Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Thiol-dependent DNA cleavage by aminomethylated Beaucage's reagent

Jiahui Zheng,[a] Xiaoqian Liu,[a] Qing Quan,[a] Yoon-Joo Shin,[b] Daekyu Sun[b] and Yixin Lu*[a,c]

[a]*Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore, 117543*
[b]*Department of Pharmacology and Toxicology, College of Pharmacy, The University of Arizona, Tucson, AZ 85721, USA*
[c]*Medicinal Chemistry Program, Life Sciences Institute, National University of Singapore*

Email: chmlyx@nus.edu.sg

Supporting Information

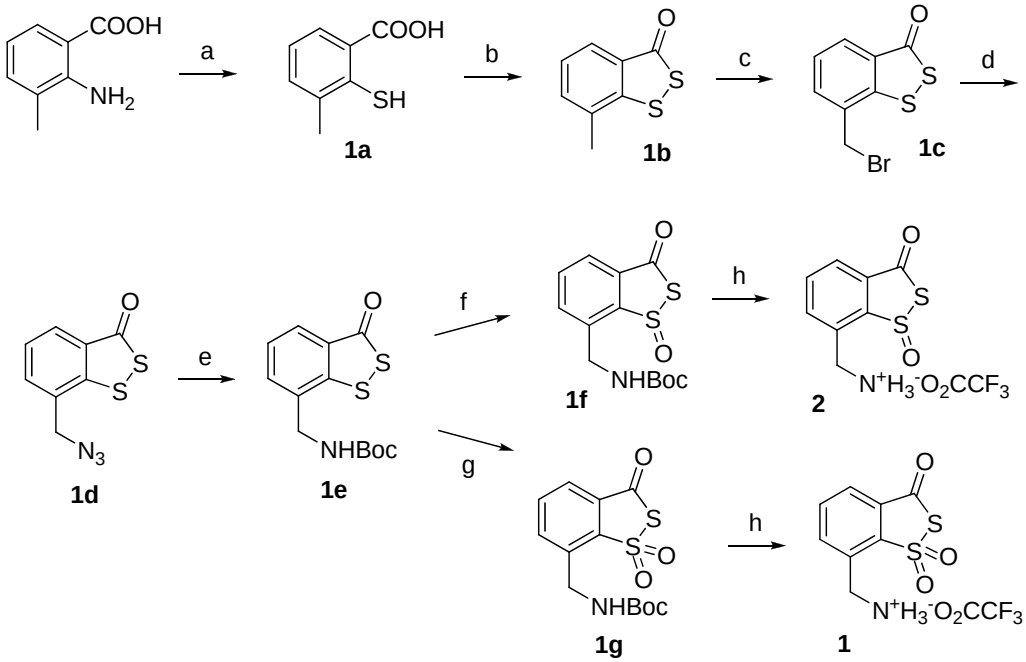
Experimental Procedures and Characterizations of Synthetic Compounds

A. General information

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF300 or DPX300 (300 MHz) or AMX500 (500 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.0). Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). Coupling constants are reported in Hertz (Hz). Low resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode, and a Finnigan/MAT 95XL-T mass spectrometer in FAB mode. All high resolution mass spectra were obtained on a Finnigan/MAT 95XL-T spectrometer. For thin-layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F_{254}) was used, and compounds were visualized with a UV light at 254 nm. Further visualization was achieved by staining with iodine, or ceric ammonium molybdate followed by heating on a hot plate. Flash chromatography separations were performed on Merck 60 (0.040 - 0.063 mm) mesh silica gel. Microwave irradiation was performed using a CEM Discover Synthesis Unit with a 0.5-2 microwave reaction vial.

