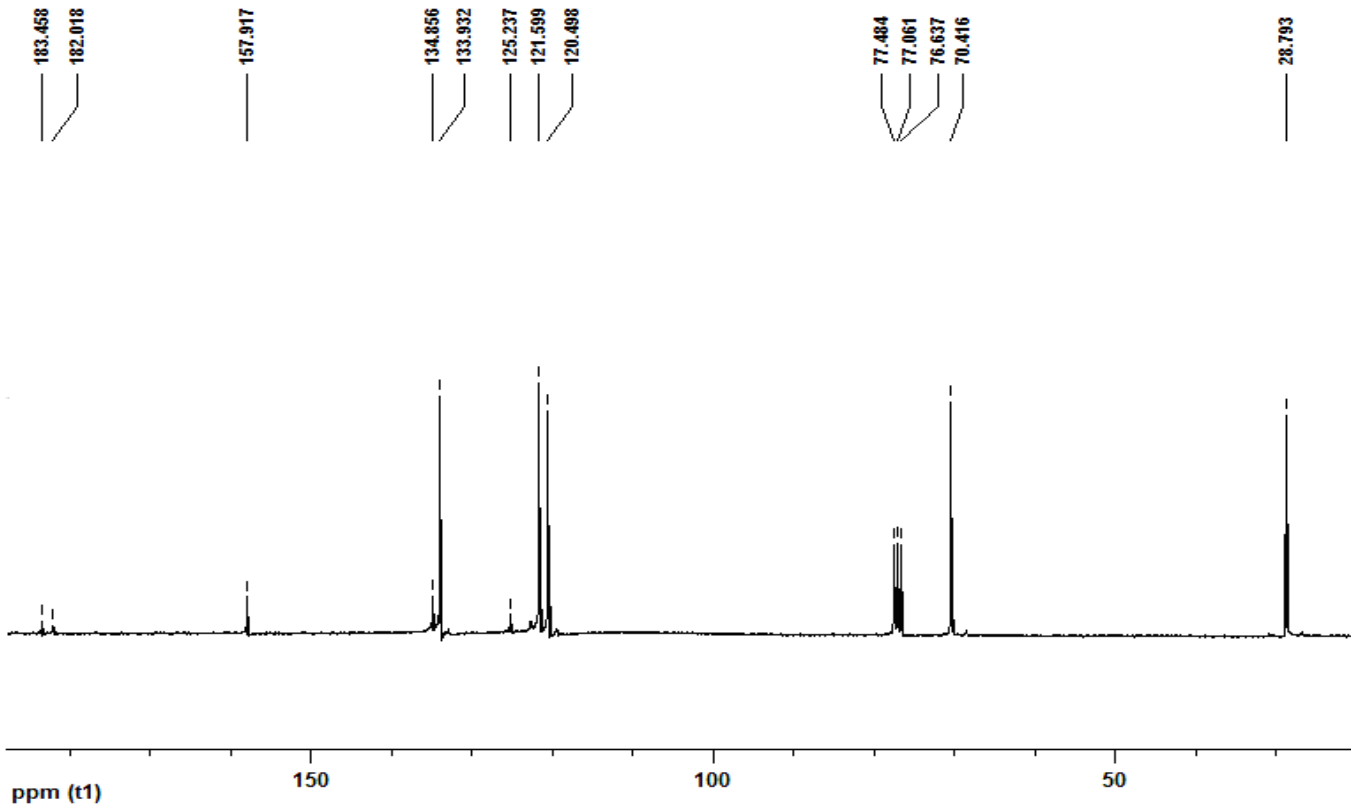


Figure S10. ^{13}C NMR (300 MHz, $CDCl_3$) of **4a**

NMR spectra of 4b

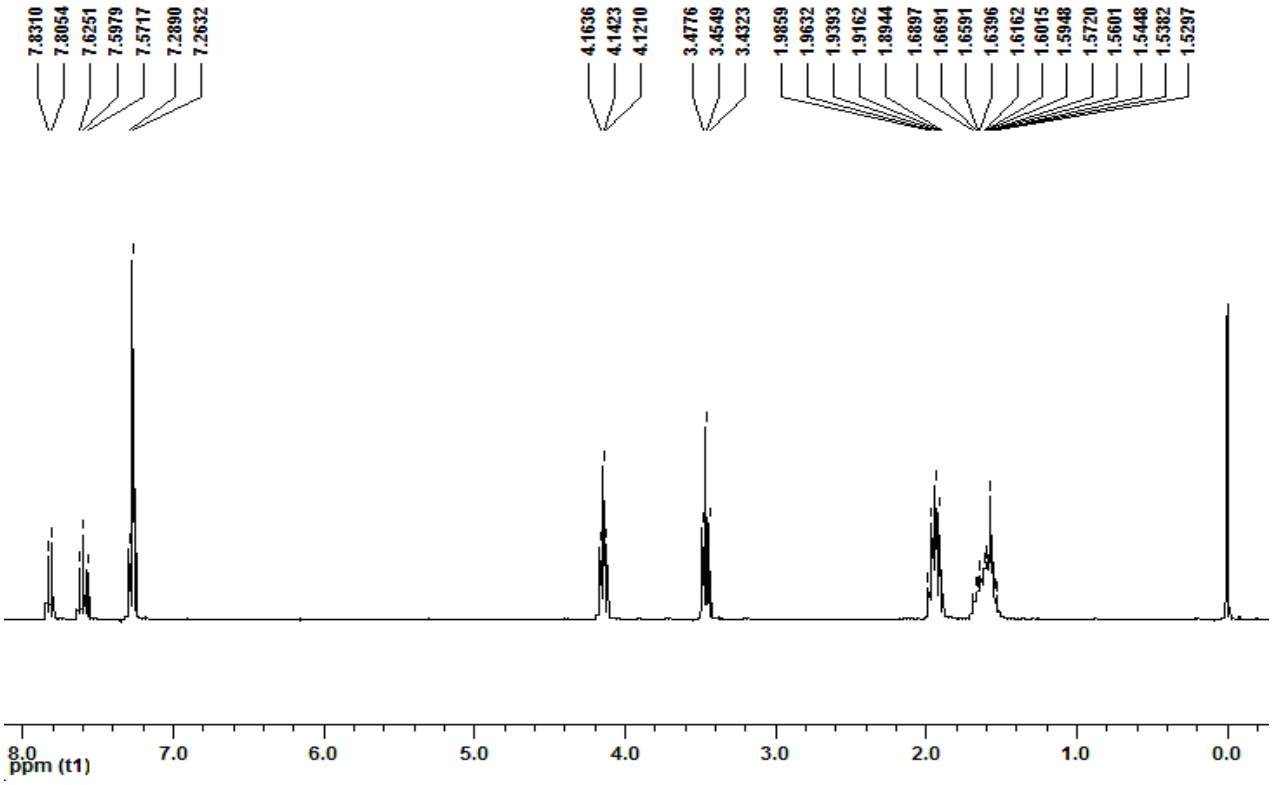


Figure S11. ^{1}H NMR (300 MHz, $CDCl_3$) of **4b**

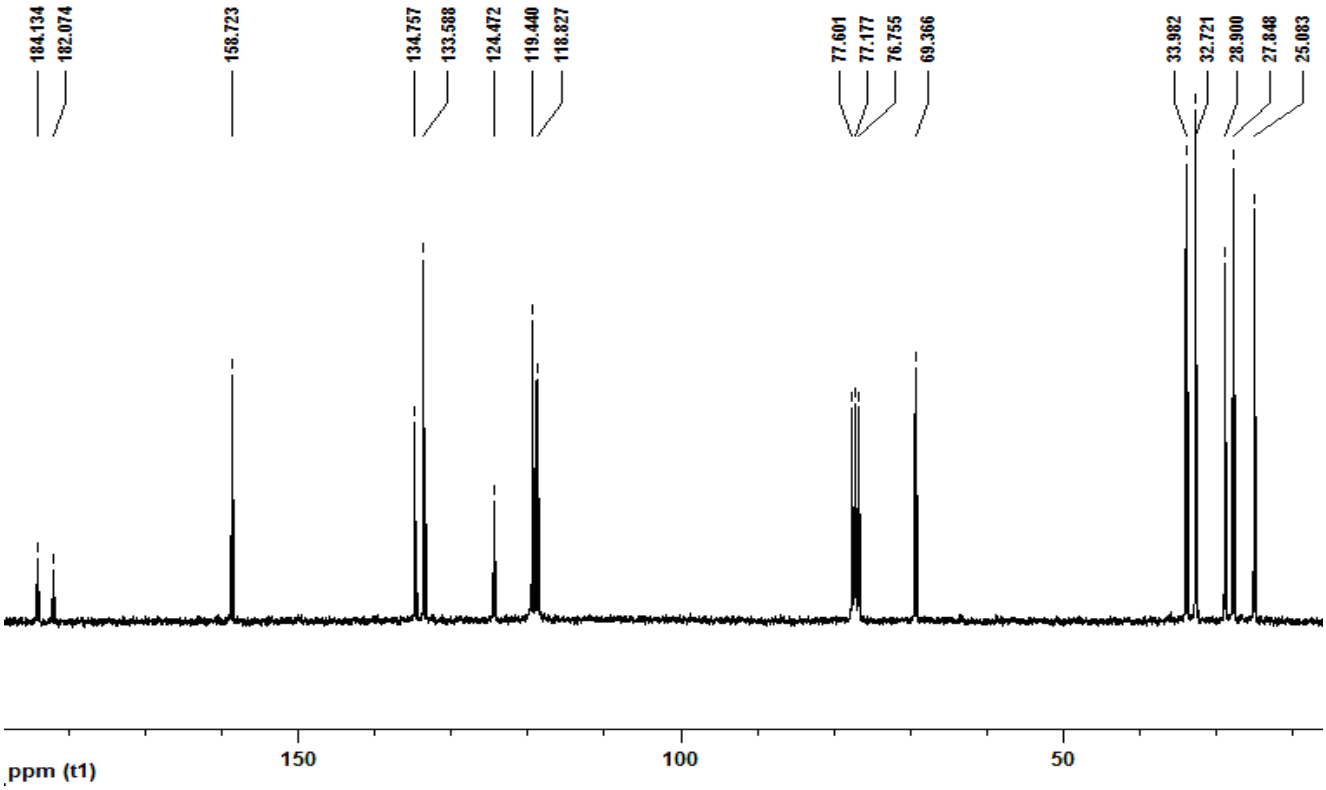


Figure S12. ^{13}C NMR (300 MHz, $CDCl_3$) of **4b**

2. Fluorescence emission spectra and the effect of ionic strength on the DNA binding

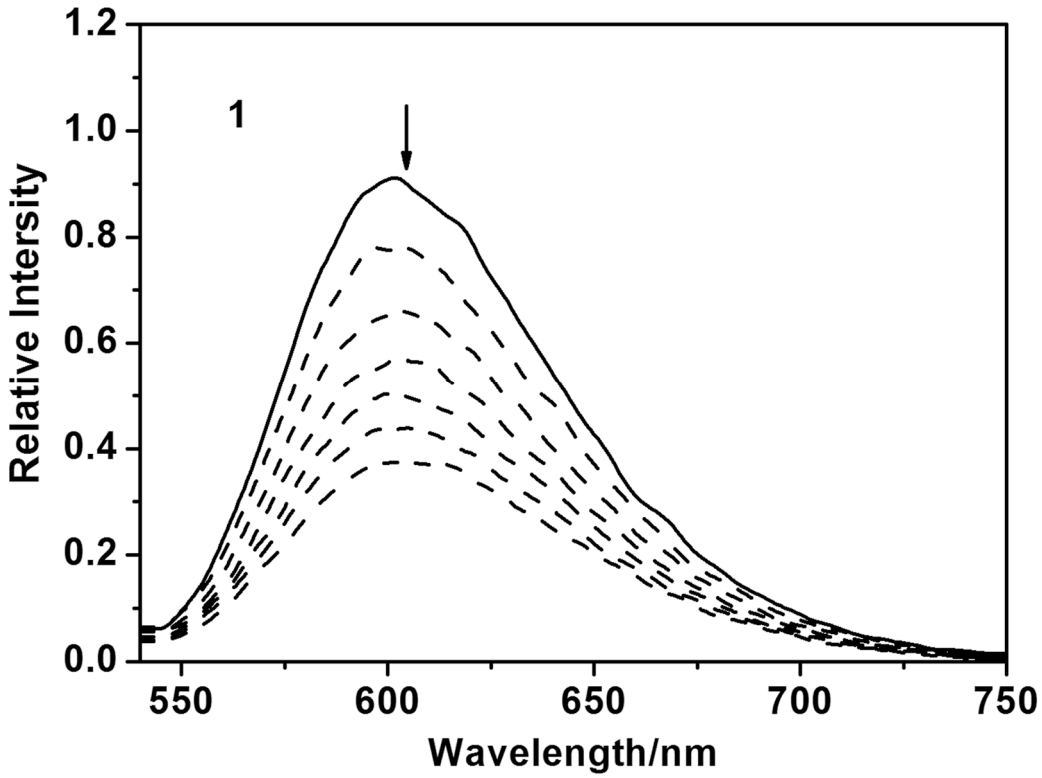


Figure S13. Emission spectra of EB bound to DNA in the absence (—) and presence (---) of **1** (r = 0, 0.05, 0.10, 0.15, 0.21, 0.26, 0.37), r = [compounds] / [CT–DNA]. [CT–DNA] = 0.039 mM. [EB] = 3.9 μM. λ_{ex} = 530 nm. Arrows show the intensity changes upon the addition of increasing concentration of **1**.

Table S1. The apparent binding constant (K_{app}) of **1** and **2** to calf thymus DNA in the presence of various concentrations of sodium chloride in EB-DNA system in 5 mM Tris–HCl buffer (pH 7.0) at room temperature.

$[Na^+]$ / mM		1	5	7.5	10	40	100
$K_{app} \times 10^{-7}$ /M^{-1}	**1**	4.05		3.61	3.54	1.84	1.22
	2	5.99	5.68		4.68	2.59	1.63

3. pH dependence

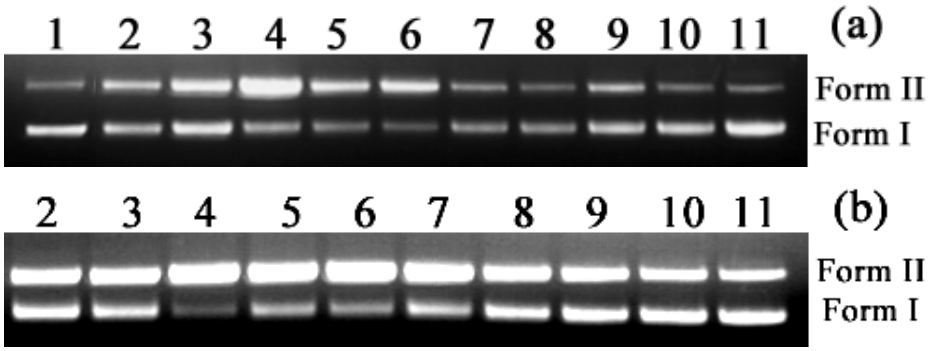


Figure S14. Agarose gel (1%) of pUC 19 DNA (0.025 mM bp) incubated for 16 h at 37°C with 0.033 mM (a) **1** and (b) **2** in different pH buffer (50 mM Tris–HCl / 10 mM NaCl). Lane 1, DNA control; Lanes 2 – 11, pH 6.50, 6.75, 7.00, 7.25, 7.50, 7.75, 8.00, 8.25, 8.50 and 9.00, respectively.

4. Concentration dependence

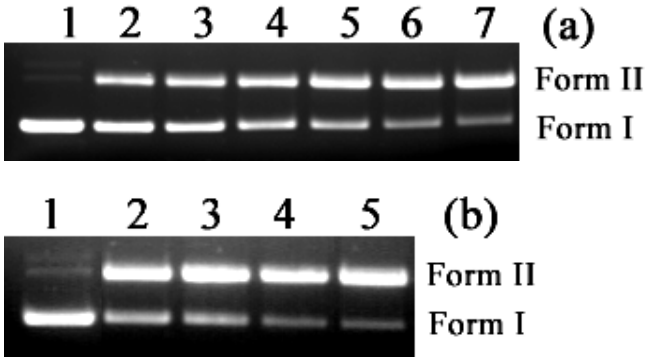


