Supplementary Information

Synthesis and Enhanced DNA Cleavage Activities of Bis-tacnorthoamide Derivatives

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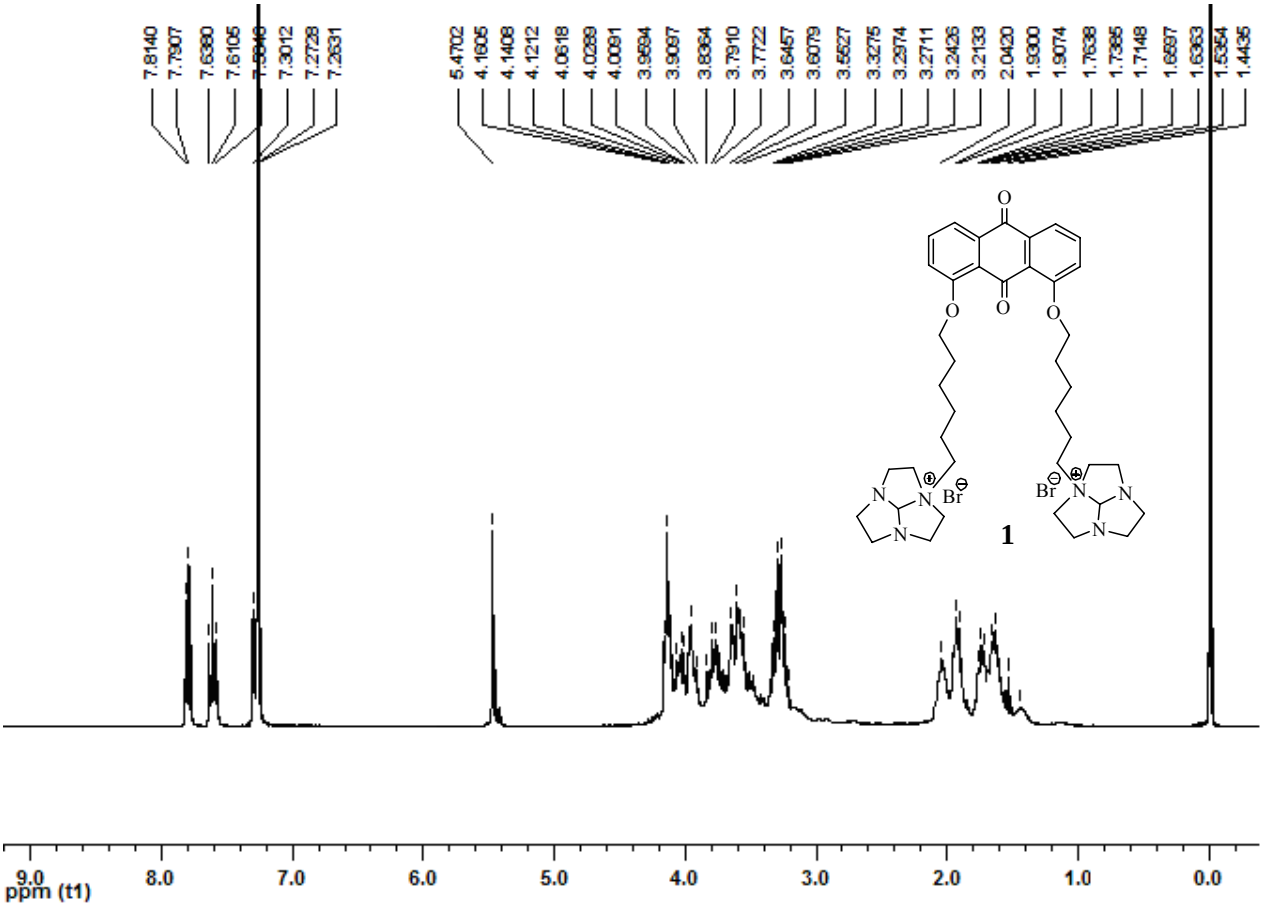
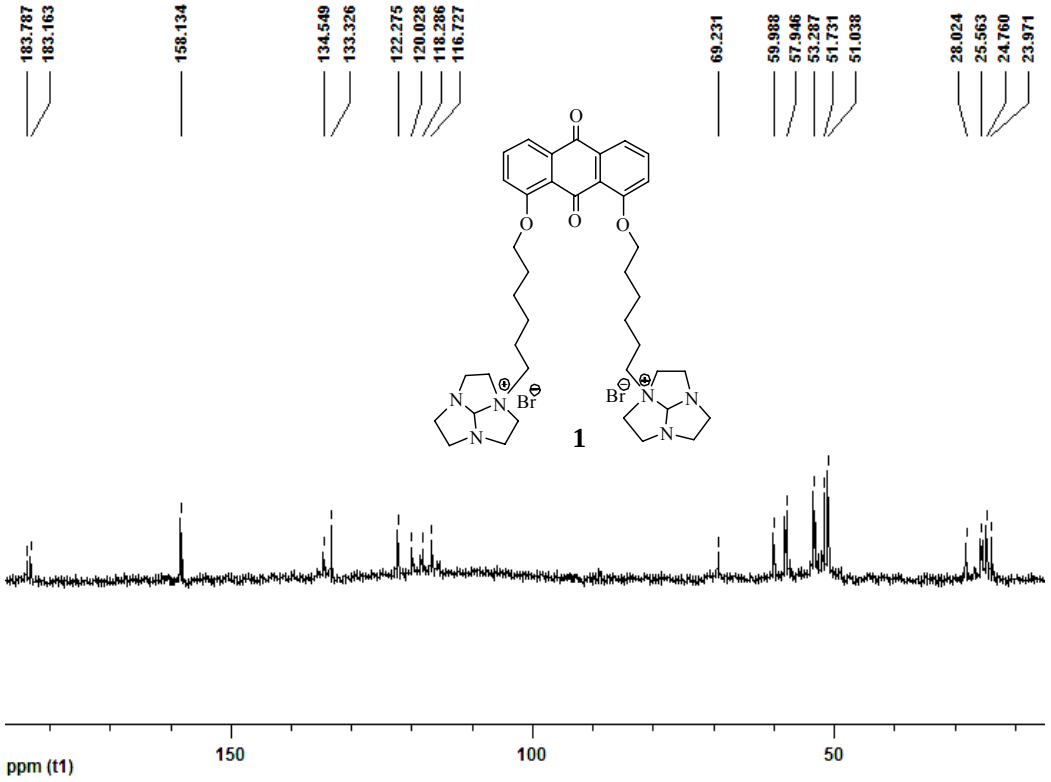
NMR and ESI-MS spectra

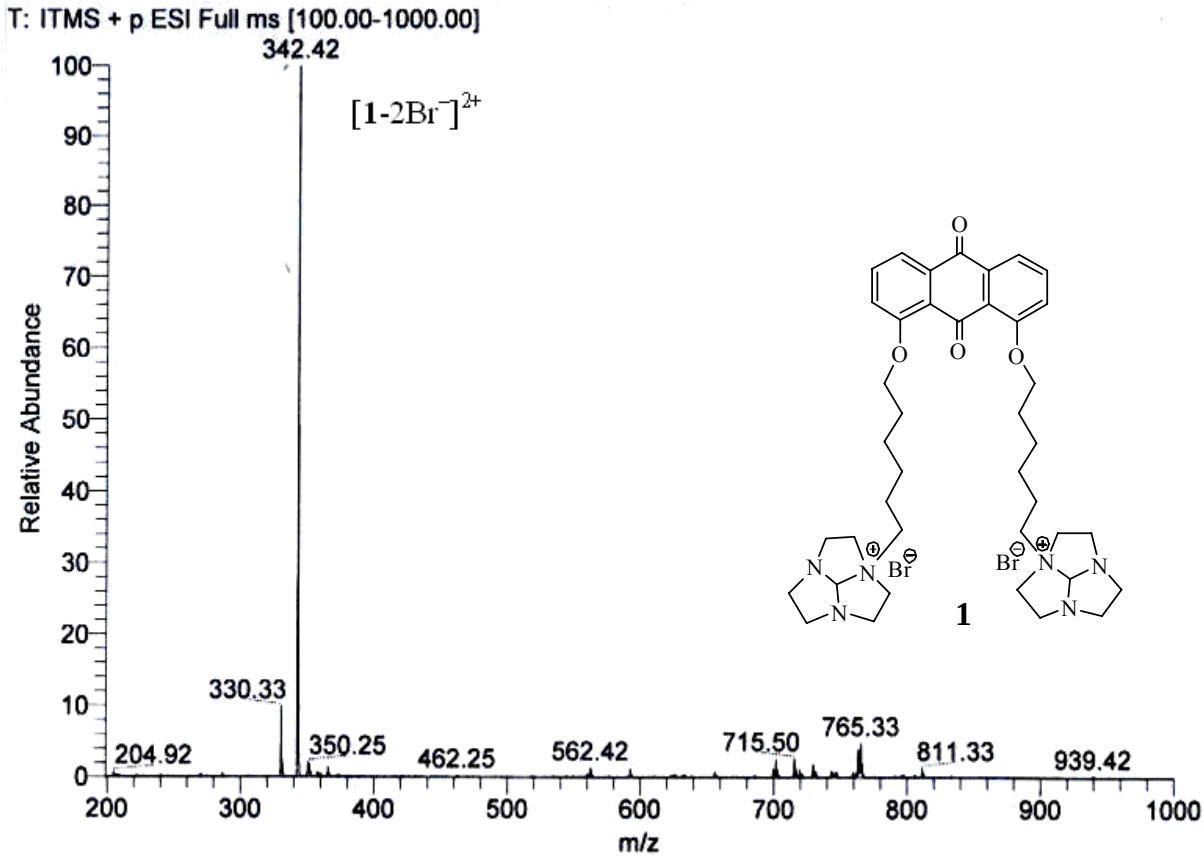


^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **1**.

Supplementary Information



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



ESI–MS spectrum of **1**.

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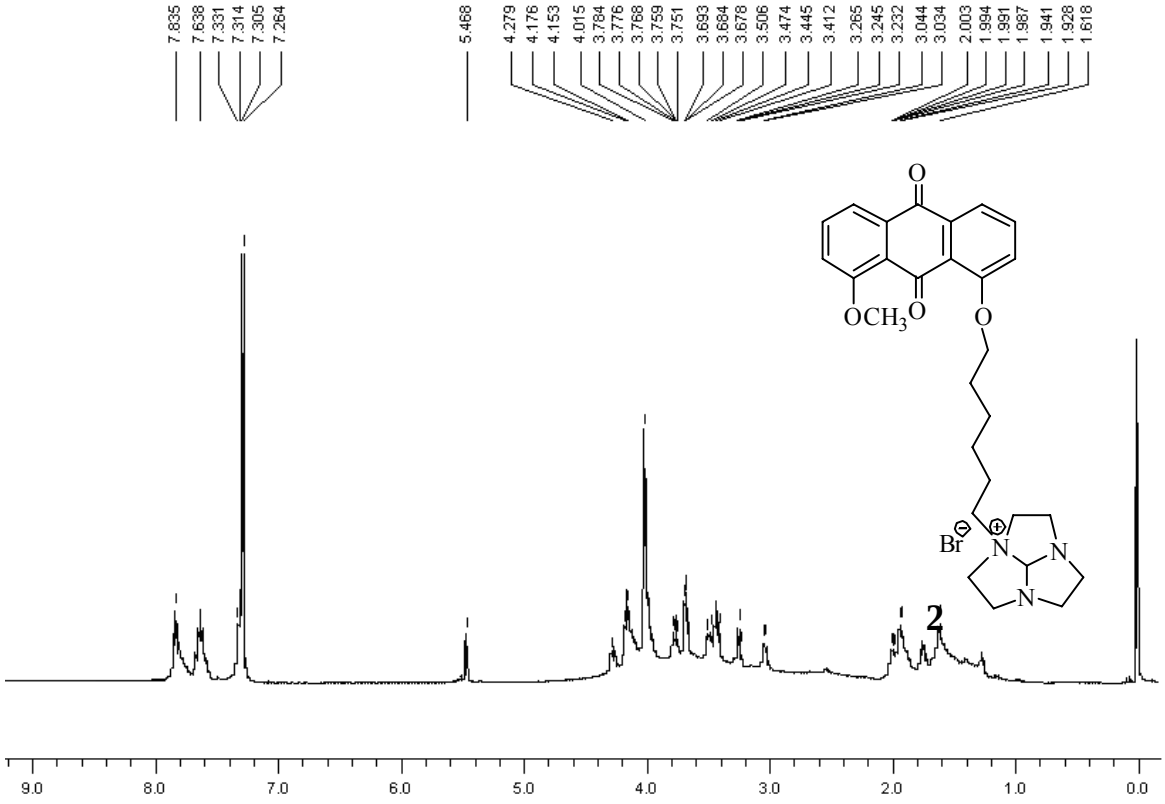
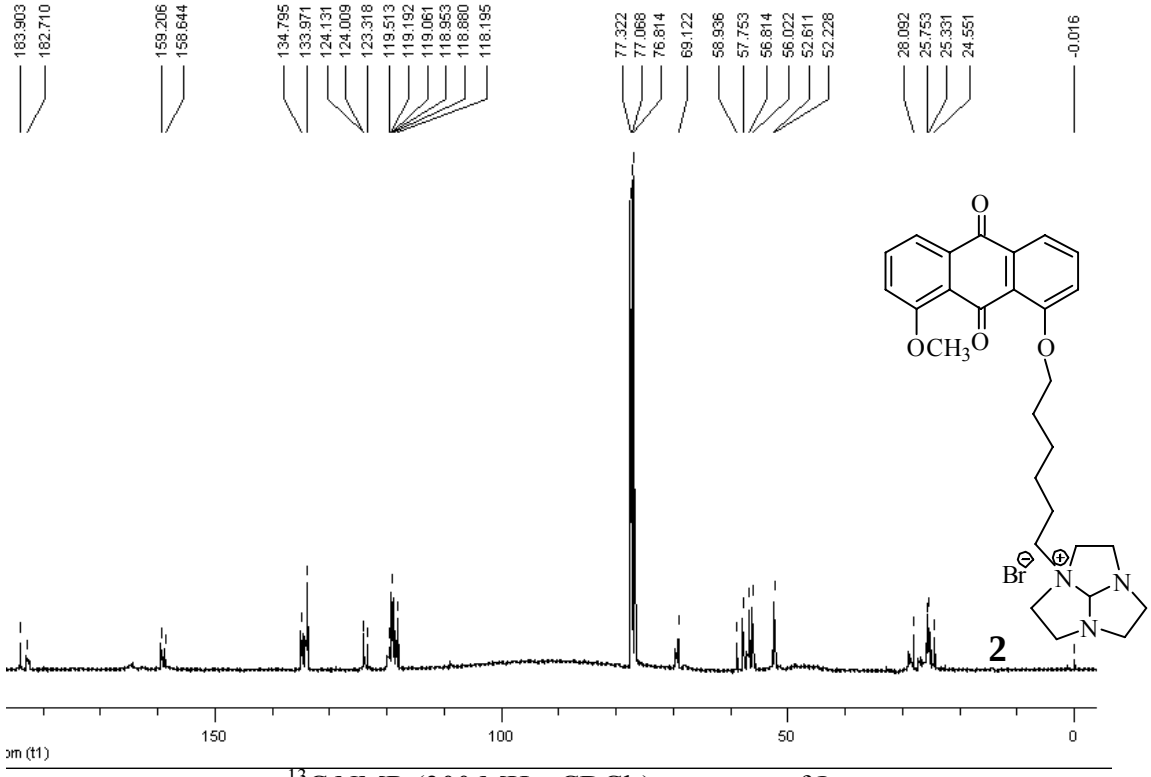