B. Experimental procedures and characterization of synthetic compounds

Synthetic Scheme:



Scheme 2: Synthesis of **1**. Reagents and conditions: (a) i. 1 eq. $NaNO_2$, conc. HCl, 0-5 ^oC; ii. 1.1 eq. Na_2S, 1.1 eq. S8, NaOH, rt, 2 h; iii. 4 eq. Zn, CH_3CO_2H, reflux, 16 h, 59 %; (b) 1 eq. CH_3COSH, conc. H_2SO_4, 50 ^oC, 2 h, 94 %; (c) 1.5 eq. NBS, 0.05 eq. AIBN, CCl_4, Reflux, 50 %; (d) 5 eq. NaN_3, DMF, 60 ^oC, 16 h, 85 %; (e) 2.1 eq. Boc_2O, 3 eq. K_2CO_3, 1.2 PPh_3, CH_2Cl_2, rt, 19 h, 35 %; (f) 1.5 eq. m-CPBA, CH_2Cl_2, -20 ^oC, 24 h, 60 %; (g) 4 eq. Oxone , 12 eq. $NaHCO_3$, acetone, 0 ^oC, 24 h, 80 %; (h) 10 eq. CF_3CO_2H, CH_2Cl_2, rt, 12 h, 100 %.

2-Mercapto-3-benzoic acid (**1a**)

To a mixture of 2-amino-3-methyl-benzoic acid (0.30 g, 1.98 mmol), water (1 mL) and concentrated HCl (0.4 mL) at 0 °C, was added a pre-cooled solution of $NaNO_2$ (0.14 g, 1.98 mmol) in water ((0.5 mL). The diazonium salt solution was tested with starch-iodine paper and a blue coloration was observed. $Na_2S{\cdot}9\ H_2O$ (0.52 g, 2.18 mmol) and powdered sulfur (0.067 g, 2.18 mmol) were dissolved in boiling water (2 mL), a solution of 10 M NaOH (0.2 mL) was then added and the resulting yellow solution was cooled in an ice bath. Along with crushed ice, the diazonium solution was added to the stirred alkaline sulfide solution over 30 minutes. The

temperature of the reaction mixture as kept below 5 °C by directly adding ice. The mixture was allowed to warm up to room temperature and stirred for another 2 hours. Concentrated HCl solution (0.24 ml) was added to adjust the pH of solution to 3. The precipitate was filtered, and washed with water. To further remove excess sulfur, the precipitate was boiled in a solution of Na_2CO_3 (0.12 g) in water (4 mL), and the mixture was filtered off while hot. The pH of the filtrate was adjusted to 3, and the precipitate was filtered, and washed with water. The crude disulfide was then mixed with zinc dust (0.5 g, 7.69 mmol) and glacial acetic acid (1 mL), and the mixture was brought to reflux for 16 h. After cooling down to room temperature, the mixture was filtered, and the precipitate was washed with water, and then suspended in water (5 mL). An aqueous solution of 33 % NaOH (0.08 mL) was added, and the mixture was boiled for 20 min to ensure complete extraction of product from the precipitate. After filtration, concentrated HCl was added to the filtrate, and the precipitate was collected and washed with water to afford desired **1a** as a pale pink powder (0.20 g, 59%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.43 (s, 3H), 6.60 (s, 1H), 7.13 (t, 1H, *J* = 7.7 Hz), 7.40 (d, 1H, *J* = 7.4 Hz), 8.08 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 21.4, 123.6, 124.4, 130.8, 134.7, 137.0, 138.9, 172.6; HRMS (IT-TOF) calcd for $[C_8H_7O_2{}^{32}S]^-$ 167.0172, found 167.0141; m.p. 195.0 °C.