Figure S15. Agarose gel (1%) of pUC 19 DNA (0.025 mM bp) incubated for 16.0 h at 37 °C with different concentrations of (a) **1**, (b) **2** in pH 7.25 buffer (50 mM Tris–HCl / 10 mM NaCl).

(a) Lanes 1 - 7, 0, 0.0067, 0.0133, 0.0200, 0.027, 0.033 and 0.0400 mM **1**, respectively.

(b) Lanes 1 - 6, 0, 0.0067, 0.0133, 0.0267 and 0.0400 mM **2**, respectively.

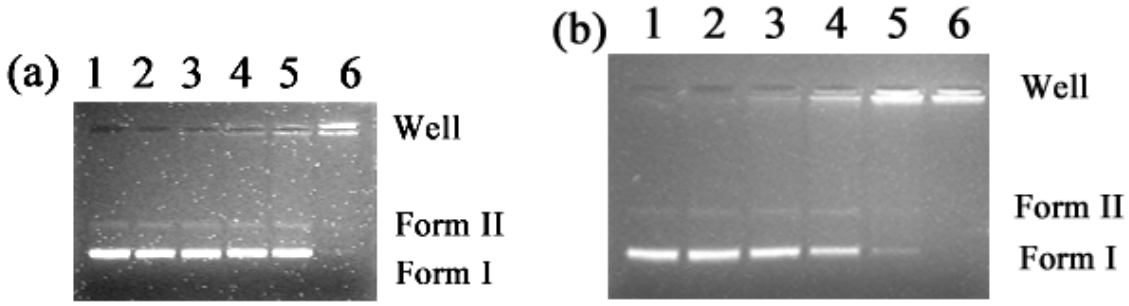


Figure S16. Agarose gel (1%) of pUC 19 DNA (0.025 mM bp) incubated for 1.5 h at 15°C in pH 7.25 buffer (50 mM Tris–HCl / 10 mM NaCl) with different concentrations of (a) **1**, (b) **2**.

(a) Lanes 1 - 6, 0, 0.0333, 0.0500, 0.1000, 0.1333, and 0.1667 mM **1**, respectively.

(b) Lanes 1 - 6, 0, 0.0333, 0.0500, 0.1000, 0.1333, and 0.1667 mM **2**, respectively.

5. DNA cleavage by 4a or 4b alone (control assays)

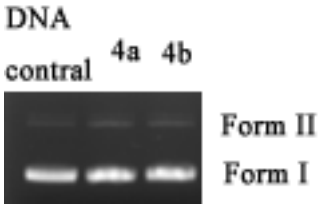


Figure S17. Agarose gel (1%) of pUC 19 DNA (0.025 mM bp) cleavage in the presence of **4a** and **4b** (0.0267 mM) (incubated for 16 h at 37 °C).

Table S2. Control assays of DNA cleavage in the presence of **4a** and **4b**.[a]

added compounds	DNA %	
	Form I	Form II
DNA control	96.79	3.21
4a	95.07	4.93
4b	95.22	4.78

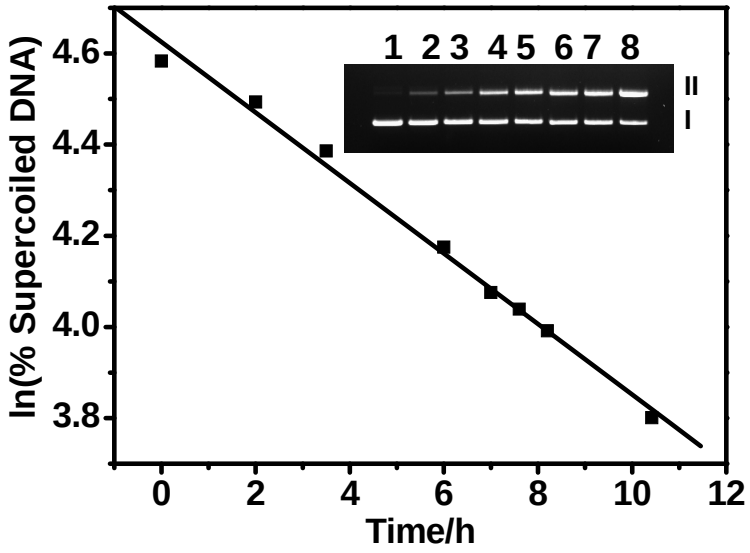
[a] Cleavage reactions were carried out in DMF - pH 7.25 Tris–HCl buffer(*v*/*v* = 2:5) for 16 h at 37 °C.

6. Kinetic data

Table S3. Apparent initial first-order rate constants of DNA cleavage reactions at various concentrations of compounds **1** and **2**. The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).