^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **2**.



^{13}C NMR (300 MHz, $CDCl_3$) spectrum of **2**

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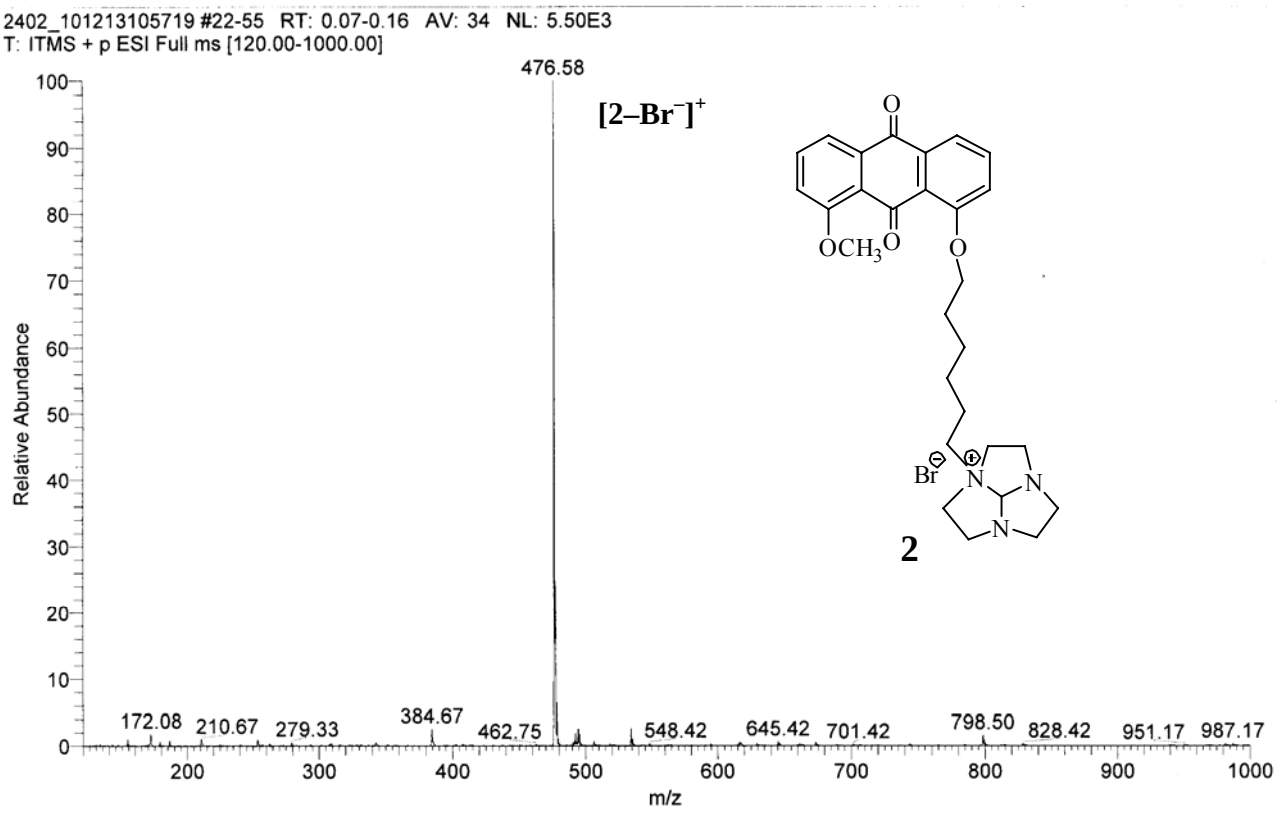
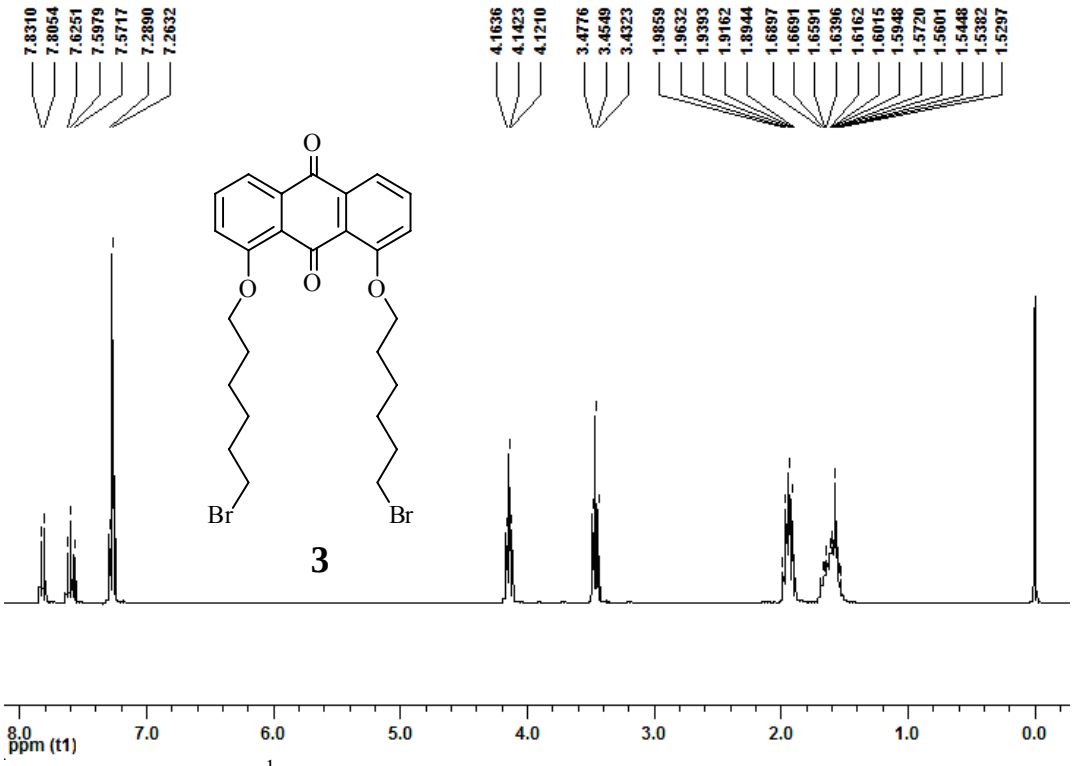


ESI–MS spectrum of **2**.



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

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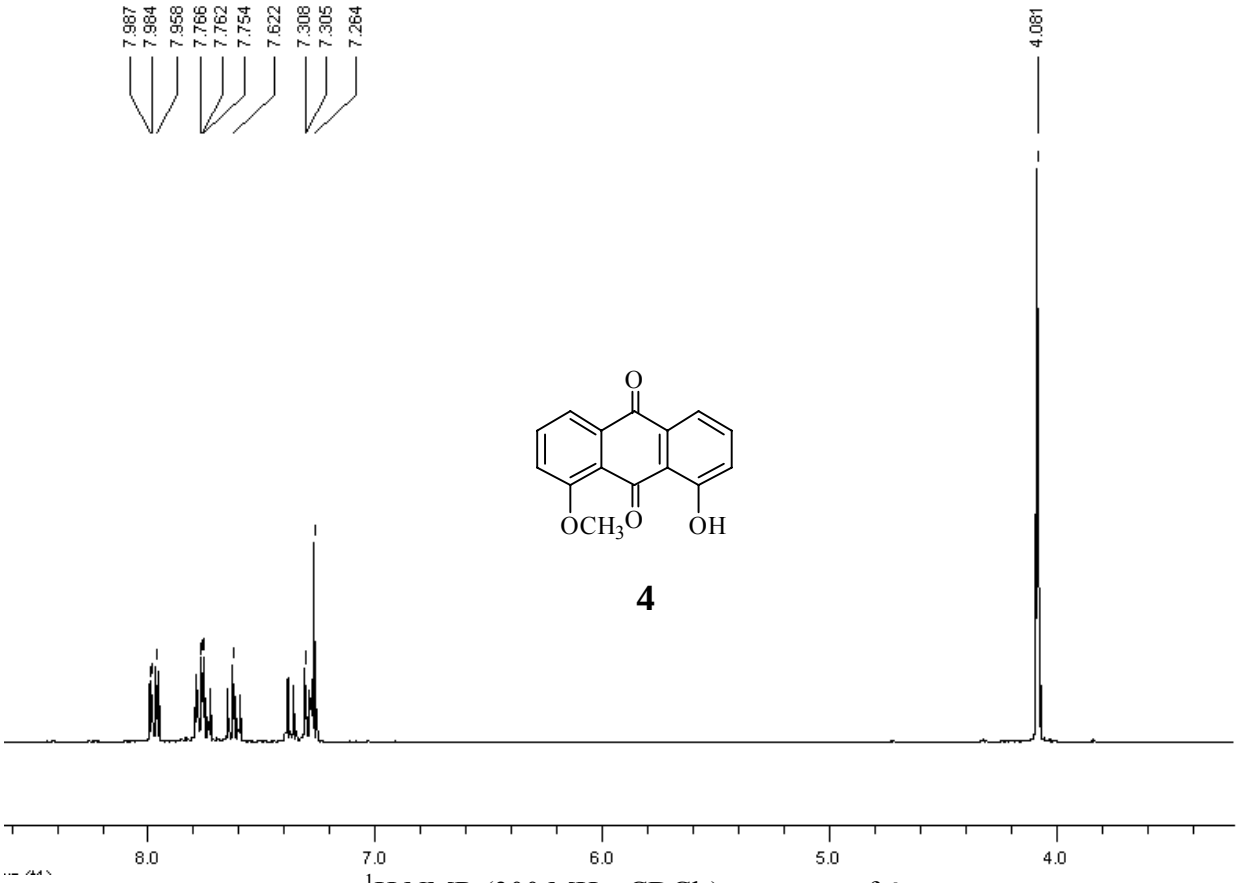
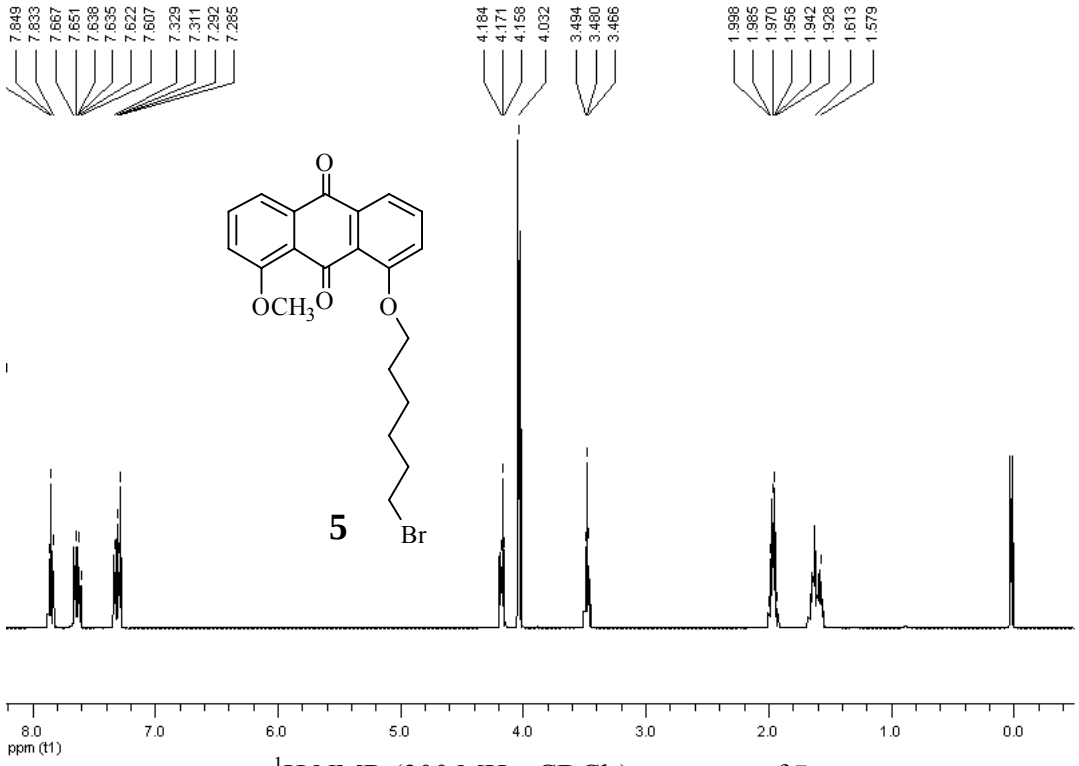


^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **4**.