7-Methyl-3H-benzo[c][1,2]dithiol-3-one (**1b**)

To a suspension of **1a** (0.14 g, 0.82 mmol) in concentrated H_2SO_4 (2 mL) at ambient temperature was added thioacetic acid (0.12 ml, 1.64 mmol) dropwise over a period of 40 minutes. The mixture was heated at 50 °C for 2 h, and then quenched by pouring the mixture onto crushed ice (300 g). The precipitate was filtered through a Büchner funnel, thoroughly washed with water

and suspended in chloroform (10 mL). The mixture was shaken with saturated aqueous sodium bicarbonate (5 mL), and solid was filtered and triturated in boiling chloroform several times, and the organic layers were combined. The organic phase was washed with saturated sodium bicarbonate (10 mL) and water (2 x 10 mL), and dried over anhydrous magnesium sulfate. After filtration and concentration, the crude was purified by column chromatography (EtOAc/hexane 1:3) to afford **1b** as a yellow needle (0.140 g, 94%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.49 (s, 3H), 7.35 (t, 1H, *J* = 7.6 Hz), 7.47 (d, 1H, *J* = 7.1 Hz), 7.80 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 18.4, 124.9, 125.9, 129.0, 133.6, 133.9, 147.9, 194.0; HRMS (EI) calcd for $C_8H_6O_1{}^{32}S_2$ 181.9860, found 181.9865; m.p. 96.0 – 97.0 °C.

7-(Bromomethyl)-3H-benzo[c][1,2]dithiol-3-one (**1c**)

To a solution of **1b** (0.14 g, 0.746 mmol) in CCl_4 (5 mL) were added *N*-bromosuccinimide (0.20 g, 1.59 mmol) and AIBN (6 mg, 0.036 mmol). The reaction mixture was then brought to reflux for 24 h, and quenched by adding cold water. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by column chromatography (EtOAc/hexane 1:20) to give **1c** as a pale yellow needle (0.097 g, 50%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 4.63 (s, 2H), 7.40 (t, 1H, *J* = 7.9 Hz), 7.67 (d, 1H, *J* = 7.9 Hz), 7.89 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.6, 126.3, 127.7, 130.2, 133.6, 134.1, 147.7, 193.1; HRMS (EI) calcd for $C_8H_5O_1{}^{79}Br_1{}^{32}S_2$ 259.8965, found 259.8962; $C_8H_5O_1{}^{81}Br_1{}^{32}S_2$ 261.8945, found 261.8942; m.p .119.0 – 120.0 °C.

7-(Azidomethyl)-3H-benzo[c][1,2]dithiol-3-one (**1d**)

To a solution of **1c** (0.25 g, 0.960 mmol) in DMF (3 mL) was added NaN_3 (0.310 g, 4.79 mmol), and the resulting mixture was stirred at 60 °C for 24 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (20 mL). The organic phase was washed with water (3 x 15 mL), and dried over anhydrous $MgSO_4$. After filtration and concentration *in vacuo*, the crude was purified by column chromatography (EtOAc/hexane 1:4) to give **1d** as a white solid (0.182 g, 85%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 4.63 (s, 2H), 7.48 (t, 1H, *J* = 7.7 Hz), 7.67 (d, 1H, *J* = 7.2 Hz), 7.97 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 52.0, 126.1, 127.5, 130.2, 131.8, 133.0, 147.2, 193.2; HRMS (EI) calcd for $C_8H_5O_1N_3{}^{32}S_2$ 222.9874, found 222.9872; m.p. 62.0 – 63.0 °C.

tert-Butyl (3-oxo-3H-benzo[c][1,2]dithiol-7-yl)methylcarbamate (**1e**)

To a solution of **1d** (0.11 g, 0.490 mmol) in CH_2Cl_2 (6 mL) were added PPh_3 (0.15 g, 0.588 mmol), Boc_2O (0.23 g, 1.03 mmol), K_2CO_3 (0.20 g, 1.76 mmol) and water (2 mL), the reaction mixture was stirred at room temperature for 24 hours. The organic and aqueous phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic extracts were washed with brine (1 x 15 mL), and dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/hexane 1:6) to give **1e** as a white solid (0.050 g, 35%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.49 (s, 9H), 4.53 (d, 2H, *J* = 5.9 Hz), 5.15 (s, 1H), 7.41 (t, 1H, *J* = 7.6 Hz), 7.62 (d, 1H, *J* = 7.2 Hz), 7.87 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ =

28.3, 42.2, 80.2, 126.0, 126.4, 129.8, 132.1, 134.9, 146.5, 155.6, 193.6; HRMS (EI) calcd for $C_{13}H_{15}O_3N_1{}^{32}S_2$ 297.0493, found 297.0494; m.p. 96.0 – 97.0 °C.