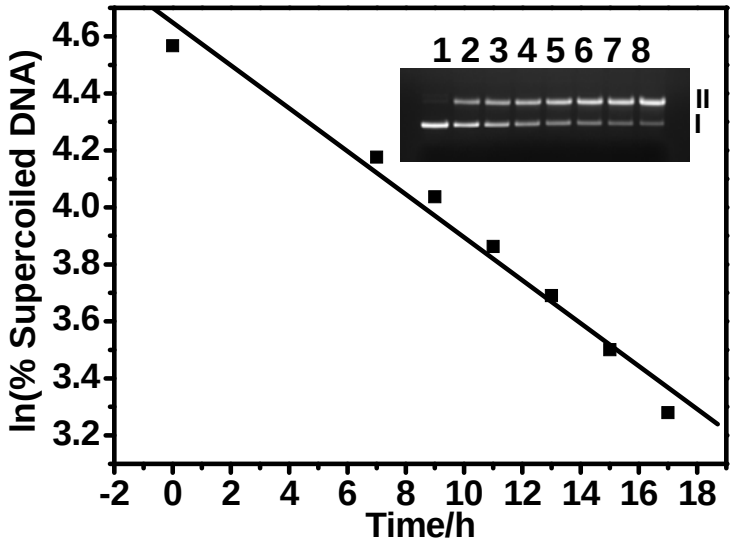
	2		**1**	
	Concentration/mM	k_{obs}/h^{-1}	Concentration/mM	k_{obs}/h^{-1}
1	0.0040	0.030 ± 0.0013	0.0067	0.040 ± 0.0008
2	0.0067	0.048 ± 0.0008	0.0133	0.052 ± 0.0028
3	0.0133	0.073 ± 0.0080	0.0200	0.053 ± 0.0024
4	0.0200	0.093 ± 0.0033	0.0267	0.065 ± 0.0022
5	0.0267	0.102 ± 0.0034	0.0333	0.071 ± 0.0008
6	0.0400	0.113 ± 0.0071	0.0400	0.075 ± 0.0050
7	0.0500	0.123 ± 0.0027	0.0500	0.077 ± 0.0028

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0500mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



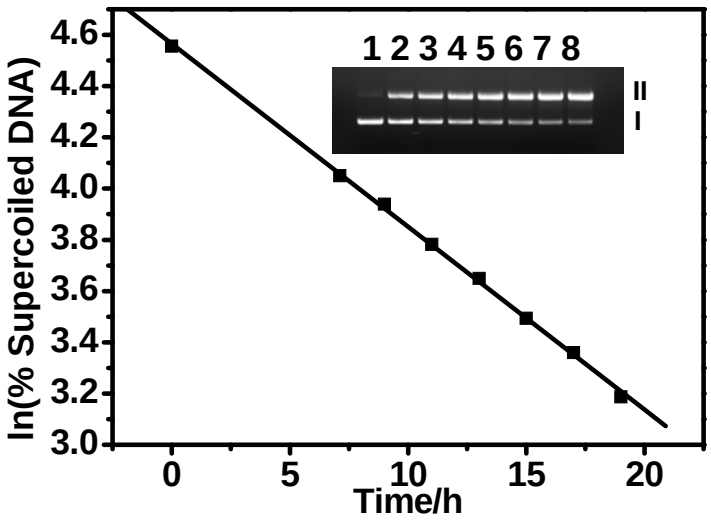
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.583	97.85	2.15
2	2.00	4.493	89.40	10.60
3	3.50	4.386	80.32	19.68
4	6.00	4.175	65.03	34.97
5	7.00	4.076	58.90	41.10
6	7.60	4.039	56.78	43.22
7	8.20	3.992	54.14	45.86
8	10.42	3.801	44.75	55.25

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0400 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



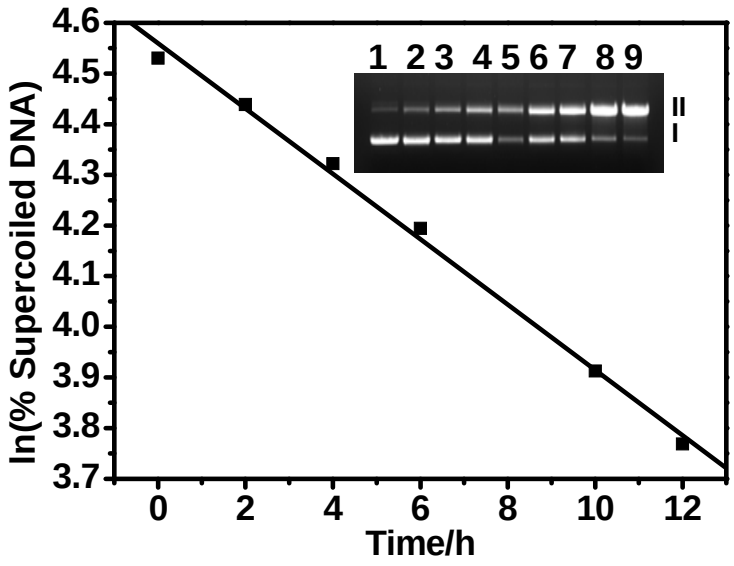
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.567	96.29	3.71
2	7.00	4.176	65.13	34.87
3	9.00	4.037	56.66	43.34
4	11.00	3.863	47.59	52.41
5	13.00	3.691	40.07	59.93
6	15.00	3.501	33.15	66.85
7	17.00	3.280	26.57	73.43
8	19.00	2.945	19.01	80.99

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0333 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



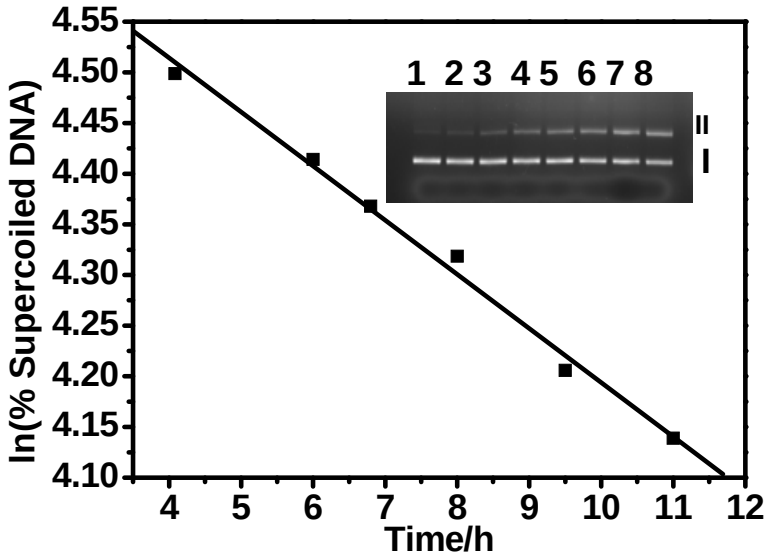
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.556	95.19	4.81
2	7.12	4.050	57.41	42.59
3	9.00	3.940	51.40	48.60
4	11.00	3.782	43.93	56.07
5	13.00	3.649	38.44	61.56
6	15.00	3.493	32.89	67.11
7	17.00	3.360	28.78	71.22
8	19.00	3.187	24.22	75.78

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0267 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



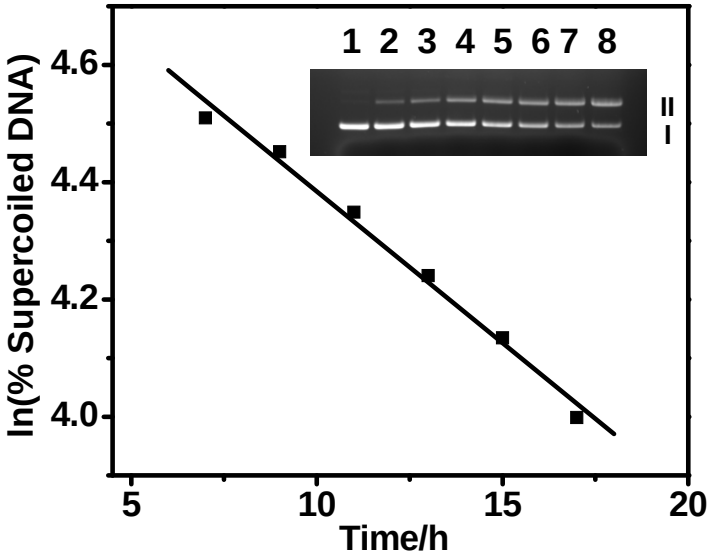
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.530	92.76	7.24
2	2.00	4.439	84.66	15.34
3	4.00	4.322	75.33	24.67
4	6.00	4.194	66.30	33.70
5	8.00	3.749	42.47	57.53
6	10.00	3.913	50.03	49.97
7	12.00	3.769	43.33	56.67
8	21.28	2.909	18.34	81.66
9	24.48	2.334	10.32	89.68

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0200 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



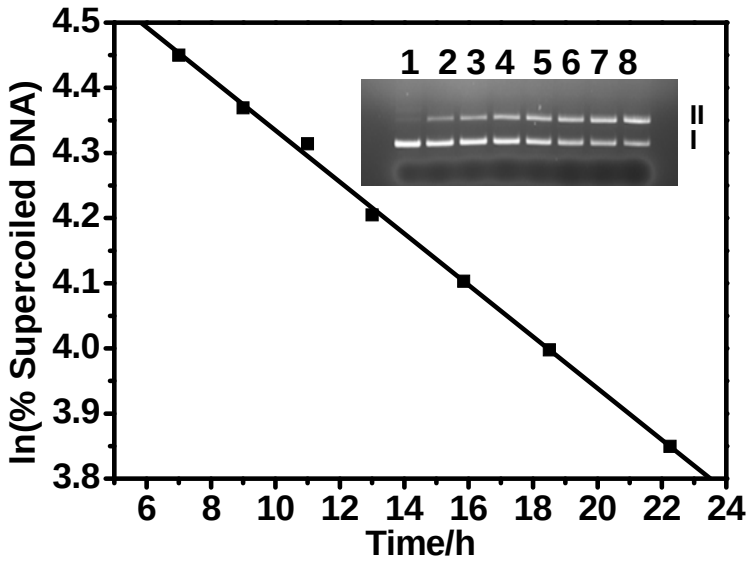
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.586	98.07	1.93
2	2.00	4.562	95.77	4.23
3	4.08	4.499	89.91	10.09
4	6.00	4.414	82.60	17.40
5	6.80	4.368	78.88	21.12
6	8.00	4.318	75.07	24.93
7	9.50	4.206	67.08	32.92
8	11.00	4.139	62.74	37.26

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0133 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