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **5**.

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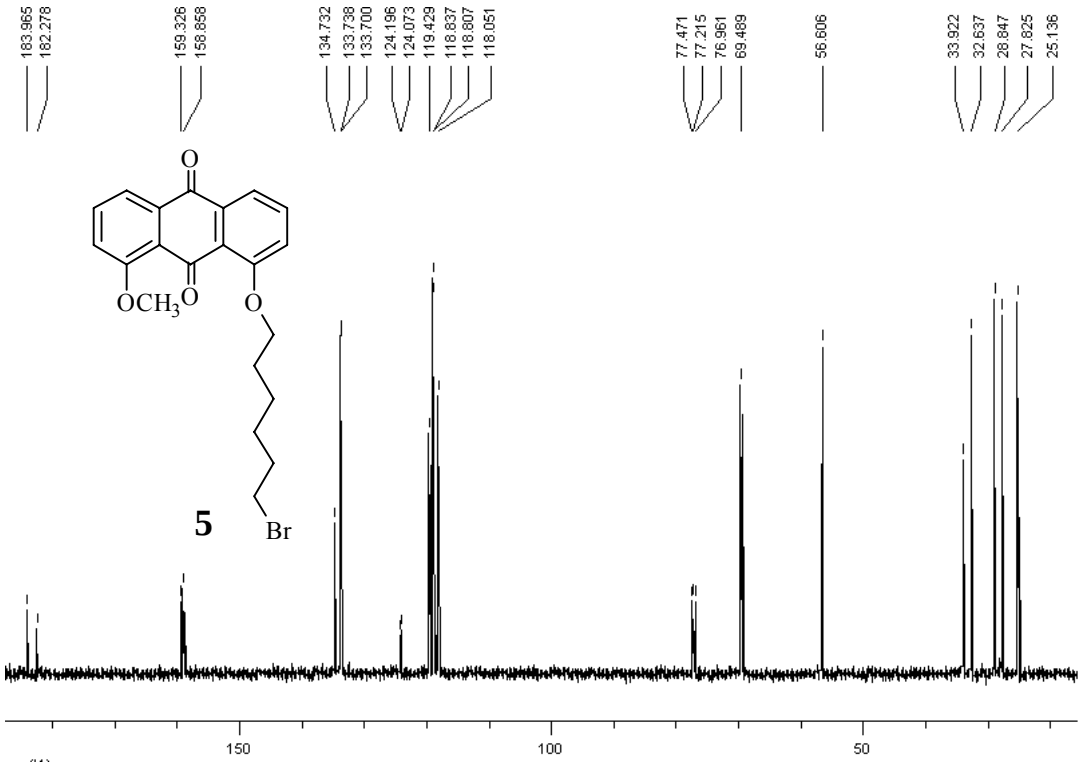
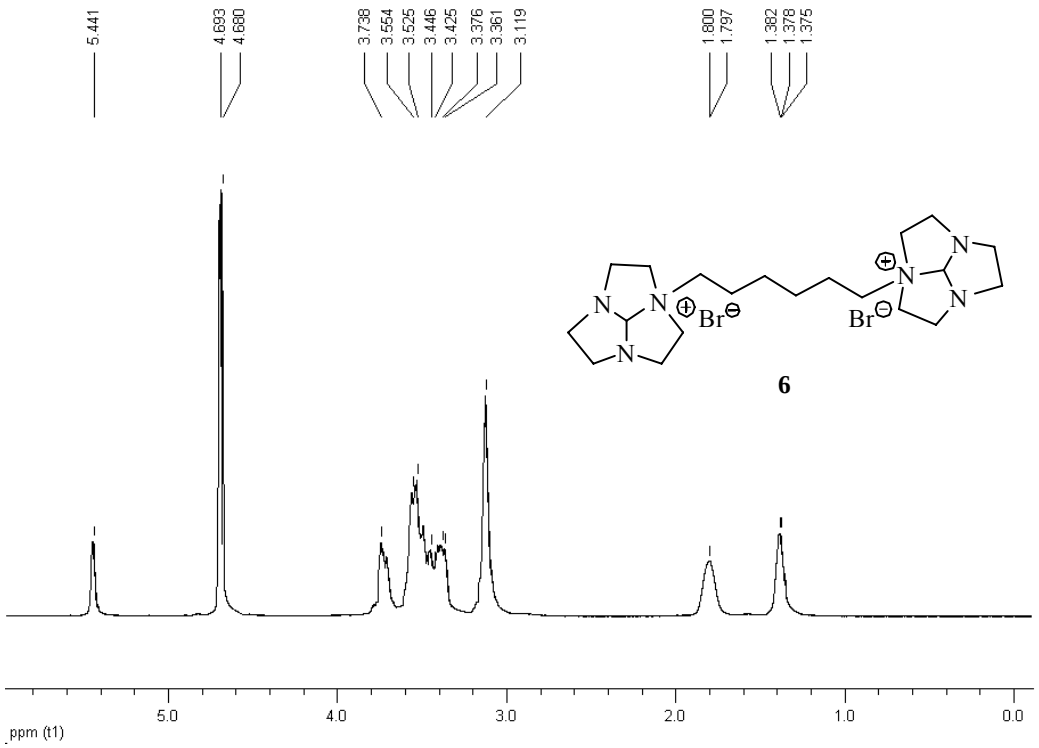
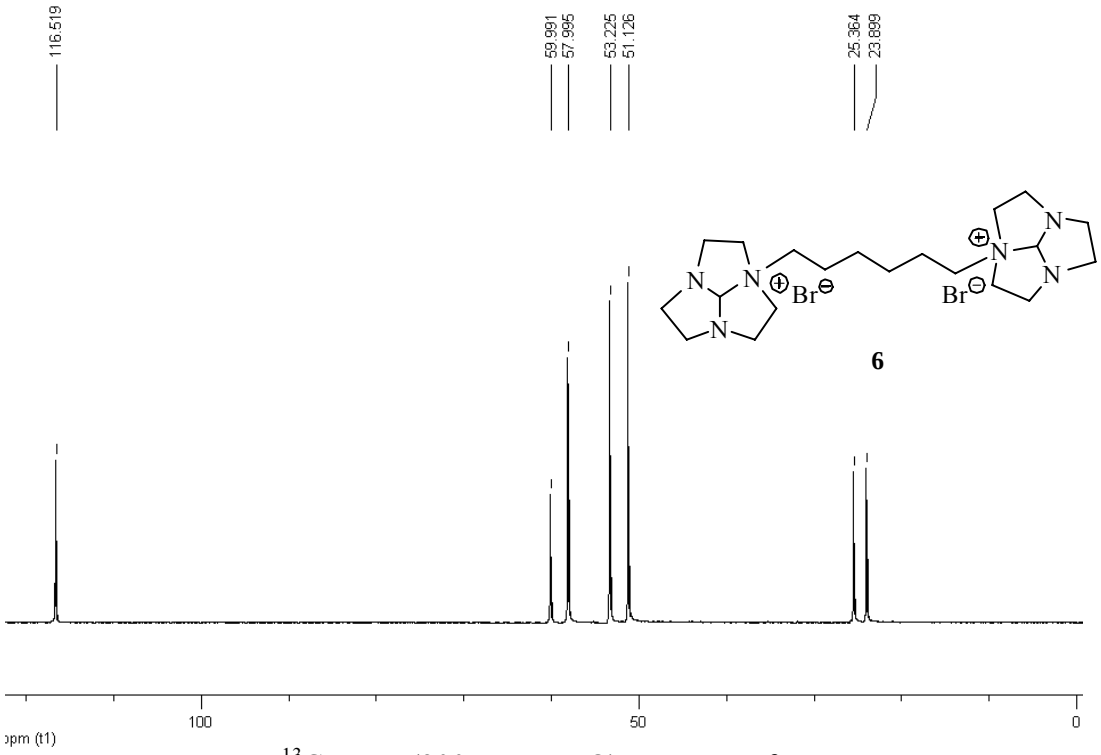


^{13}C NMR (300 MHz, $CDCl_3$) spectrum of **5**.

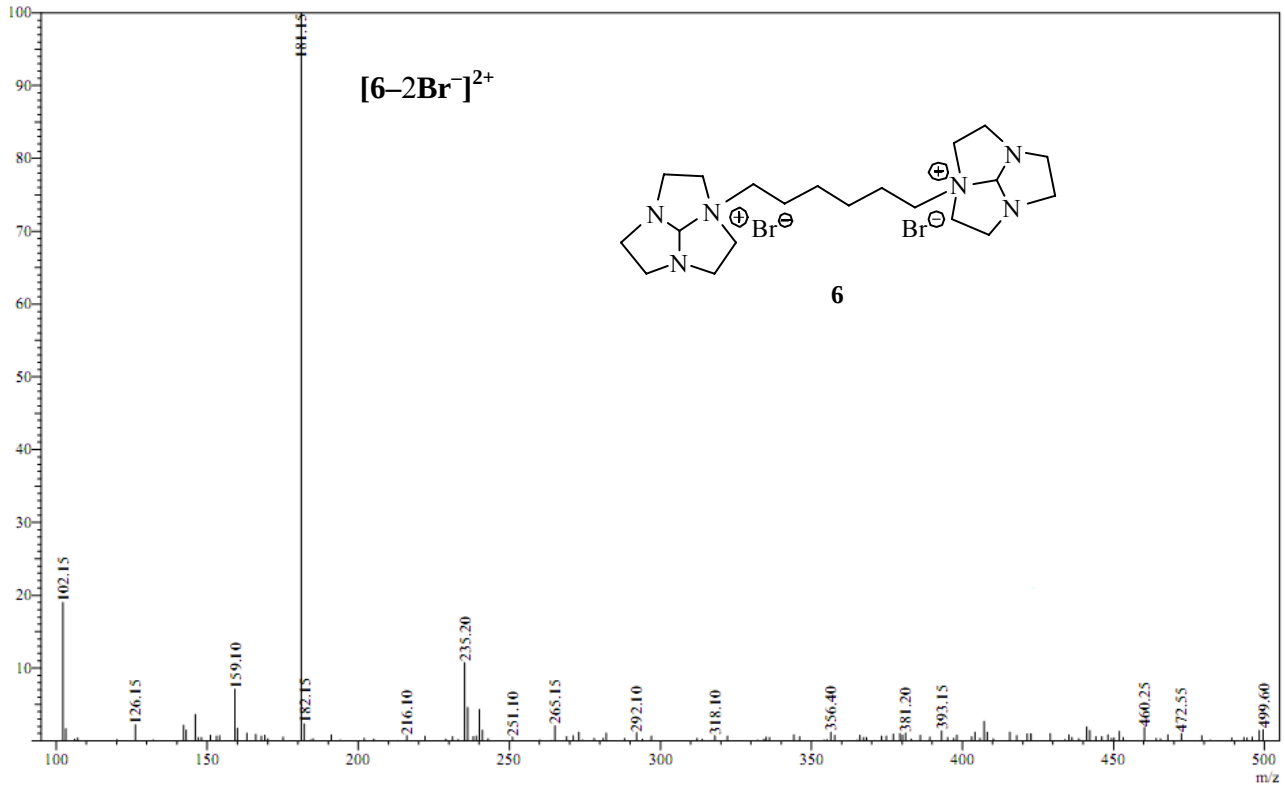


^{1}H NMR (300 MHz, D_2O) spectrum of **6**.