7-(*N*-Boc-aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1-oxide (**1f**)

To a stirred solution of **1e** (0.020 g, 0.067 mmol) in CH_2Cl_2 (2 mL) at -20 °C was added slowly a solution of *m*-chloroperoxybenzoic acid (0.040 g, 0.202 mmol) in CH_2Cl_2 (2 mL). The mixture was kept stirring at below -30 °C for 45 minutes, and then at room temperature for 24 hours. The reaction mixture was diluted with CH_2Cl_2 and washed with aqueous $Na_2S_2O_3$ (2 x 5 mL) and $NaHCO_3$ (1 x 5 mL). The organic phase was concentrated *in vacuo* and purified by column chromatography (EtOAc/hexane 1:5) to give **1f** as a white solid (0.013 g, 60%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.47 (s, 9H), 4.52 (d, 2H, *J* = 5.9 Hz), 4.99 (s, 1H), 7.41 (t, 1H, *J* = 7.9 Hz), 7.61 (d, 1H, *J* = 6.8 Hz), 7.88 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.4, 42.3, 80.4, 126.1, 126.6, 129.9, 132.2, 135.0, 146.7, 155.6, 193.6; HRMS (ESI) calcd for $C_{13}H_{15}O_4N_1{}^{32}S_2{}^{23}Na_1$ 336.0335, found 336.0323; m.p. 94.0 – 95.0 °C.

7-(*N*-Boc-aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1,1-dioxide (**1g**)

To a stirred solution of **1e** (10 mg, 0.034 mmol) in acetone (1.5 mL) at 0 °C was added Oxone™ (83 mg, 0.135 mmol) and $NaHCO_3$ (33.6 mg, 0.404 mmol) over 20 minutes. The reaction mixture was then allowed to warm up to room temperature. Upon completion of reaction as judged by TLC, solvent was removed *in vacuo*. The residue was taken up in CH_2Cl_2, and the mixture was filtered. Concentration of the filtrate under reduced pressure yielded **1g** as a white solid (9 mg, 80%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.43 (s, 9H), 4.57 (dd, 1H, *J* = 4.6 Hz and 15.6 Hz), 4.90 (dd, 1H, *J* = 7.7 Hz and 15.5 Hz), 5.27 (s, 1H), 7.77 (t, 1H, *J* = 7.6 Hz), 7.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.4, 41.4, 80.6, 126.6, 129.9, 130.3, 131.9, 134.0, 136.6, 140.8, 192.7; HRMS (IT-TOF) calcd for $C_{13}H_{15}O_5N_1{}^{32}S_2Na_1$ 352.0289, found 352.0254; m.p. 192 .0 °C.

7-(Aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1-oxide (**2**)

To a solution of **1f** (15 mg, 0.051 mmol) in CH_2Cl_2 (10 mL) was added CF_3COOH, and the resulting solution was stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure and co-evaporated with CH_2Cl_2 several times to afford **2** as a pale yellow syrup (10 mg, 100 %).

Compound **1** was obtained from **1g** following similar procedure.