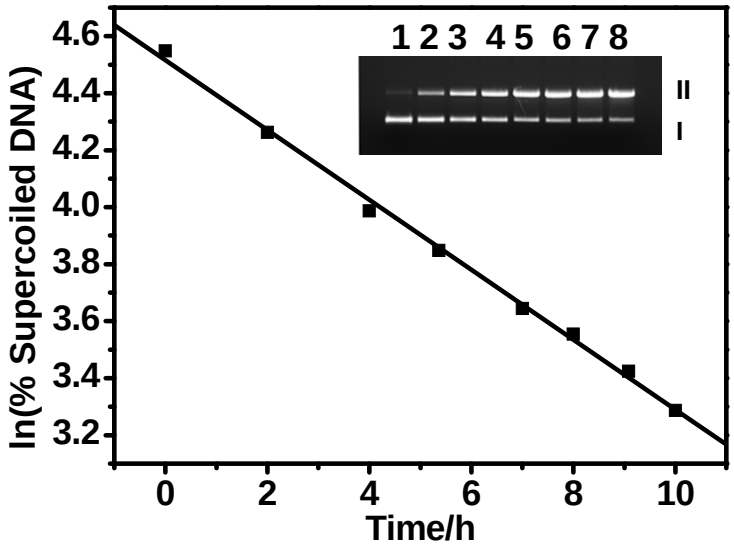
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.597	99.17	0.83
2	7.00	4.510	90.90	9.10
3	9.00	4.452	85.81	14.19
4	11.00	4.349	77.40	22.60
5	13.00	4.241	69.46	30.54
6	15.00	4.135	62.46	37.54
7	17.00	3.999	54.53	45.47
8	19.00	3.798	44.62	55.38

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.0067 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



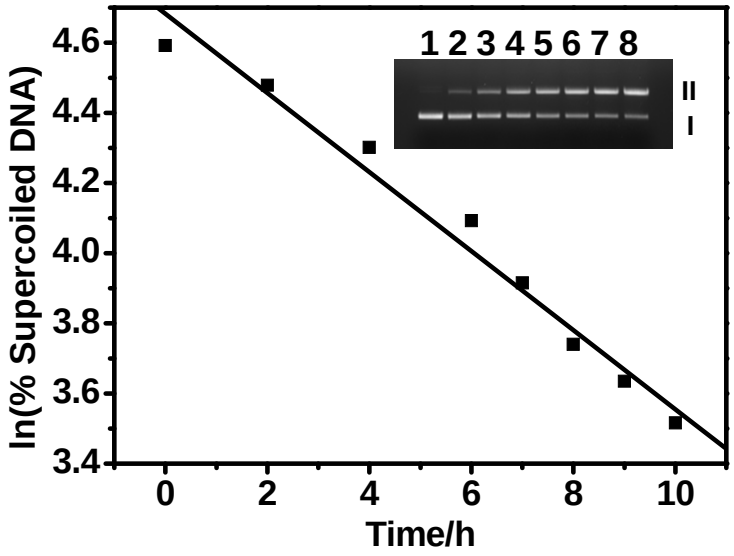
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0	4.587	98.24	1.76
2	7	4.450	85.65	14.35
3	9	4.369	78.98	21.02
4	11	4.314	74.74	25.26
5	13	4.205	67.01	32.99
6	15.85	4.103	60.53	39.47
7	18.51	3.998	54.47	45.53
8	22.25	3.850	46.98	53.02

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0500 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



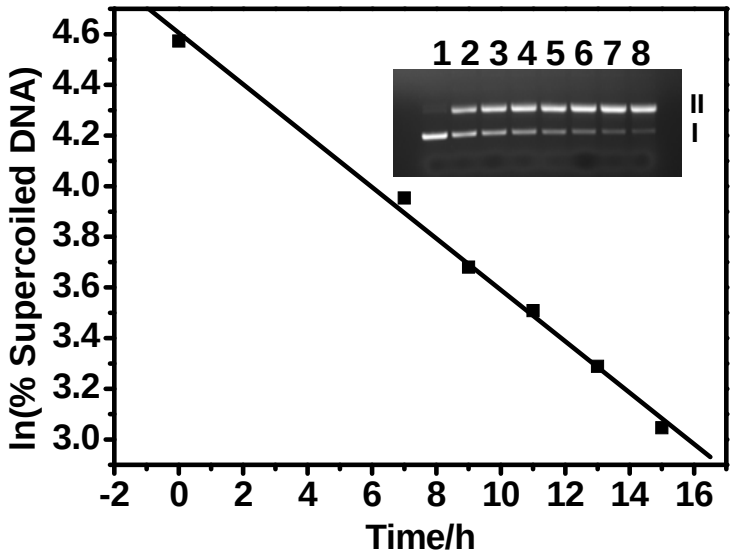
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.549	94.50	5.50
2	2.00	4.262	70.98	29.02
3	4.00	3.987	53.90	46.10
4	5.37	3.849	46.94	53.06
5	7.00	3.644	38.25	61.75
6	8.00	3.554	34.97	65.03
7	9.08	3.424	30.70	69.30
8	10.00	3.287	26.75	73.25

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0400 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



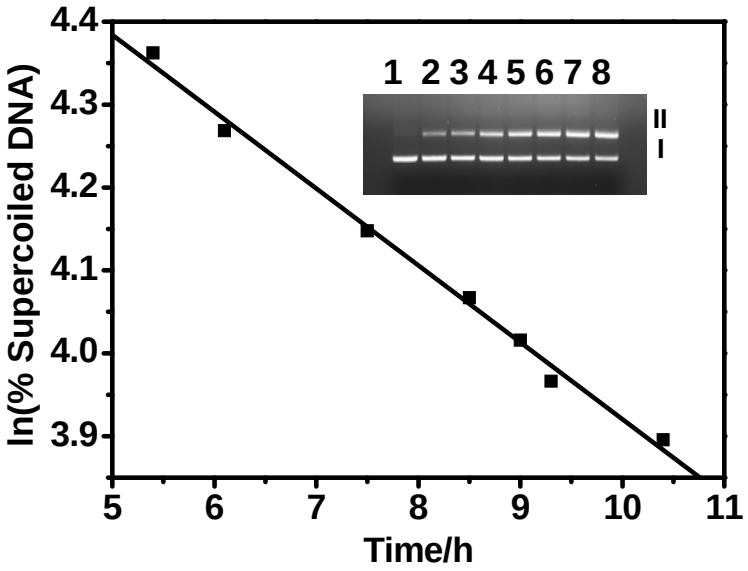
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.592	98.69	1.31
2	2.00	4.479	88.16	11.84
3	4.00	4.302	73.83	26.17
4	5.50	4.092	59.88	40.12
5	7.00	3.916	50.17	49.83
6	8.00	3.740	42.10	57.90
7	9.00	3.635	37.91	62.09
8	10.00	3.516	33.66	66.34

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0267 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



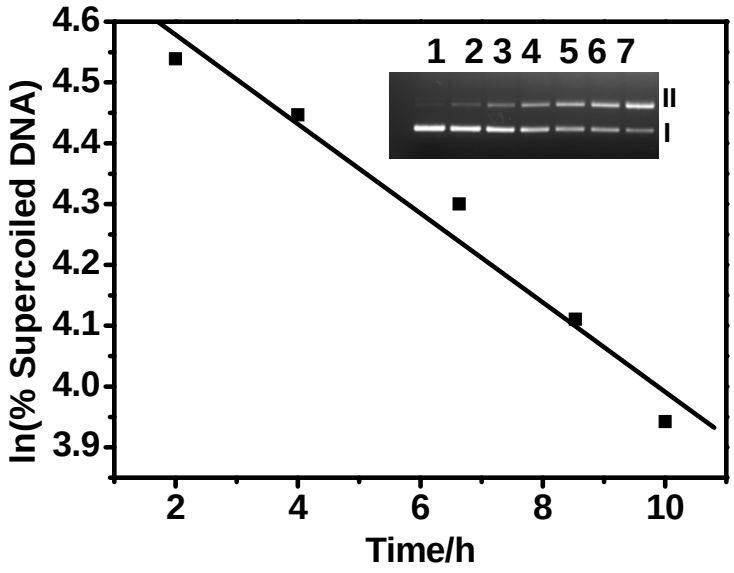
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.573	96.85	3.15
2	7.00	3.953	52.12	47.88
3	9.00	3.680	39.65	60.35
4	11.00	3.509	33.40	66.60
5	13.00	3.289	26.82	73.18
6	15.00	3.046	21.04	78.96
7	17.00	2.669	14.43	85.57
8	19.00	2.286	9.83	90.17

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0200 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



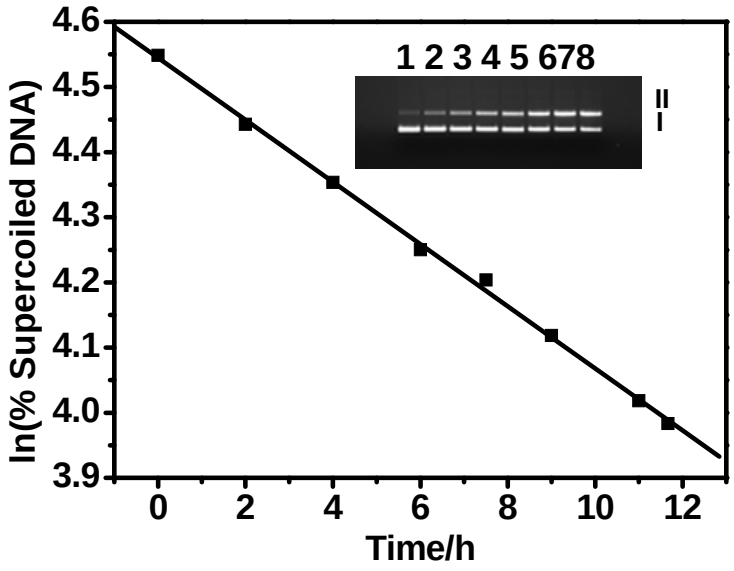
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.588	98.32	1.68
2	5.40	4.362	78.43	21.57
3	6.10	4.268	71.40	28.60
4	7.50	4.148	63.29	36.71
5	8.50	4.067	58.39	41.61
6	9.00	4.016	55.48	44.52
7	9.30	3.966	52.80	47.20
8	10.40	3.896	49.20	50.80

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0133 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