Supplementary Information

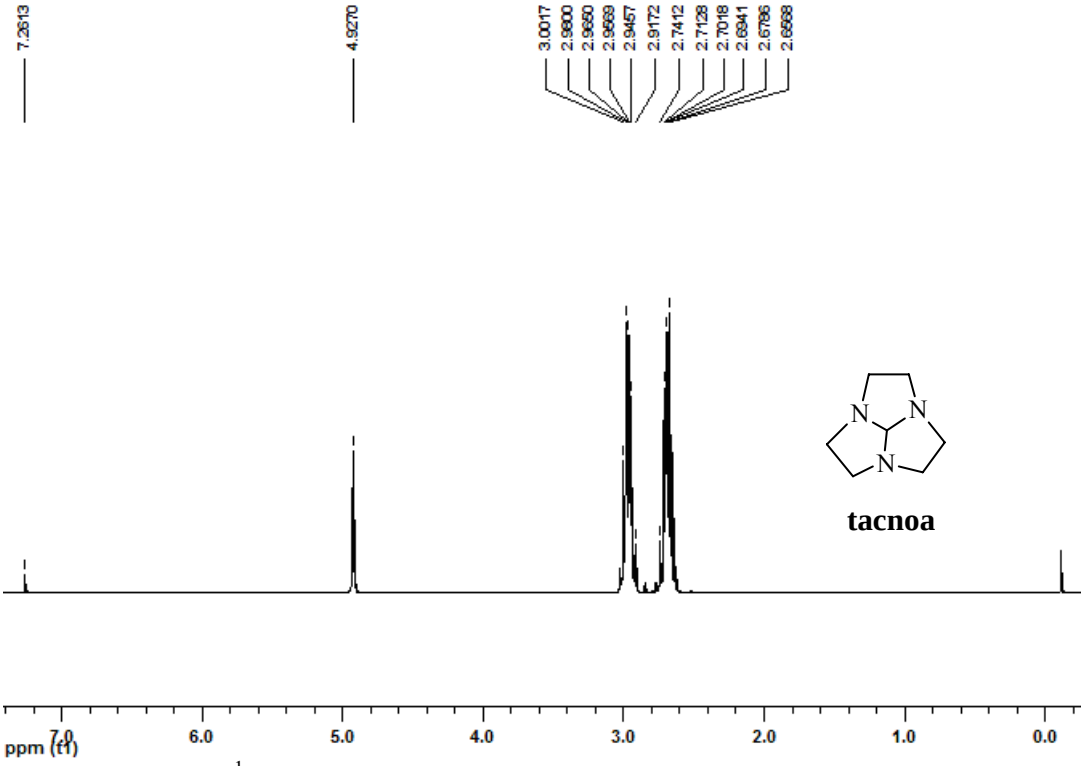


^{13}C NMR (300 MHz, D_2O) spectrum of **6**.

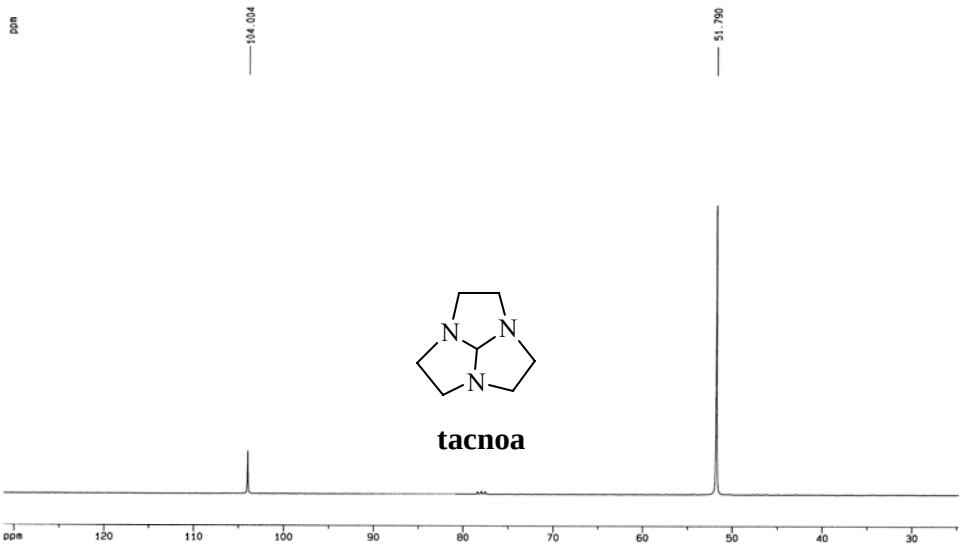


ESI–MS spectrum of **6**.

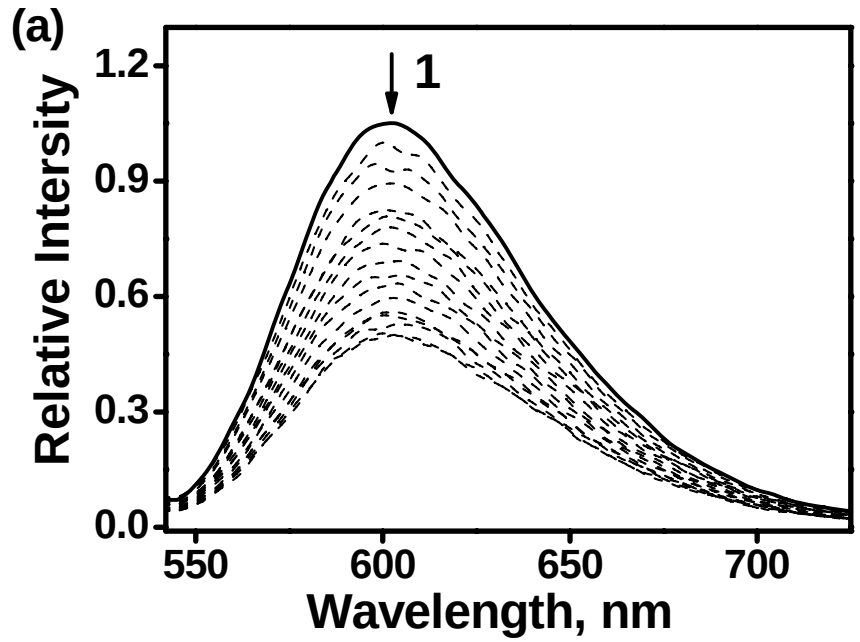
Supplementary Information

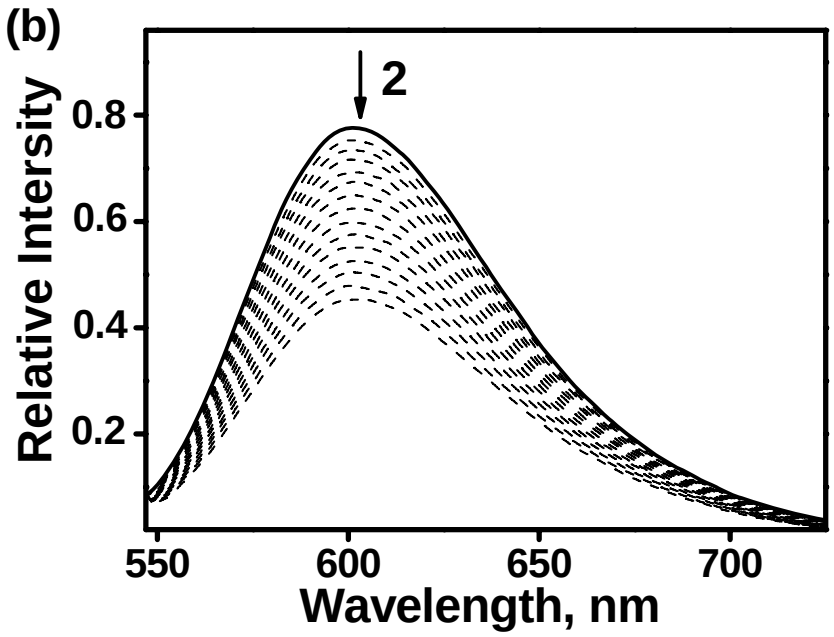


^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **tacnoa**.



^{13}C NMR (300 MHz, $CDCl_3$) spectrum of **tacnoa**.





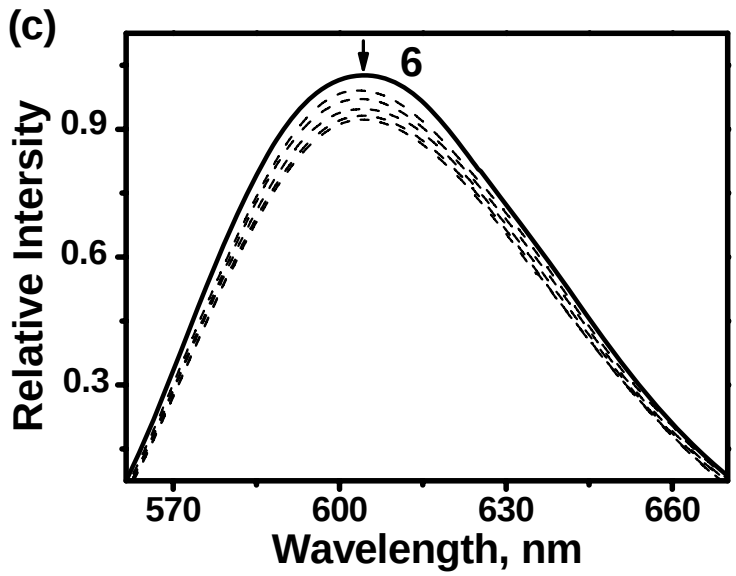


Figure S1. Emission spectra of EB bound to DNA in the absence (—) and presence (---) of (a) **1** (r = 0, 0.05, 0.10, 0.15, 0.21, 0.26, 0.36, 0.41, 0.46, 0.51, 0.56, 0.62, 0.67, 0.72, 0.77, 0.82, 0.92), (b) **2** (r = 0, 0.04, 0.07, 0.10, 0.15, 0.19, 0.25, 0.31, 0.37, 0.44, 0.51, 0.60, 0.68, 0.78, 0.89), (c) **6** (r = 0, 0.3, 0.5, 0.7, 0.85, 0.95), from top to bottom . (r = [compound] / [CT–DNA]; [CT–DNA] = 39 μM; [EB] = 3.9 μM; λ_{ex} = 530 nm)

Supplementary Information

pH dependence

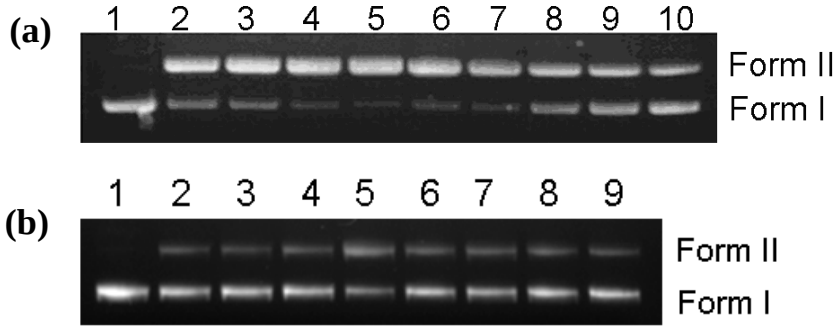


Figure S2. Agarose gel (1%) of pUC19 DNA (0.025 mM bp) incubated at 37 °C for 16 h with 0.033 mM (a) **1** and (b) **2** in buffer of different pH values (50 mM Tris-HCl). Lane 1, DNA control; (a) Lanes 2-10, pH 6.0, 6.50, 6.75, 7.25, 7.75, 8.00, 8.25, 8.50 and 9.00; (b) Lanes 2-9, pH 6.0, 6.50, 6.75, 7.25, 7.75, 8.0, 8.5 and 9.00, respectively.

DNA cleavage by compound 3 alone (control assays)

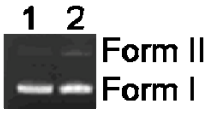


Figure S3. Agarose gel (1%) of pUC19 DNA (0.025 mM bp) incubated at 37°C for 16 h in the presence of 1,8-Bis(6-bromohexyloxy)anthraquinone (**3**) (0.027 mM). Lane 1, DNA control; Lane 2, 0.027 mM **3**.

Table S1. Analysis of DNA cleavage in the presence of **3**[a]

compound	DNA %	
	Form I	Form II
DNA control	96.79	3.21
3	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.