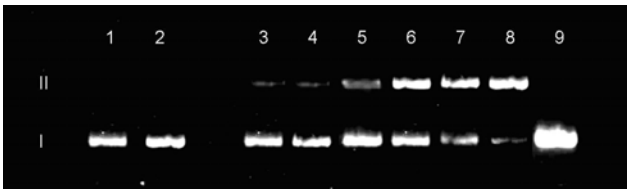
^{1}H NMR (CD_3OD, 300 MHz) δ = 4.38 (s, 2H), 7.63 (t, 1H, *J* = 7.9 Hz), 7.87 (d, 1H, *J* = 7.2 Hz), 8.01 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ = 70.5, 127.1, 128.1, 130.1, 130.4, 134.2, 161.6, 183.3; HRMS (IT-TOF) calcd for $[C_8H_8N_1O_2{}^{32}S_2]^+$ 213.9996, found 213.9963.

7-(Aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1,1-dioxide (**1**)

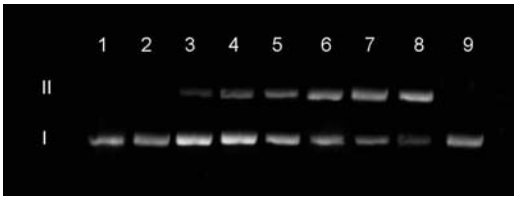
A light yellow powder; ^{1}H NMR (CD_3OD, 300 MHz) δ = 4.64 (dd, *J* = 14.5 Hz and 26.6 Hz), 8.00 (t, 1H, *J* = 7.6 Hz), 8.10 (m, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ = 70.5, 128.2, 130.1, 134.5, 134.8, 137.8, 148.8, 180.5; HRMS (IT-TOF) calcd for $[C_8H_8N_1O_3{}^{32}S_2]^+$ 229.9946, found 229.9912.

C. Figures of DNA cleavage experiments and quantifications of extents of cleavages

Full sets of Figures

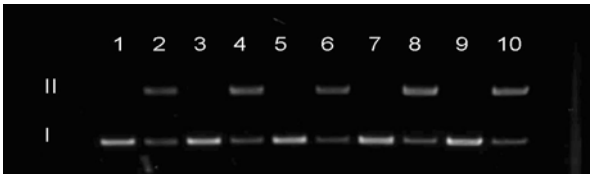


1st

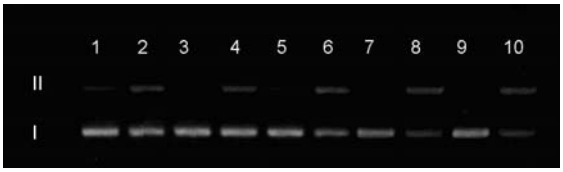


2nd

Fig. 1 Thiol-dependent DNA cleavage by **1** with various concentrations at pH 7.0. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **1** (5 μM); Lane 3, **1** (5 μM) + thiol (25 μM); Lane 4, **1** (10 μM) + thiol (50 μM); Lane 5, pBR322 DNA with **1** (20 μM) + thiol (100 μM); Lane 6, pBR322 DNA with **1** (30 μM) + thiol (150 μM); Lane 7, pBR322 DNA with **1** (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with **1** (80 μM) + thiol (400 μM); Lane 9, pBR322 DNA with **1** (100 μM).

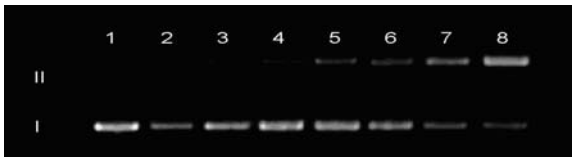


1st



2nd

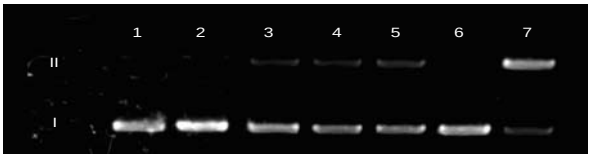
Fig. a Thiol-dependent DNA cleavage by compound **1**. Assays were performed in 50 mM sodium phosphate buffer with different pH containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **1** (50 μM) and 2-mercaptoethanol (250 μM). The total volume of reaction was 20 μL. The reaction time was 12 h. Lane 1/2, pH 6.0 without/with thiol; Lane 3/4, pH 6.5 without/with thiol; Lane 5/6, pH 7.0 without/with thiol; Lane 7/8, pH 7.5 without/with thiol; Lane 9/10, pH 8.0 without/with thiol.