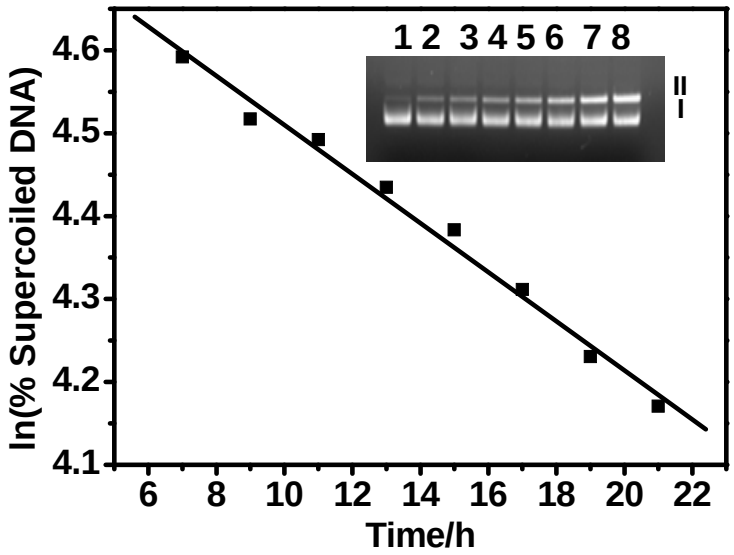
Lane	Time /h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.595	99.01	0.99
2	2.00	4.539	93.58	6.42
3	4.00	4.447	85.36	14.64
4	6.63	4.300	73.71	26.29
5	8.53	4.111	61.00	39.00
6	10.00	3.942	51.53	48.47
7	12.00	3.492	32.84	67.16

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0067 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.549	94.50	5.50
2	2.00	4.443	85.02	14.98
3	4.00	4.354	77.75	22.25
4	6.00	4.250	70.11	29.89
5	7.50	4.204	66.94	33.06
6	9.00	4.118	61.46	38.54
7	11.00	4.018	55.61	44.39
8	11.67	3.983	53.70	46.30

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.0040 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 10 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.588	98.31	1.69
2	7.00	4.592	98.69	1.31
3	9.00	4.517	91.59	8.41
4	11.00	4.492	89.32	10.68
5	13.00	4.435	84.33	15.67
6	15.00	4.384	80.12	19.88
7	17.00	4.311	74.55	25.45
8	19.00	4.231	68.77	31.23
9	21.00	4.171	64.77	35.23

Table S4. Apparent initial first-order rate constants of DNA cleavage reactions at various concentrations of compounds **3**. The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).

	Compound **5**	
	Concentration/mM	k_{obs}/h^{-1}
1	0.05	0.0015 ± 3.4152E-5
2	0.1	0.0016 ± 2.5787E-5
3	0.15	0.0018 ± 8.2484E-5
4	0.2	0.0020 ± 5.8992E-5
5	0.25	0.0021 ± 7.7566E-5

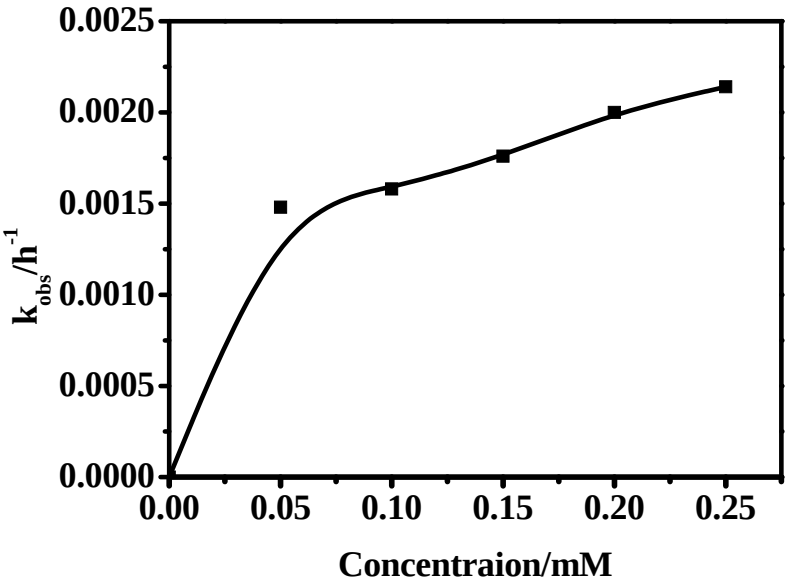
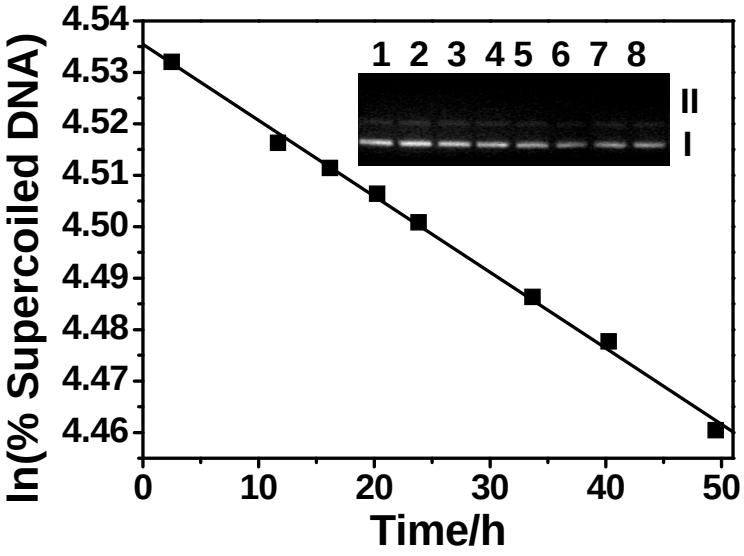


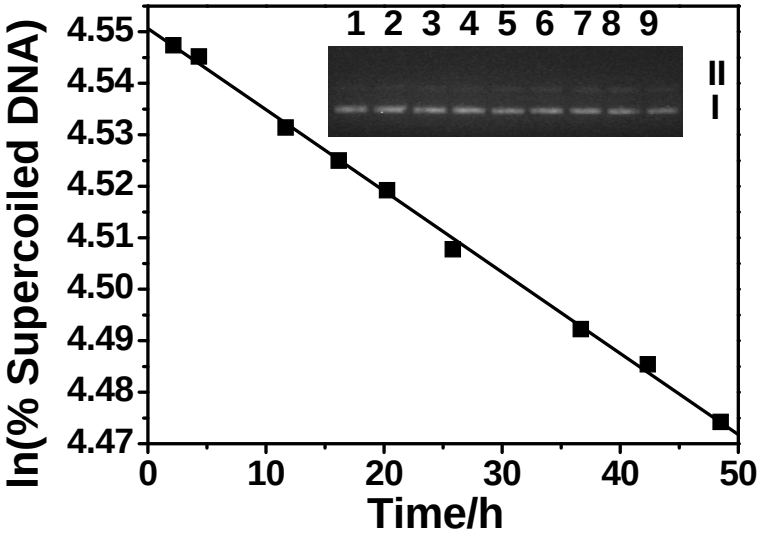
Figure S18. Kinetics plot of k_{obs} vs various concentrations of **3**.

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **3** (0.050 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).



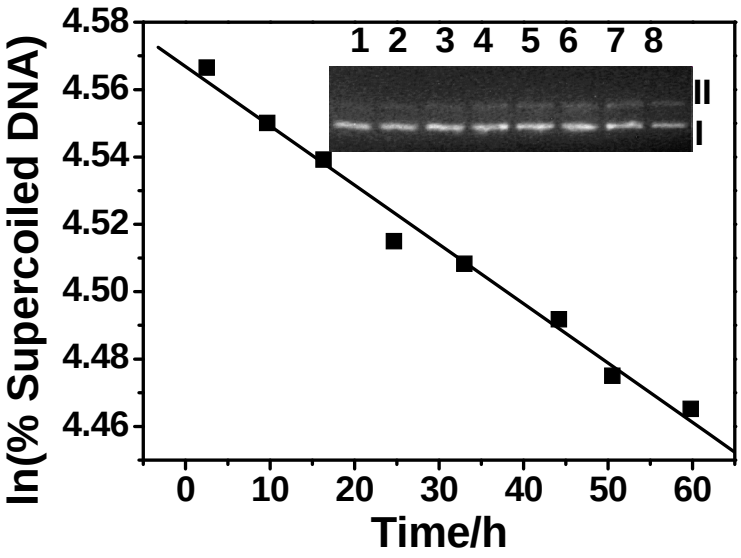
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	2.50	4.532	92.95	7.05
2	11.67	4.516	91.50	8.50
3	16.17	4.511	91.05	8.95
4	20.25	4.506	90.60	9.40
5	23.83	4.501	90.09	9.91
6	33.67	4.486	88.80	11.20
7	40.25	4.478	88.03	11.97
8	49.50	4.460	86.52	13.48

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **3** (0.100 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).



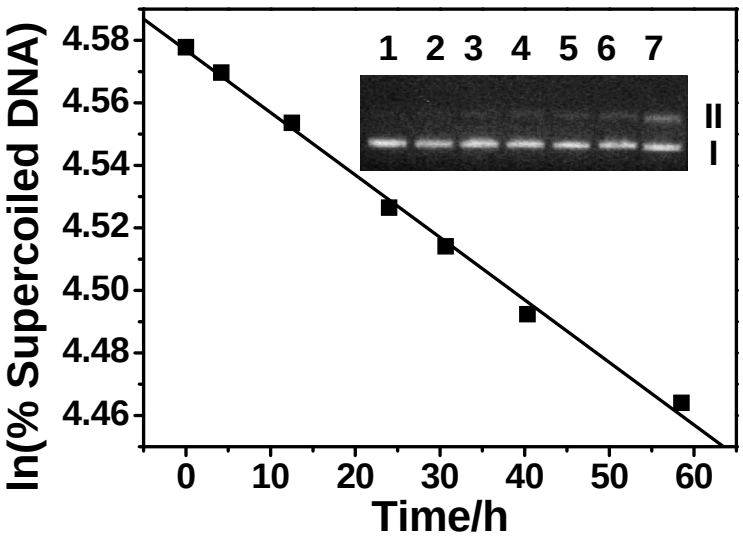
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	2.17	4.547	94.38	5.62
2	4.33	4.545	94.18	5.82
3	11.67	4.531	92.89	7.11
4	16.17	4.525	92.30	7.70
5	20.25	4.519	91.76	8.24
6	25.83	4.508	90.72	9.28
7	36.67	4.492	89.32	10.68
8	42.33	4.485	88.72	11.28
9	48.50	4.474	87.73	12.27

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **3** (0.150 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).