Supplementary Information

Ionic strength dependence

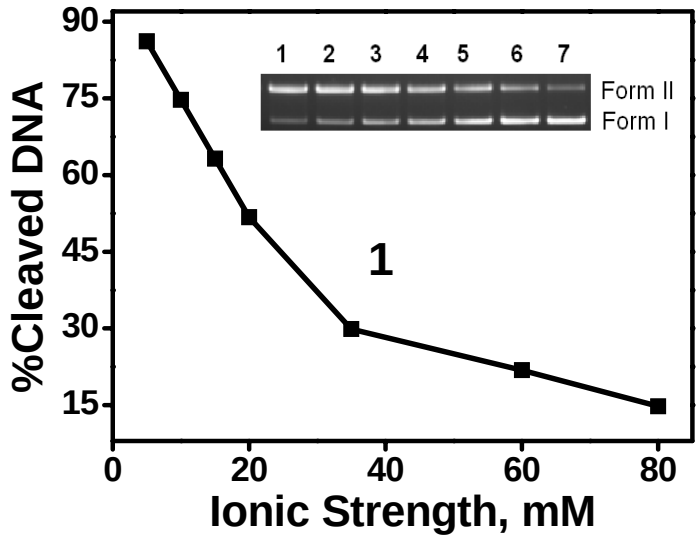
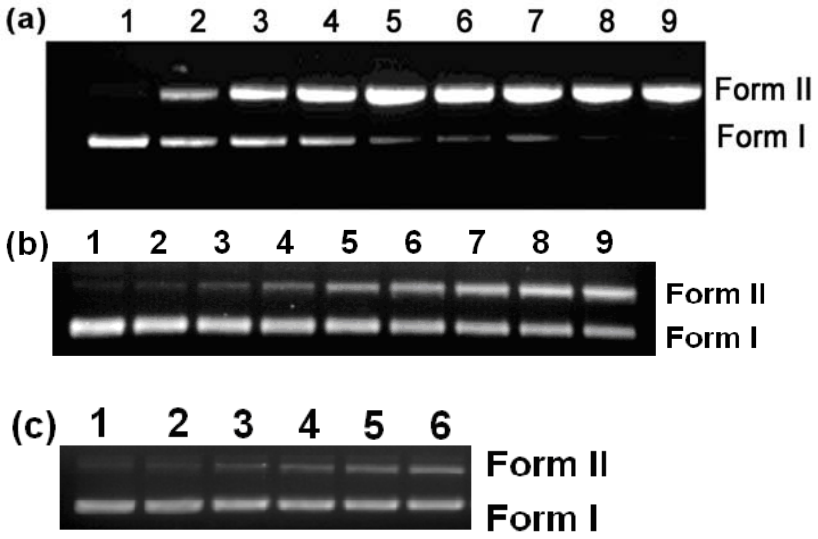


Figure S4. Ionic strength-dependent profile for DNA cleavage promoted by compound **1** (0.033 mM). The inset shows agarose gel (1%) of the DNA cleavage reaction products promoted by compound **1** (0.033 mM) for 16 h at 37 °C and pH 7.25 (50 mM Tris-HCl) under different ionic strength conditions: lanes 1-7, ionic strength of 5, 10, 15, 20, 35, 60 and 80 mM, respectively.

Concentration dependence



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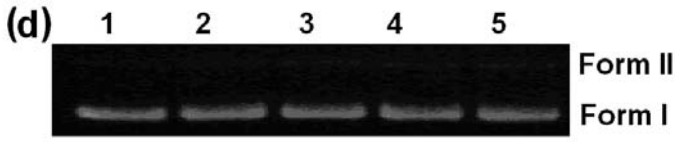


Figure S5. Agarose gel (1%) of pUC19 DNA (0.025 mM bp) incubated for 16.0 h at 37 °C with different concentrations of (a) **1** (b) **2** (c) **6** (d) tacnoa in pH 7.25 buffer (50 mM Tris–HCl / 5 mM NaCl). (a) Lanes 1 - 9, 0, 0.0006, 0.002, 0.006, 0.013, 0.027, 0.040, 0.053, 0.067 mM **1**, respectively; (b) Lanes 1 - 9, 0, 0.0006, 0.002, 0.006, 0.013, 0.027, 0.040, 0.053, 0.067 mM **2**, respectively; (c) Lanes 1 - 6, 0, 0.006, 0.027, 0.034, 0.053 and 0.067 mM **6**, respectively. (d) Lanes 1 - 5, 0, 0.025, 0.034, 0.053 and 0.067 mM tacnoa, respectively.

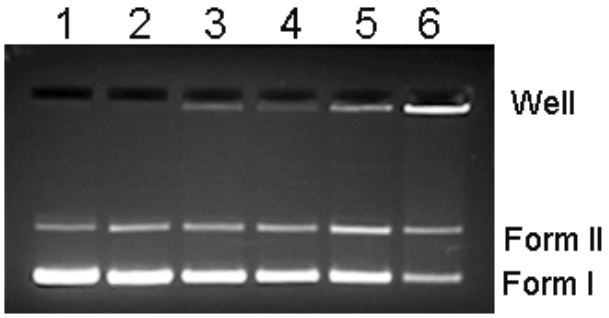


Figure S6. Agarose gel (1%) of pUC19 DNA (0.025 mM bp) incubated for 1.5 h at 20°C in pH 7.25 buffer (50 mM Tris–HCl / 5 mM NaCl) with different concentrations of **1**. Lanes 1 - 6, 0, 0.050, 0.067, 0.133, 0.267 and 0.400 mM **1**, respectively.

Supplementary Information

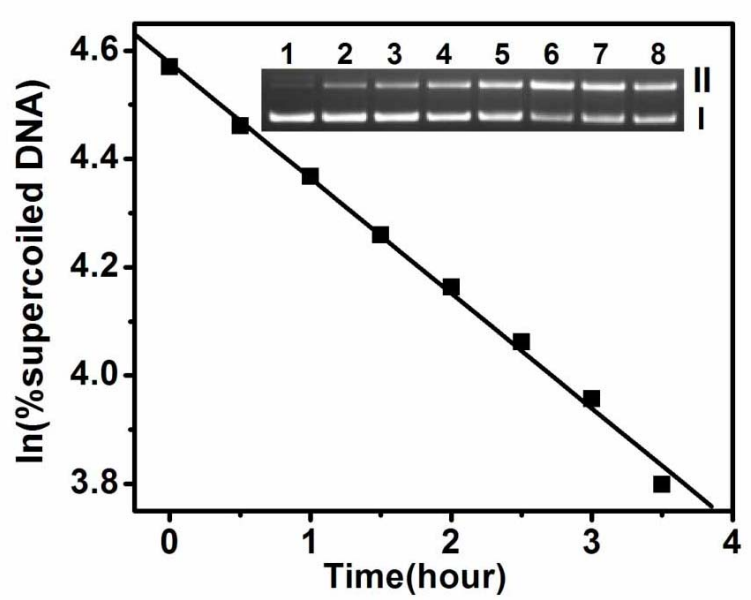
Kinetics data

Table S2. Apparent initial first-order rate constants of DNA cleavage at various concentrations of compounds **1**, **2** and **6**. The reactions were carried out at 37 °C in 50 mM Tris-HCl/ 5 mM NaCl buffer (pH 7.25).

	1		**2**		**6**	
	[1]/mM	k_{obs}/h^{-1}	**[2]**/mM	k_{obs}/h^{-1}	**[6]**/mM	k_{obs}/h^{-1}
1	0.002	0.031±0.0012	0.006	0.021 ± 0.0016	0.012	0.015 ± 0.0009
2	0.006	0.064±0.0020	0.012	0.031 ± 0.0012	0.027	0.029± 0.0013
3	0.012	0.102±0.0021	0.027	0.050 ± 0.0024	0.033	0.035± 0.0021
4	0.027	0.170±0.0083	0.033	0.058 ± 0.0022	0.053	0.040± 0.0007
5	0.033	0.183±0.0105	0.053	0.062 ± 0.0024		
6	0.040	0.195±0.0122				
7	0.053	0.213± 0.0055				

Supplementary Information

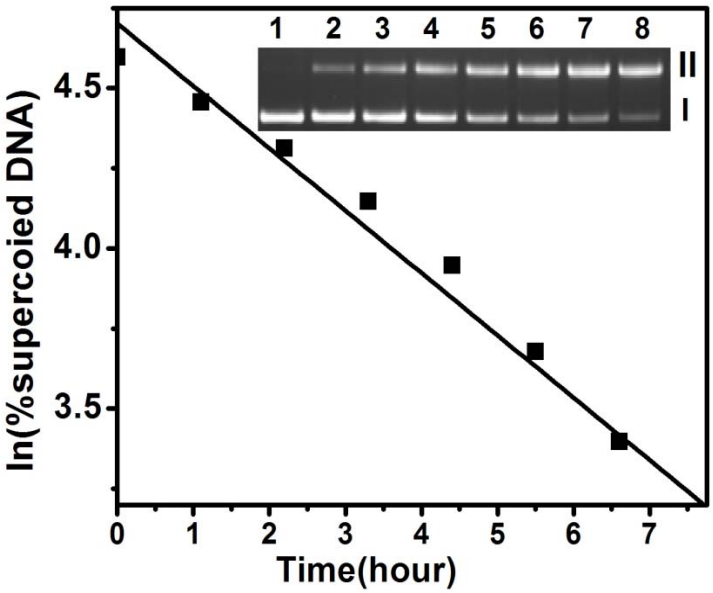
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.053mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.57	96.53	3.47
2	0.50	4.46	86.56	13.44
3	1.00	4.37	78.85	21.15
4	1.50	4.26	70.81	29.19
5	2.00	4.16	64.28	35.72
6	2.50	4.06	44.67	55.33
7	3.00	3.96	52.34	47.66
8	3.50	3.80	58.08	41.92

Supplementary Information

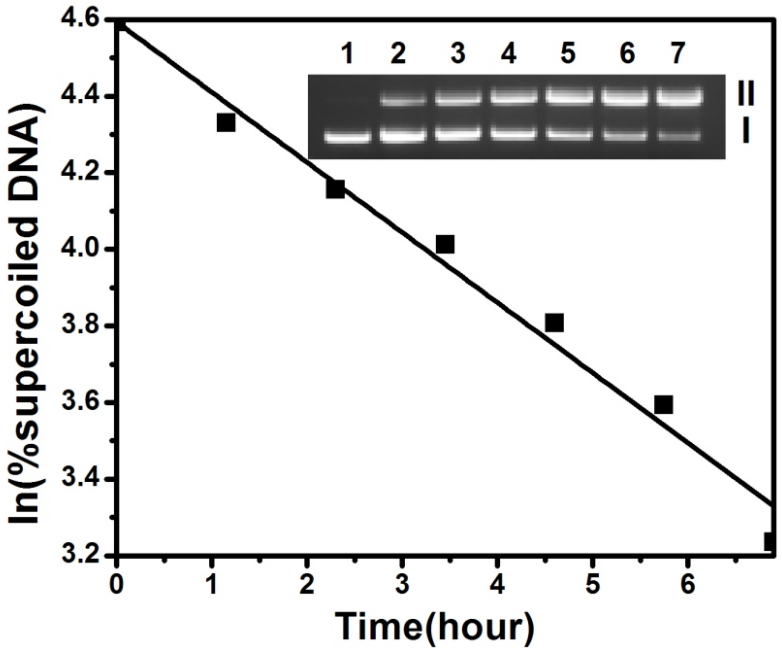
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.040mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0	4.60	99.14	0.86
2	1.1	4.46	86.17	13.83
3	2.2	4.31	74.72	25.28
4	3.3	4.15	63.21	36.79
5	4.4	3.95	51.76	48.24
6	5.5	3.68	39.58	60.42
7	6.6	3.40	29.88	70.12
8	7.7	3.08	21.86	78.14

Supplementary Information

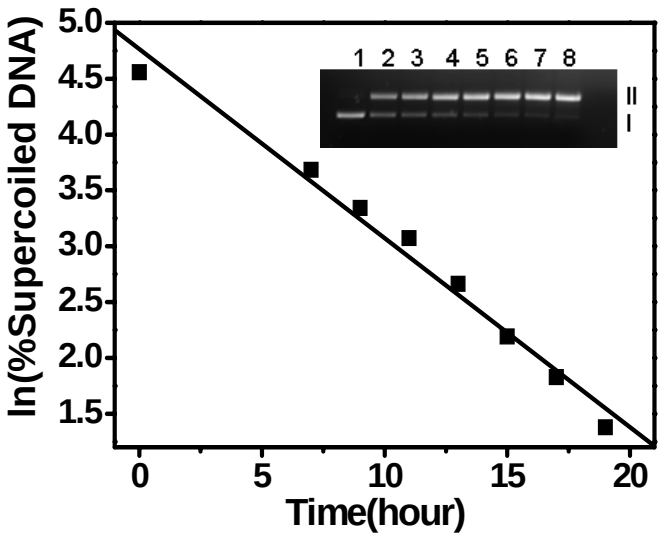
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.033mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.59	98.87	1.13
2	1.15	4.33	76.03	23.97
3	2.30	4.16	63.86	36.14
4	3.45	4.01	55.28	44.72
5	4.60	3.81	45.09	54.91
6	5.75	3.59	36.36	63.64
7	6.90	3.24	25.44	74.56