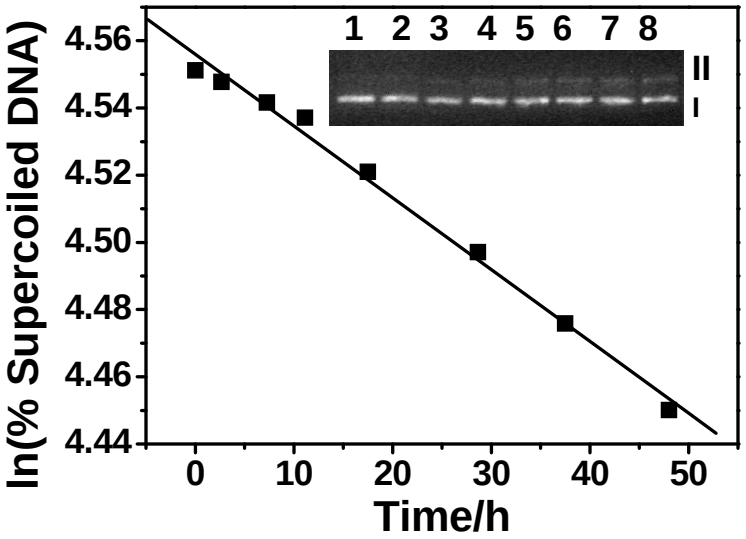
Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	2.50	4.567	96.21	3.79
2	9.67	4.550	94.64	5.36
3	16.33	4.539	93.62	6.38
4	24.67	4.515	91.37	8.63
5	33.00	4.508	90.77	9.23
6	44.17	4.492	89.28	10.72
7	50.50	4.475	87.80	12.20
8	59.80	4.465	86.94	13.06

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **3** (0.200 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0	4.578	97.30	2.70
2	4.17	4.570	96.51	3.49
3	12.50	4.554	94.97	5.03
4	24.00	4.526	92.43	7.57
5	30.67	4.514	91.29	8.71
6	40.33	4.492	89.33	10.67
7	58.50	4.464	86.84	13.16

Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **3** (0.250 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0	4.551	94.75	5.25
2	2.67	4.548	94.42	5.58
3	7.25	4.542	93.84	6.16
4	11.13	4.537	93.42	6.58
5	17.50	4.521	91.92	8.08
6	28.67	4.497	89.76	10.24
7	37.50	4.476	87.87	12.13
8	48.00	4.450	85.64	14.36

7. Radical scavengers' and EDTA' inhibition

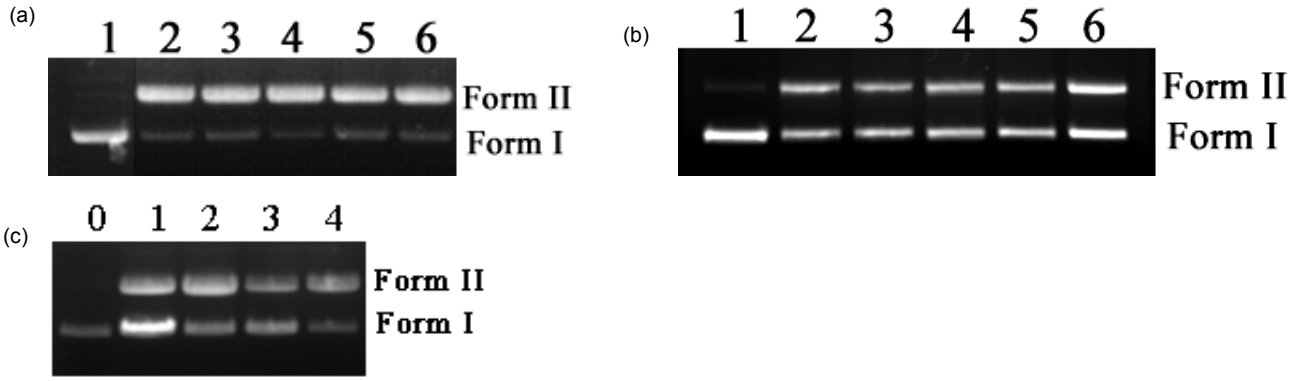


Figure S19. Agarose gel (1%) of pUC19 plasmid DNA (0.025 mM bp) cleaved by (a) **2** (0.0400 mM) and (b) **1** (0.0180 mM) in the presence of standard radical scavengers incubated for 18 h at 37 °C in pH 7.25 buffer (50 mM Tris–HCl / 10 mM NaCl). Lane 1, DNA control; lane 2, no scavengers; lanes 3 – 6, in the presence of NaN_3, DMSO, *t*-BuOH and KI, respectively;
(c) **2** (0.040 mM) and **1** (0.021 mM) in the presence of EDTA (10 mM) incubated for 10 h at 37 °C in pH 7.25 buffer (50 mM Tris–HCl / 10 mM NaCl). Lane 0, DNA control; lane 1, **1** without EDTA; lanes 2, **2** without EDTA; lane 3, **1** in the presence EDTA; lane 4, **2** in the presence EDTA.

Table S5. DNA cleavage promoted by (a) **2** (0.0400 mM) and (b) **1** (0.0180 mM) in the presence of standard radical scavengers.

	% DNA			
	2		**1**	
	Form I	Form II	Form I	Form II
DNA control	98.58	1.42	96.12	3.88
1 or **2** only	14.33	85.67	53.87	46.13
NaN_3	8.16	91.84	62.71	37.29
DMSO	18.72	81.28	58.29	41.71
t-BuOH	16.36	83.64	58.76	41.24
KI	11.58	88.42	56.51	43.49

Table S6. DNA cleavage promoted by (a) **2** (0.040 mM) and (b) **1** (0.021 mM) in the presence of 10 mM EDTA.

	% DNA			
	2		**1**	
	Form I	Form II	Form I	Form II
DNA control	95.63	4.37	95.63	4.37
1 or **2** only	34.35	65.65	64.89	35.11
EDTA	29.78	70.22	60.50	39.50

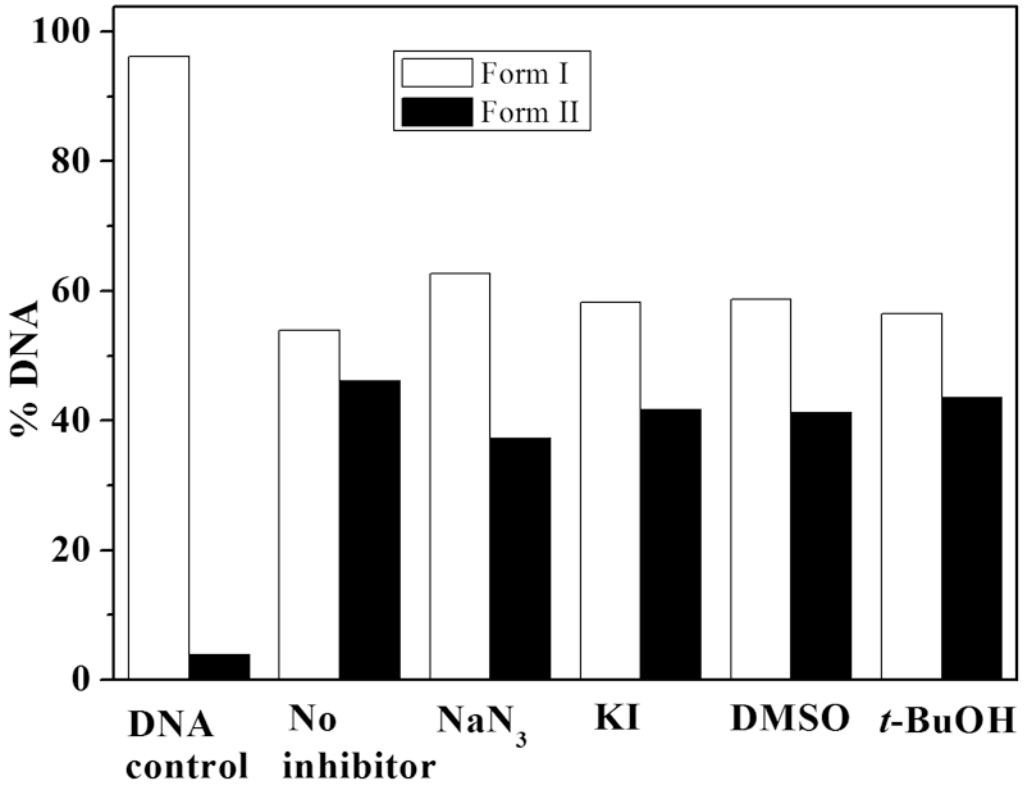
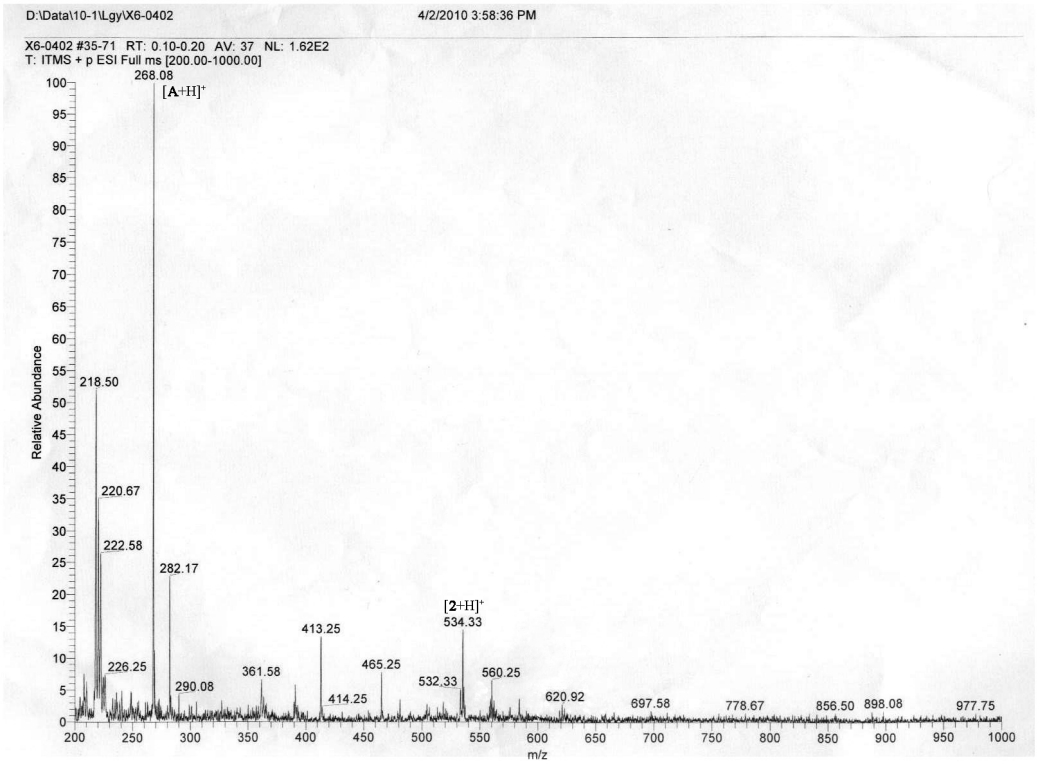
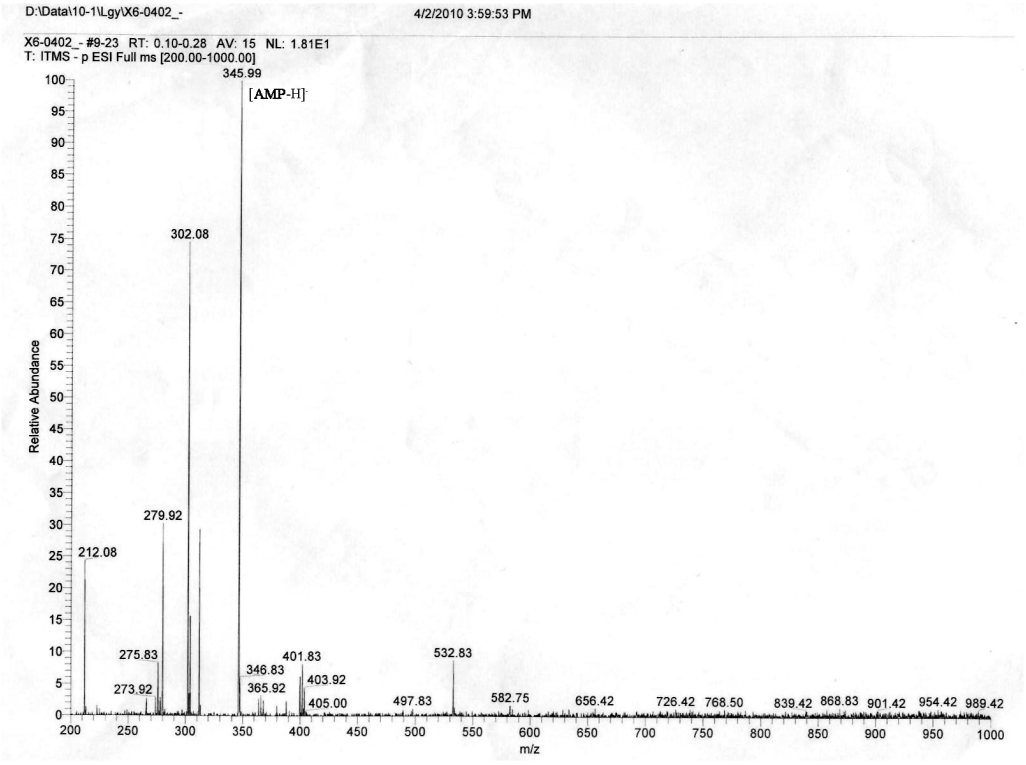


Figure S20. Histogram representing of pUC19 plasmid DNA (0.025 mM bp) cleaved by **1** (0.0180 mM) in the presence of standard radical scavengers incubated for 18 h at 37 °C in pH 7.25 buffer (50 mM Tris–HCl / 10 mM NaCl).

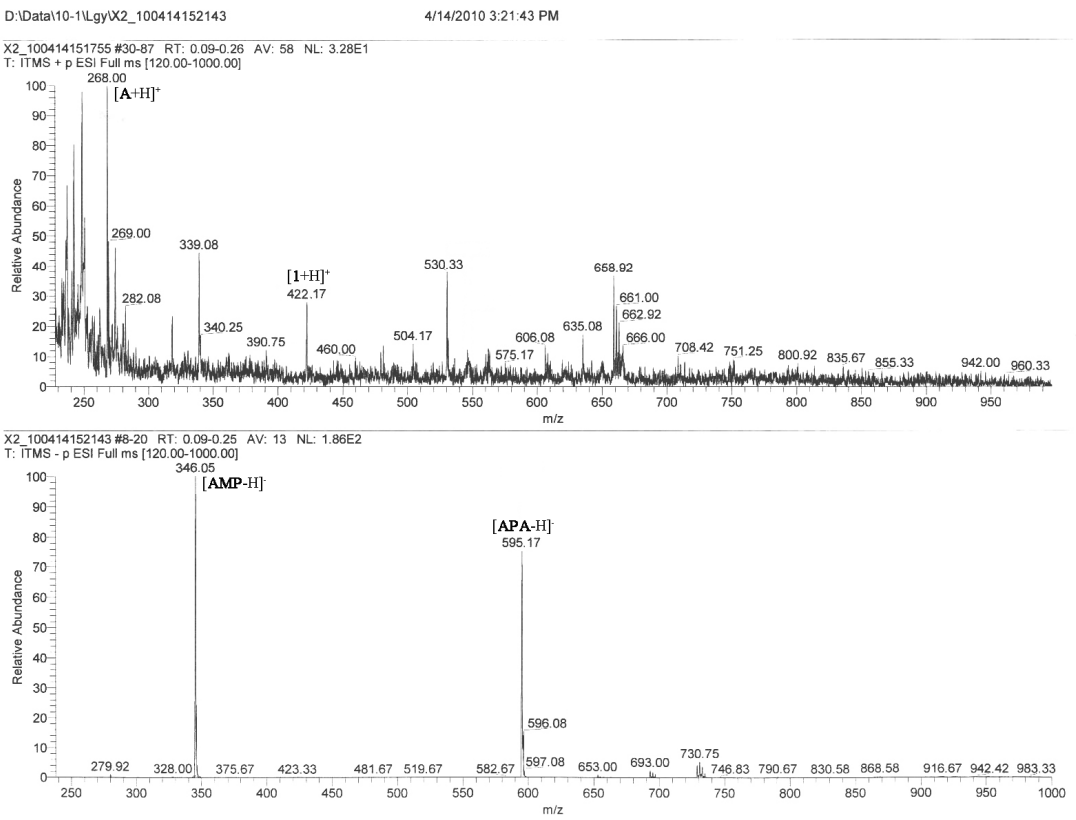
8. ESI-MS spectra of ApA

(a)





(b)


D:\Data\10-1\Lgy\X2_100414152143
4/14/2010 3:21:43 PM
X2_100414151755 #30-87 RT: 0.09-0.26 AV: 58 NL: 3.28E1
T: ITMS + p ESI Full ms [120.00-1000.00]
[A+H]+
[1+H]+
Relative Abundance
m/z
X2_100414152143 #8-20 RT: 0.09-0.25 AV: 13 NL: 1.86E2
T: ITMS - p ESI Full ms [120.00-1000.00]
[AMP-H]-
[APA-H]-
Relative Abundance
m/z

(c)

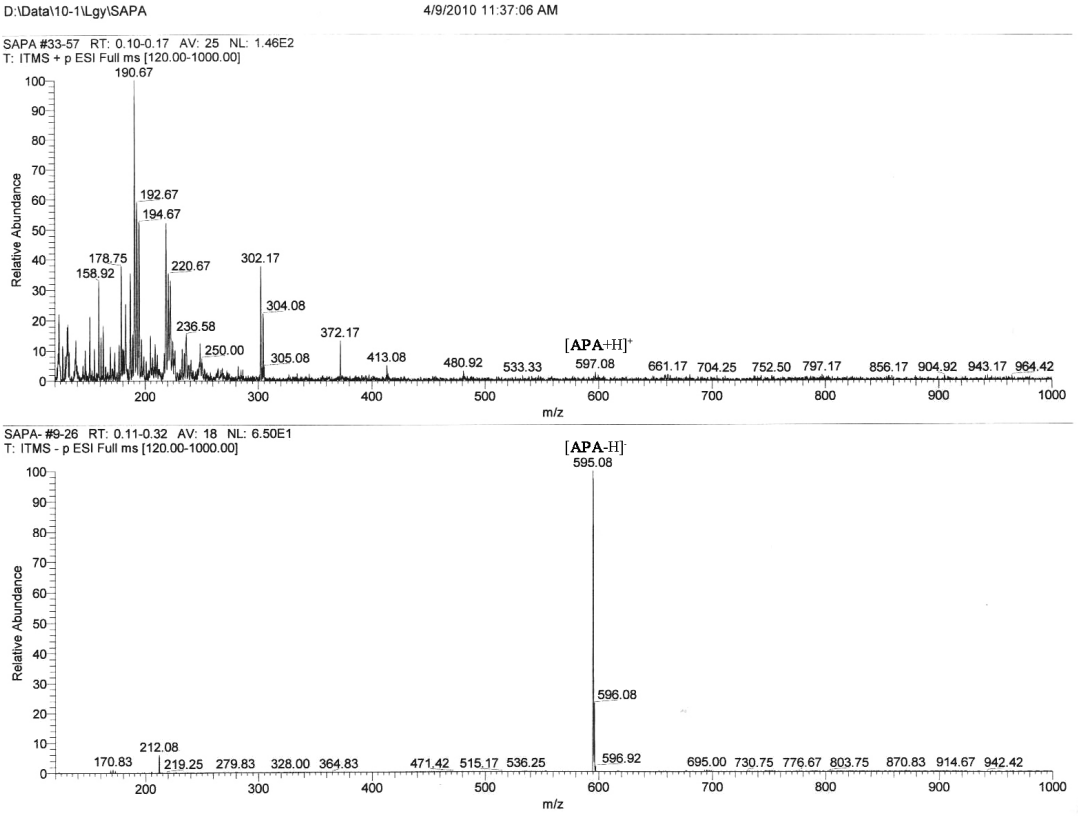


Figure S21. ESI–MS analysis of ApA after treatment with (a) **2**, (b) **1** and (c) ApA alone for 16 h at 37 °C.