Supplementary Information

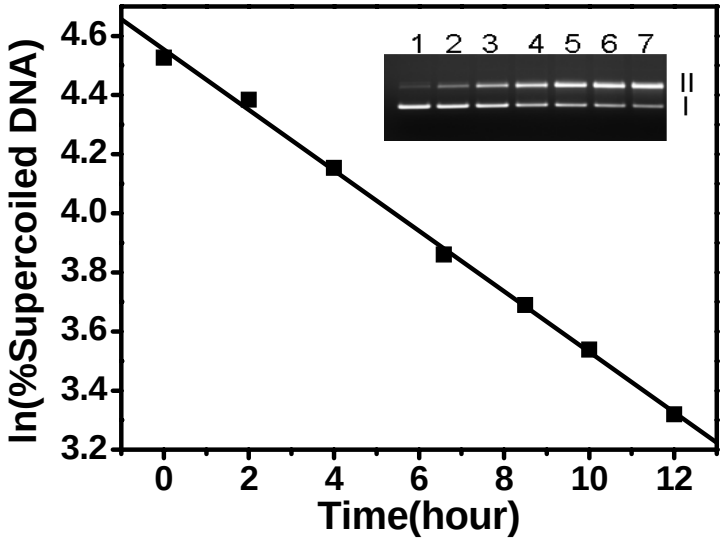
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.027mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.56	95.34	4.66
2	7.00	3.68	39.78	60.22
3	9.00	3.34	28.30	71.70
4	11.00	3.07	21.57	78.43
5	13.00	2.66	14.35	85.65
6	15.00	2.19	8.95	91.05
7	17.00	1.83	6.21	93.79
8	19.00	1.38	3.97	96.03

Supplementary Information

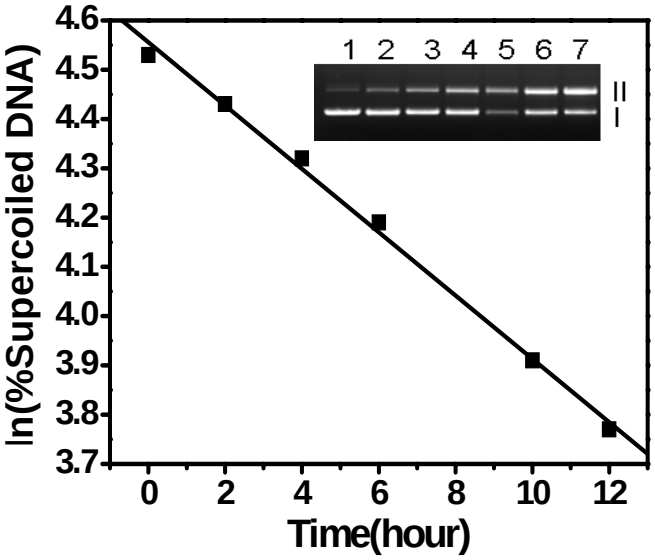
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.012mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.53	92.46	7.54
2	2.00	4.38	80.16	19.84
3	4.00	4.15	63.66	36.34
4	6.58	3.86	47.46	52.54
5	8.50	3.69	40.02	59.98
6	10.00	3.54	34.43	65.57
7	12.00	3.32	27.64	72.36

Supplementary Information

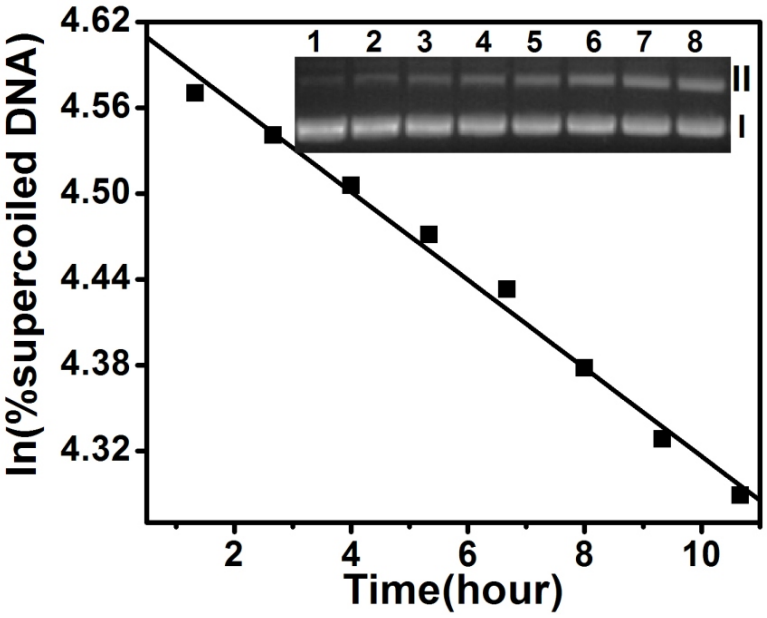
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.006mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.53	92.53	7.47
2	2.00	4.43	83.83	16.17
3	4.00	4.32	75.30	24.70
4	6.00	4.19	66.31	33.69
5	8.00	3.75	42.59	57.41
6	10.00	3.91	49.92	50.08
7	12.00	3.77	43.28	56.72

Supplementary Information

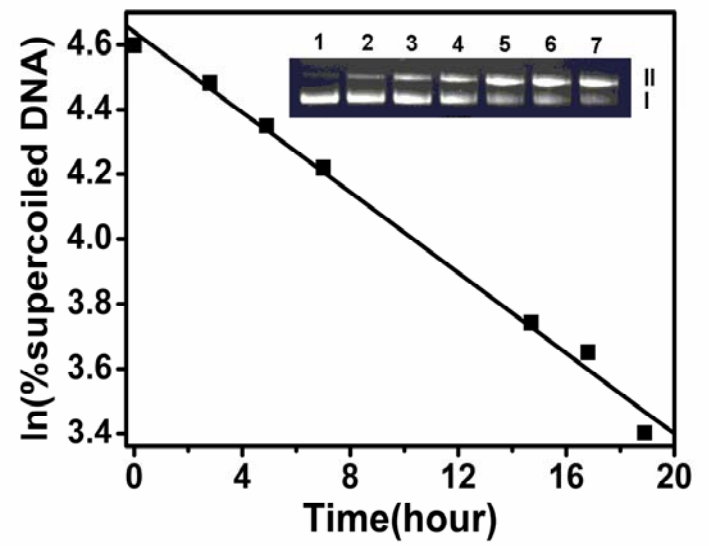
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **1** (0.002mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	1.33	4.57	96.56	3.44
2	2.67	4.54	93.78	6.22
3	4.00	4.51	90.52	9.48
4	5.33	4.47	87.46	12.54
5	6.67	4.43	84.18	15.82
6	8.00	4.38	79.70	20.30
7	9.33	4.33	75.83	24.17
8	10.67	4.29	72.89	27.11

Supplementary Information

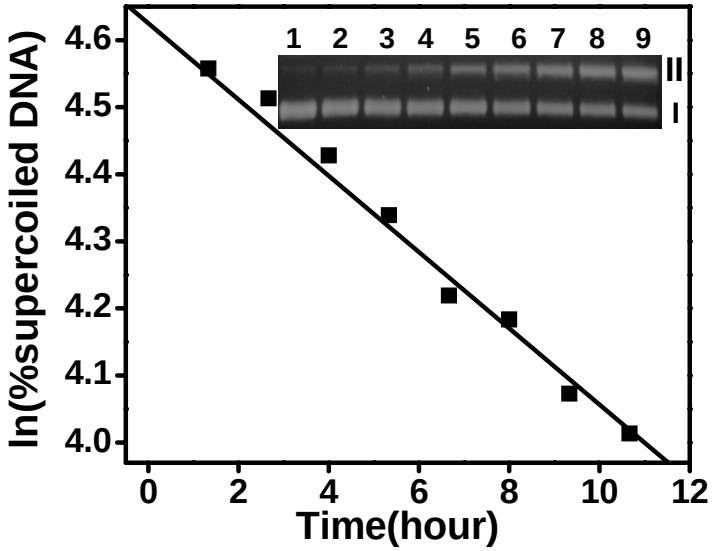
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.053mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0	4.60	99.32	0.68
2	3	4.48	88.40	11.60
3	5	4.35	77.36	22.64
4	7	4.22	67.96	32.04
5	15	3.74	42.19	57.81
6	17	3.65	38.53	61.47
7	19	3.40	30.00	70.00

Supplementary Information

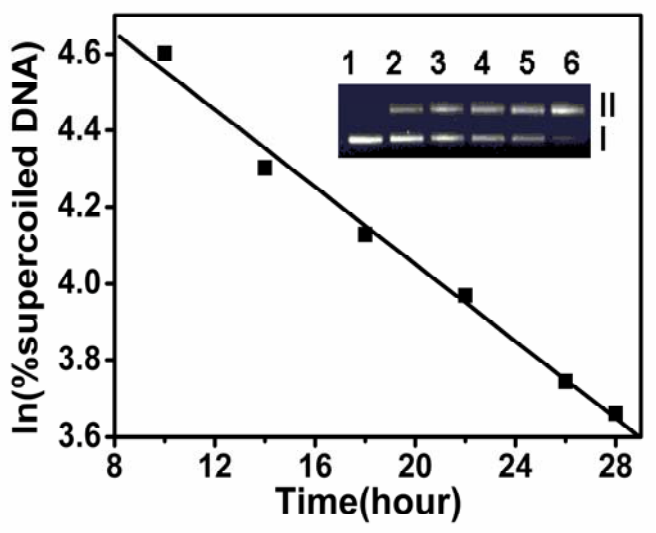
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.033mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	1.33	4.56	95.35	4.65
2	2.67	4.51	91.20	8.80
3	4.00	4.43	83.75	16.25
4	5.33	4.34	76.64	23.36
5	6.67	4.22	67.97	32.03
6	8.00	4.18	65.60	34.40
7	9.33	4.07	58.73	41.27
8	10.67	4.01	55.34	44.66

Supplementary Information

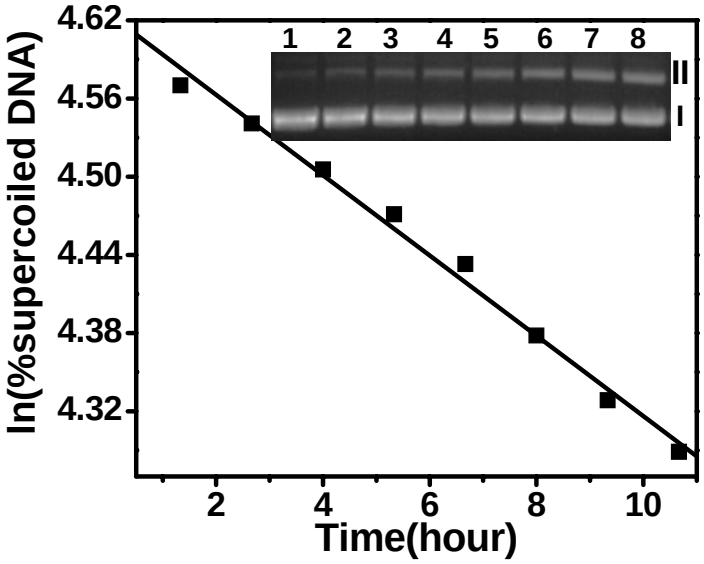
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.027mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	10	4.60	99.62	0.38
2	14	4.30	73.90	26.10
3	18	4.13	62.11	37.89
4	22	3.97	52.91	47.09
5	26	3.74	42.29	57.71
6	28	3.64	38.09	61.91

Supplementary Information

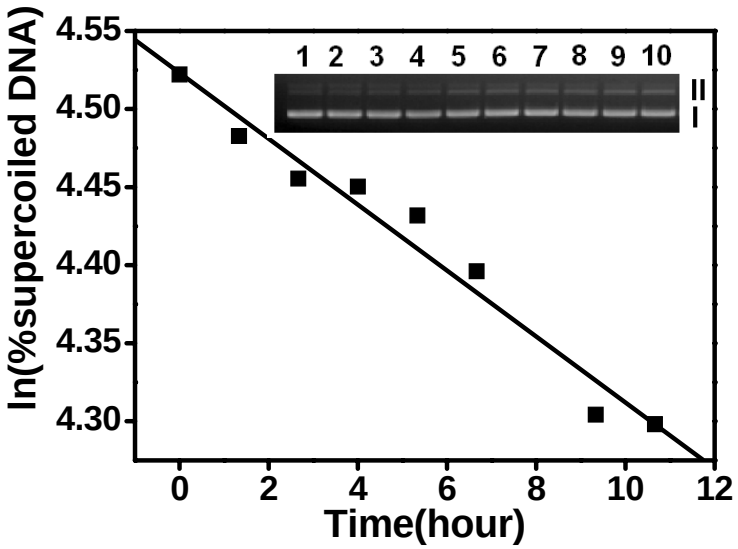
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.012mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	1.33	4.57	96.56	3.44
2	2.67	4.54	93.78	6.22
3	4	4.50	90.52	9.48
4	5.33	4.47	87.46	12.54
5	6.67	4.43	84.18	15.82
6	8	4.38	79.70	20.30
7	9.33	4.32	75.83	24.17
8	10.67	4.29	72.89	27.11