9. UV-visible spectra and fluorescence emission spectra of compounds 1 and 2

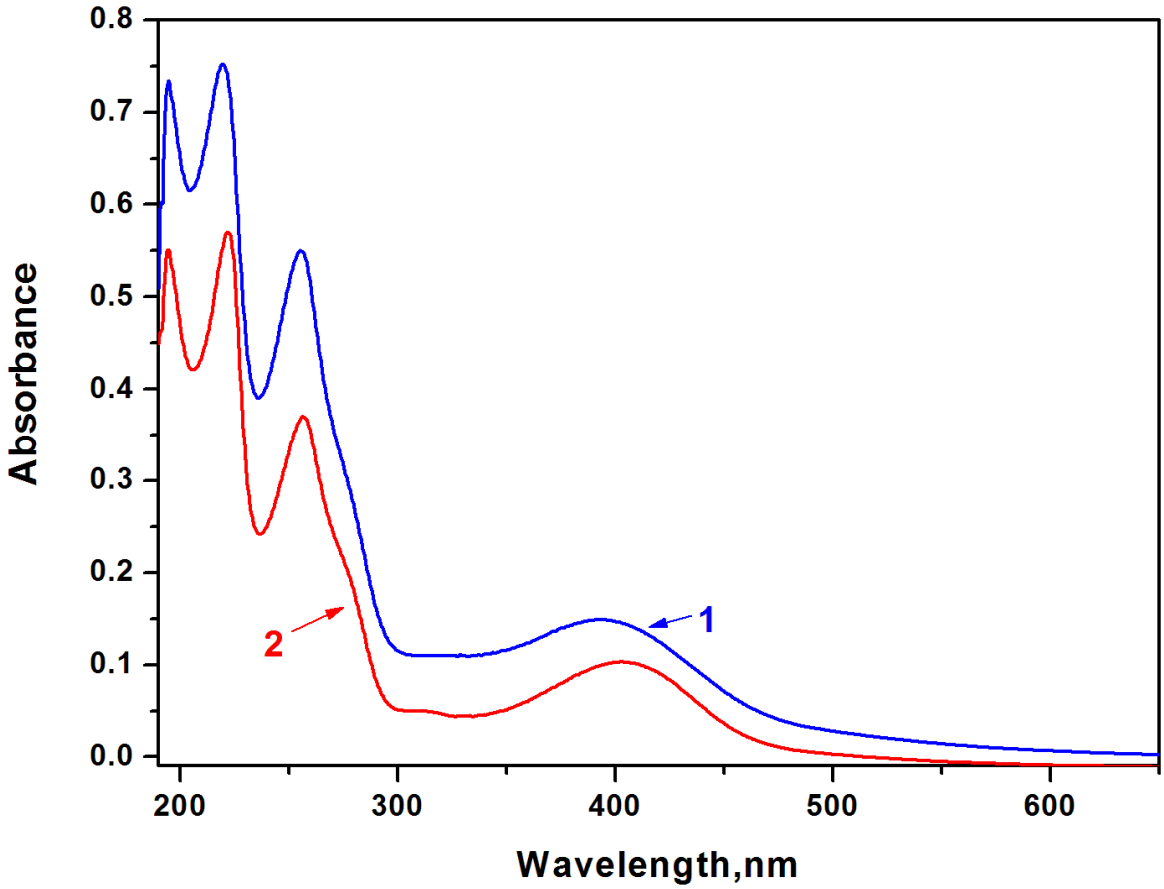


Figure S22. Absorption spectra of compounds **1** (27μM) and **2** (20μM) in pH 7.0 buffer (5mM Tris-HCl) at room temperature.

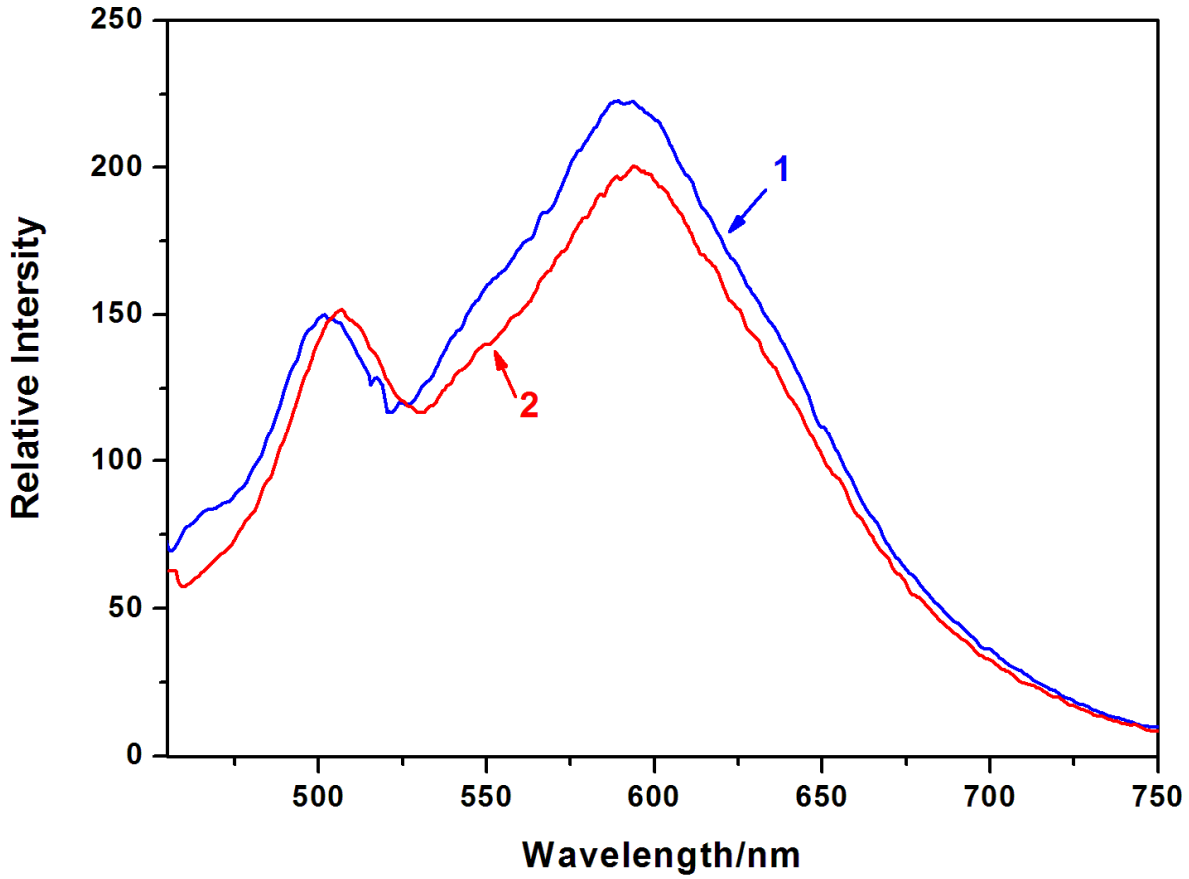


Figure S23. Emission spectra of compounds **1** (6.7μM) or **2** (5μM) in 5 mM Tris–HCl buffer (pH 7.0) at room temperature. λ_{ex} = 430 nm. The photomultiplier voltage is 900. Excitation slit width: 15nm; Emission slit width: 15nm.[b]

[b] The fluorescent spectral studies were performed on an a Perkin-Elmer LS55 luminescence spectrometer.

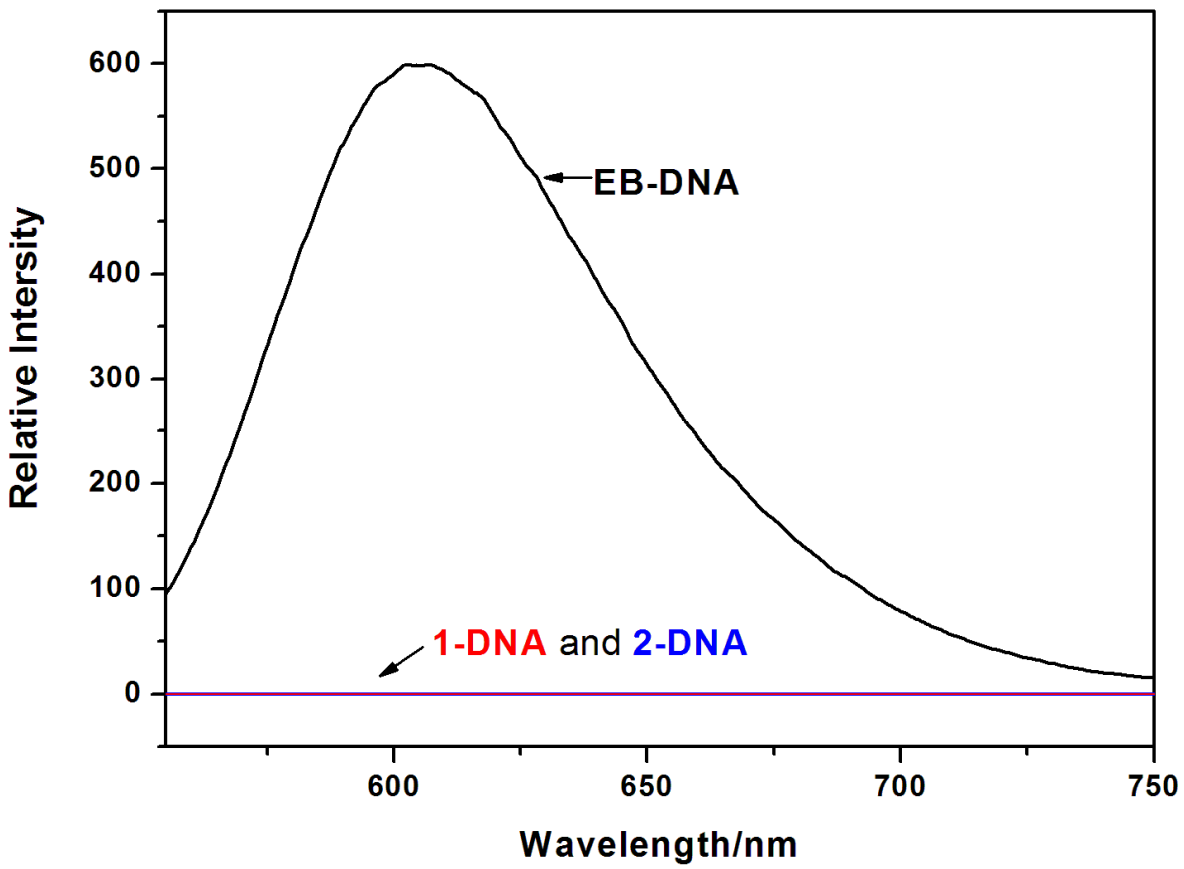


Figure S24. Emission spectra of EB-DNA (3.9 μM), compounds **1**-DNA (6.7μM) or **2**-DNA (5μM) in 5 mM Tris–HCl buffer (pH 7.0) at room temperature. λ_{ex} = 530 nm. The photomultiplier voltage is 785. Excitation slit width: 10nm; Emission slit width: 10nm.[b]

[b] The fluorescent spectral studies were performed on an a Perkin-Elmer LS55 luminescence spectrometer.