Supplementary Information

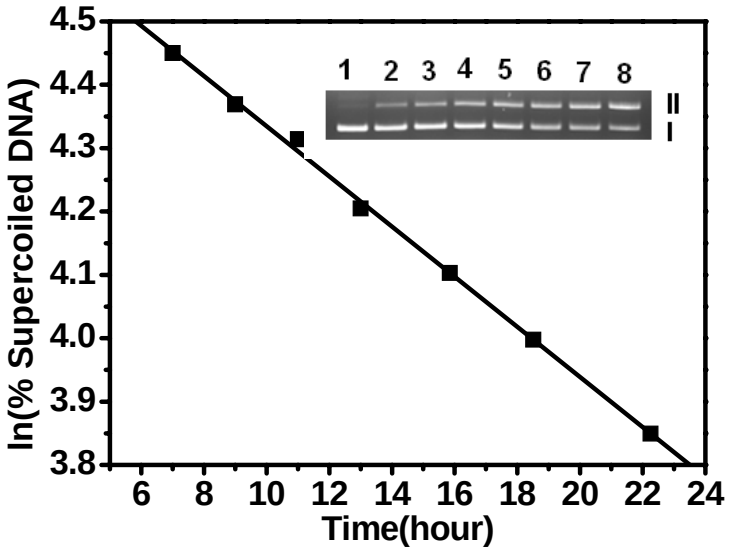
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.006mM). The reaction was carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time/h	Ln (% Form I)	% DNA	
			Form I	Form II
1	0.00	4.52	92.03	7.97
2	1.33	4.48	88.48	11.52
3	2.67	4.46	86.10	13.90
4	4.00	4.45	85.65	14.35
5	5.33	4.43	84.08	15.92
6	6.67	4.39	81.13	18.87
7	9.33	4.30	74.01	25.99
8	10.67	4.29	73.56	26.44

Supplementary Information

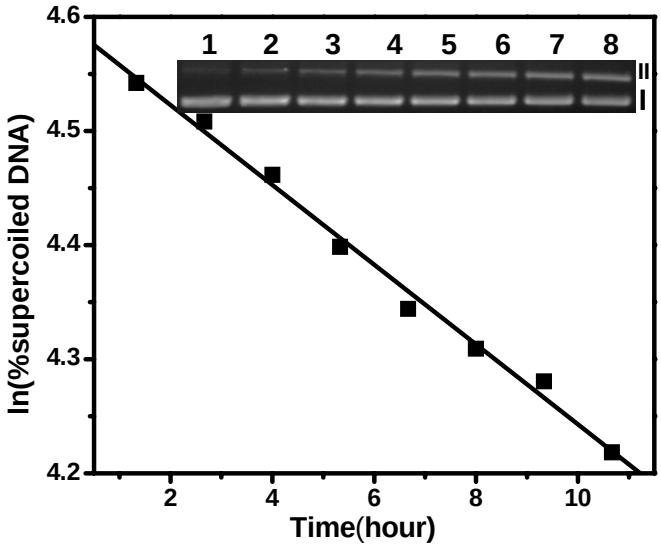
Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **6** (0.053 mM). The reactions were carried out at 37 °C in 50 mM Tris-HCl / 5 mM NaCl buffer (pH 7.25).



Lane	Time (h)	Ln(% Form I)	% DNA	
			Form I	Form II
1	0	4.58	98.24	1.76
2	7	4.45	85.65	14.35
3	9	4.36	78.98	21.02
4	11	4.31	74.74	25.26
5	13	4.20	67.01	32.99
6	16	4.10	60.53	39.47
7	18.5	3.99	54.47	45.53
8	22	3.85	46.98	53.02

Supplementary Information

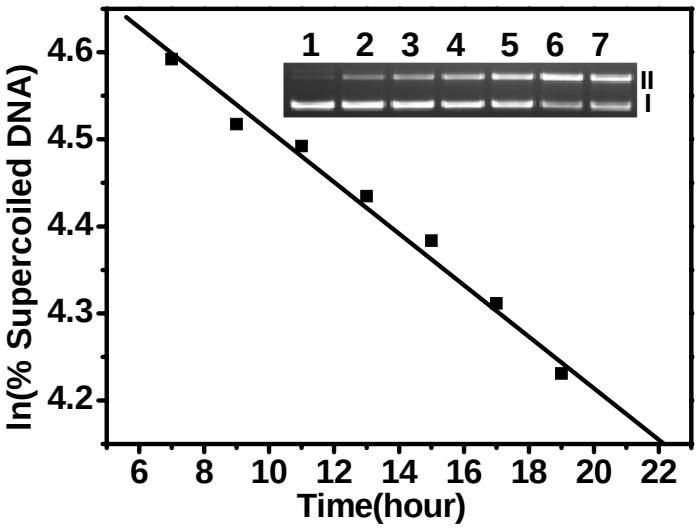
Time course of pUC 19 DNA (0.05 mM bp) cleavage promoted by **6** (0.033 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/5 mM NaCl buffer (pH 7.25).



Lane	Time (h)	Ln(% Form I)	% DNA	
			Form I	Form II
1	1.33	4.54	93.89	6.11
2	2.67	4.51	90.77	9.23
3	4	4.46	86.63	13.37
4	5.33	4.40	81.33	18.67
5	6.67	4.34	77.02	22.98
6	8	4.31	74.38	25.62
7	9.33	4.28	72.28	27.72
8	10.67	4.22	67.92	32.08

Supplementary Information

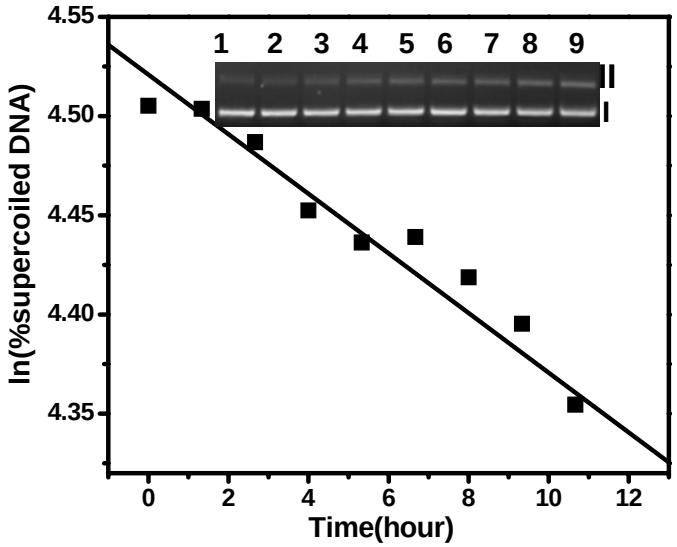
Time course of pUC 19 DNA (0.05 mM bp) cleavage promoted by **6** (0.027 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/5 mM NaCl buffer (pH 7.25).



Lane	Time (h)	Ln(% Form I)	% DNA	
			Form I	Form II
1	7.00	4.59	98.69	1.31
2	9.00	4.51	91.59	8.41
3	11.00	4.49	89.32	10.68
4	13.00	4.43	84.33	15.67
5	15.00	4.38	80.12	19.88
6	17.00	4.31	74.55	25.45
7	19.00	4.231	68.77	31.23

Supplementary Information

Time course of pUC 19 DNA (0.05 mM bp) cleavage promoted by **6** (0.012 mM)**.** The reactions were carried out at 37 °C in 50 mM Tris-HCl/5 mM NaCl buffer (pH 7.25).



Lane	Time (h)	Ln(% Form I)	% DNA	
			Form I	Form II
1	0	4.51	91.29	8.71
2	1.33	4.50	90.34	9.66
3	2.67	4.49	88.84	11.16
4	4	4.45	85.84	14.16
5	5.33	4.44	84.46	15.54
6	6.67	4.43	83.69	15.31
7	8	4.42	82.99	17.01
8	9.33	4.40	81.07	18.93
9	10.67	4.35	77.83	22.17

Supplementary Information

Radical scavengers' inhibition

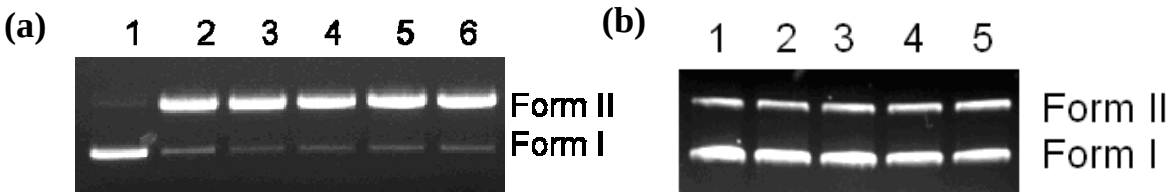


Figure S7. Agarose gel (1%) of pUC19 plasmid DNA (0.025 mM bp) cleaved by (a) **1** and (b) **2** (0.04 mM) in the presence of standard radical scavengers incubated for 16 h at 37 °C in pH 7.25 buffer (50 mM Tris–HCl / 5 mM NaCl). (a) Lane 1, DNA control; lane 2, no scavengers; lanes 3 - 6, in the presence of NaN_3, DMSO, *t*-BuOH and KI, respectively; (b) Lane 1, no scavengers; lanes 2 - 5, in the presence of NaN_3, DMSO, *t*-BuOH and KI, respectively.

Table S3. DNA cleavage promoted by **1** or **2** (0.04 mM) in the presence of standard radical scavengers.

	% DNA			
	1		**2**	
	Form I	Form II	Form I	Form II
DNA control	96.86	3.14	96.86	3.14
Compound only	8.79	91.21	58.54	41.46
NaN_3	4.81	95.19	60.64	39.36
DMSO	5.89	94.11	62.87	39.13
t-BuOH	6.20	93.80	61.97	38.03
KI	7.11	92.89	62.58	37.42

DNA cleavage in the presence of Nucleosides monophosphates or BDNPP

Figure S8. Agarose gel (1%) of pUC 19 DNA (0.05 mM bp) cleavage promoted by 0.05 mM **1** (incubated for 10 h at 37 °C in pH 7.25 (50 mM Tris-HCl)). Lane 1, DNA control; Lane 2, DNA + **1**; Lane 3, DNA + **1** + 0.05 mM adenosine; Lane 4, DNA + **1** + 0.05 mM uridine; Lane 5, DNA + **1** + 0.05 mM guanosine; Lane 6, DNA + **1** + 0.05 mM cytidine; Lane 7, DNA + **1** + 0.10 mM BDNPP; Lane 8, DNA + **1** + 0.20 mM BDNPP.

Supplementary Information

ESI-MS spectra of ApA

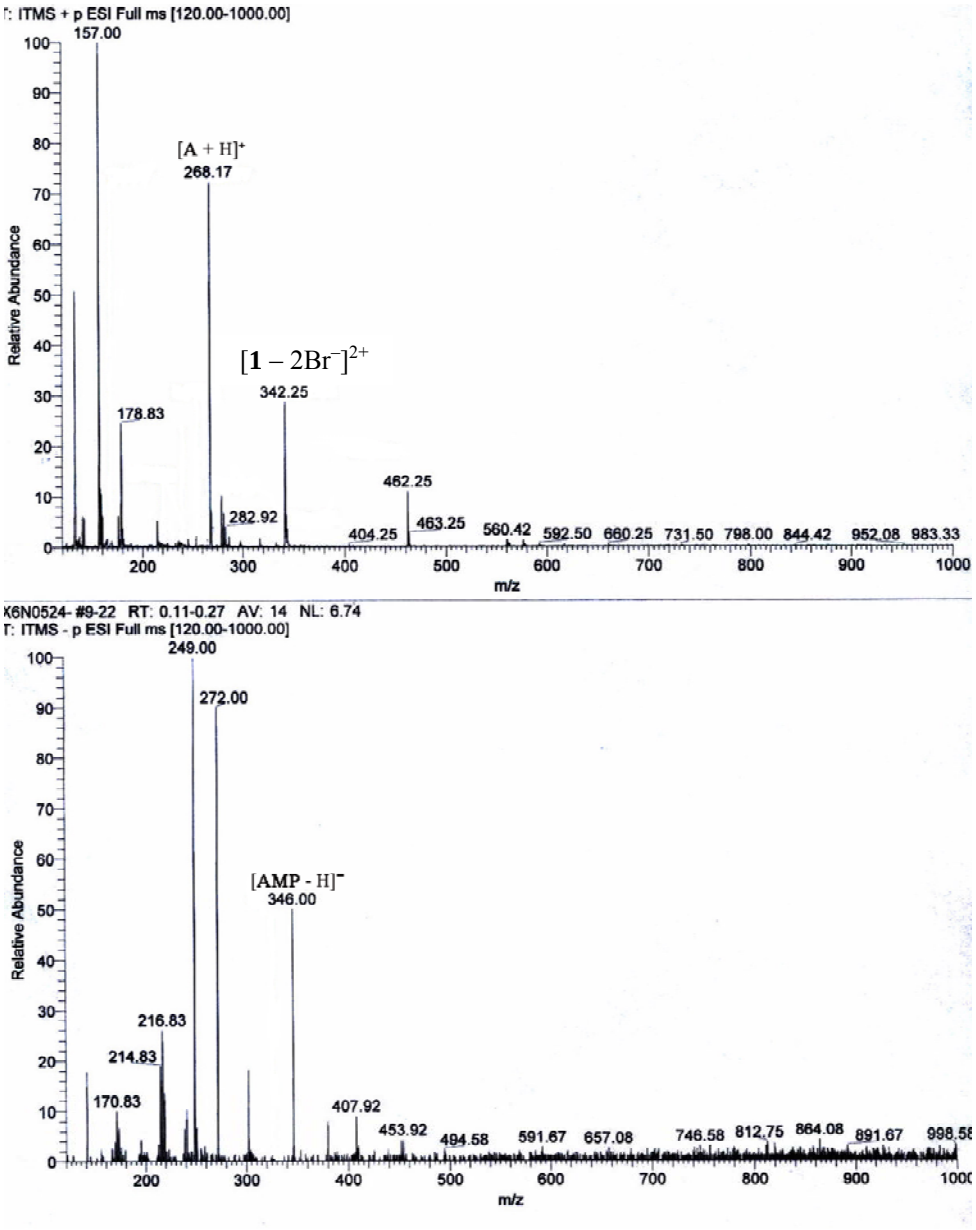


Figure S9(1). ESI–MS analysis of ApA after treatment with compound **1** and ApA for 16 h at 37 °C.

Supplementary Information

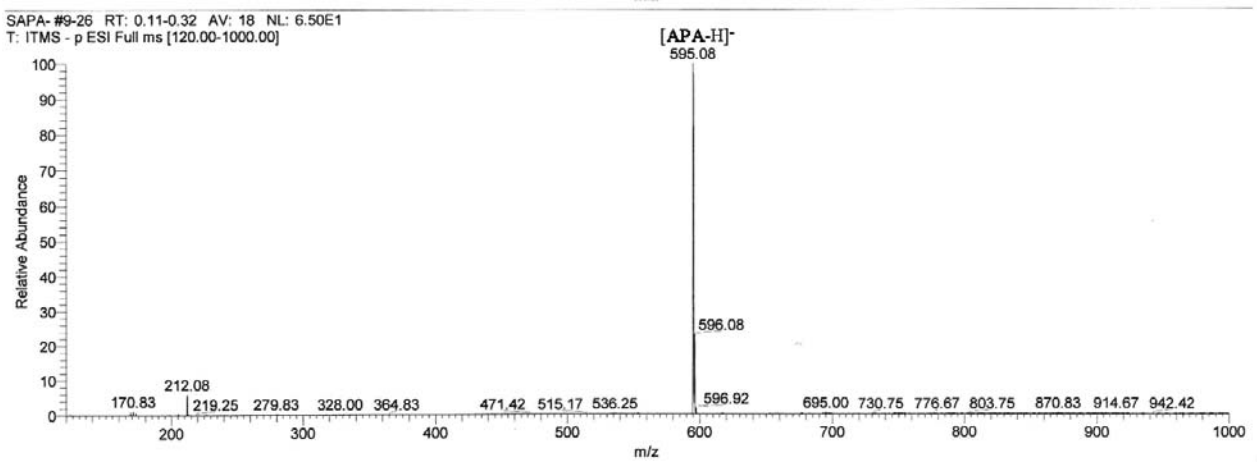


Figure S9(2). ESI–MS analysis of ApA alone incubated for 16 h at 37 °C.

Supplementary Information

ESI-MS spectra of reaction of BDNPP with compound 1

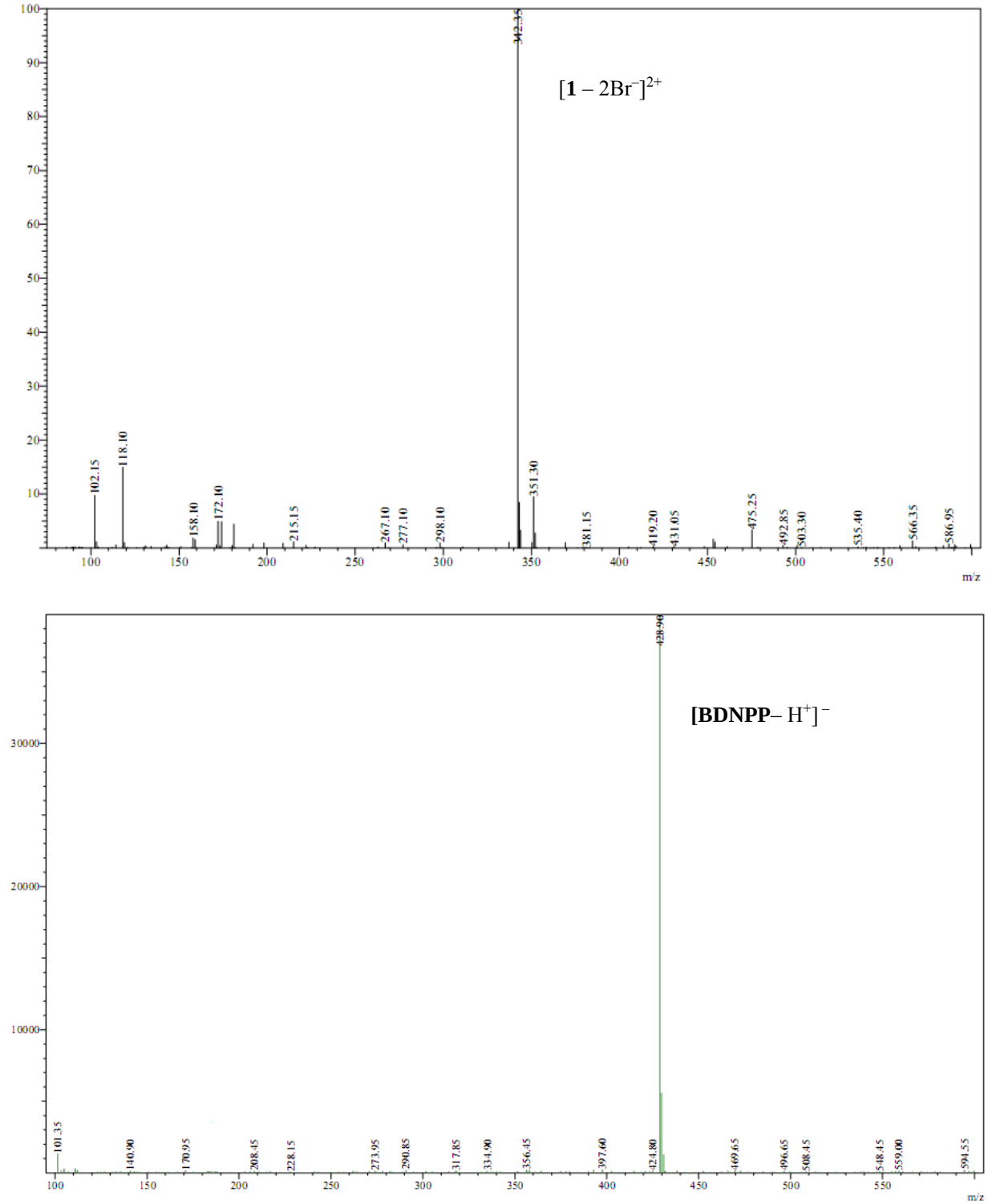


Figure S10(1). ESI–MS analysis of solution after treatment with compound **1** and BDNPP for 0 h at room temperature.

Supplementary Information

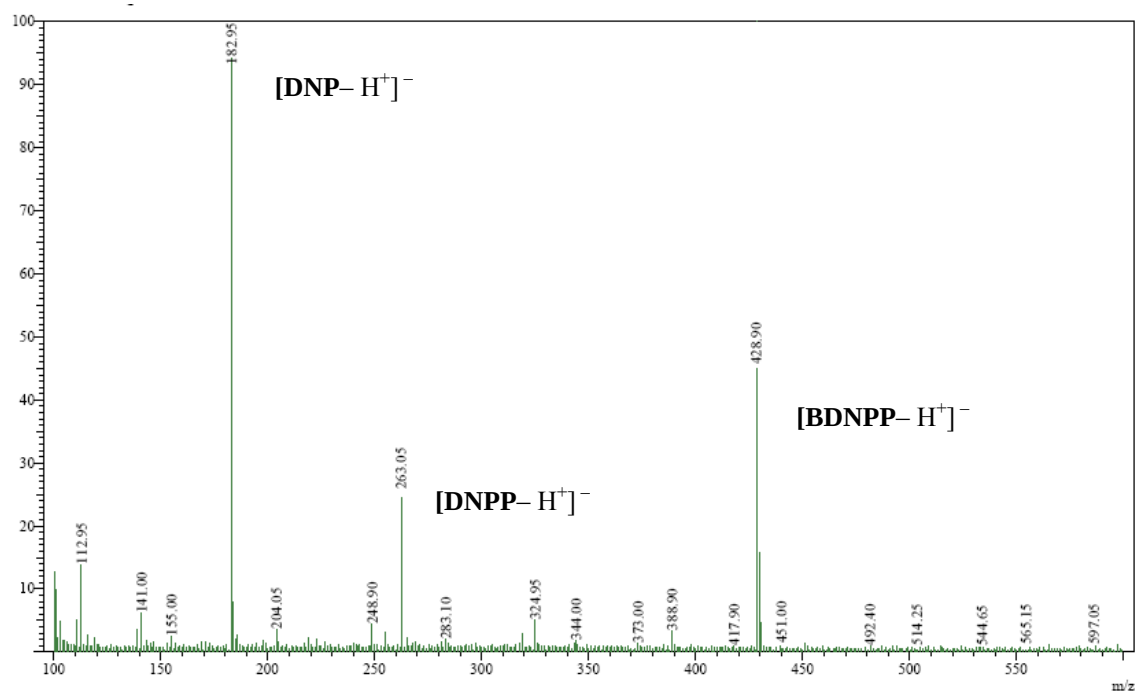


Figure S10(2). ESI–MS analysis of solution after treatment with compound **1** and BDNPP for 3 h at room temperature.

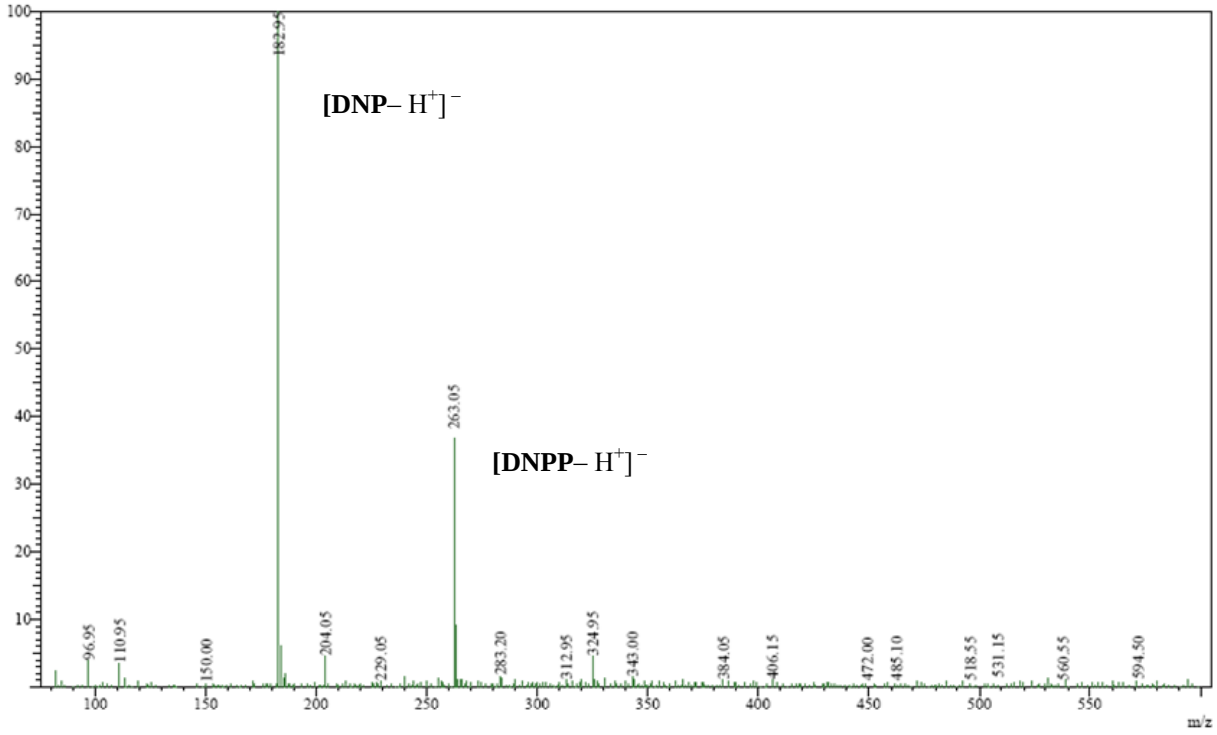


Figure S10(3)**.** ESI–MS analysis of solution after treatment with compound **1** and BDNPP for 5 h at room temperature.

Supplementary Information

DNA cleavage in the presence of EDTA

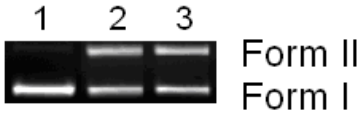


Figure S11. Agarose gel (1%) of pUC19 plasmid DNA (0.025 mM bp) cleaved by **1** (0.03 mM) in the absence or presence of EDTA (10 mM) incubated for 5 h at 37 °C in pH 7.25 buffer (50 mM Tris–HCl / 5 mM NaCl). Lane 1, DNA control; lane 2, only **1**; lane 3, **1** + EDTA.

Table S4. DNA cleavage promoted by **1** (0.030 mM) in the presence of 10 mM EDTA.

Added compound	DNA %	
	Form I	Form II
DNA control	99.35	0.65
1 only	50.29	49.71
1 + EDTA	49.70	50.30