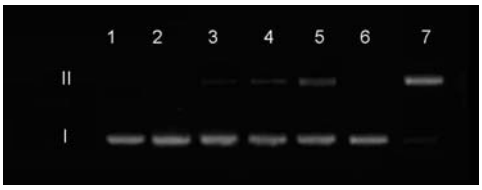
1st

2nd

Fig. b Time dependence of DNA cleavage by compound **1** (30 μM). Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **1** and 2-mercaptoethanol (total volume 20 μL). The reaction times corresponding to lanes 2 to 8 were 2, 4, 6, 8, 10, 12, 24 hours, respectively.

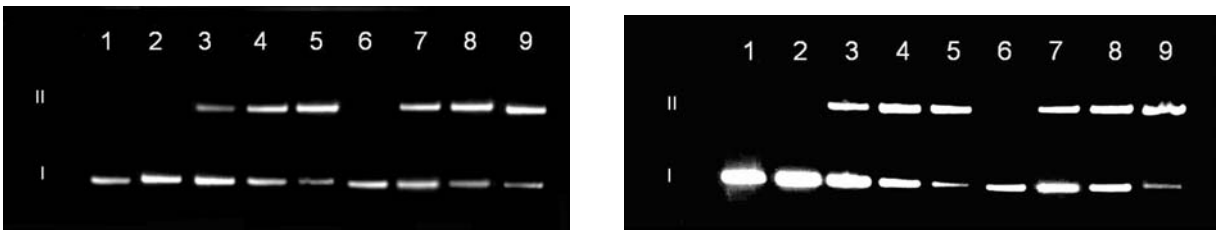


1st



2nd

Fig. c Thiol-dependent DNA cleavage by various concentrations of compound **2** compared to compound **1**. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **2** (5 μM) + thiol (25 μM); Lane 3, **2** (100 μM) + thiol (500 μM); Lane 4, **2** (300 μM) + thiol (1.5 mM); Lane 5, pBR322 DNA with **2** (1 mM) + thiol (5 mM); Lane 6, pBR322 DNA with **1** (5 μM) + thiol (25 μM); Lane 7, pBR322 DNA with **1** (100 μM) + thiol (500 μM).



1st **2nd**

Fig. 2 Thiol dependent cleavage by various concentrations of **1** at pH 7.0 compared to Beaucage's reagent (BR) at different concentrations. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **1** (80 μM) without thiol; Lane 3, **1** (5 μM) + thiol (25 μM); Lane 4, **1** (30 μM) + thiol (150 μM); Lane 5, pBR322 DNA with **1** (80 μM) + thiol (400 μM); Lane 6, pBR322 DNA with BR (250 μM) but without thiol; Lane 7, pBR322 DNA with BR (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with BR (100 μM) + thiol (500 μM); Lane 9, pBR322 DNA with BR (250 μM) + thiol (1.25 mM).

Quantifications of extents of cleavages

Fig. 1

	Sum intensity				Sum intensity							
Entry	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	116408	0	100	0	168814	0	100	0	100	0	0	0
2	119369	0	100	0	165865	0	100	0	100	0	0	0
3	192432	27148	87.6	12.4	166339	18114	90.2	9.8	88.9	11.1	1.8	1.8
4	194185	58639	76.8	23.2	133427	18908	87.6	12.4	82.2	17.8	7.6	7.6
5	132248	66976	66.4	33.6	211213	54324	79.5	20.5	73.0	27.0	9.3	9.3
6	109848	106571	50.8	49.2	166993	149468	52.8	47.2	51.8	48.2	1.4	1.4
7	65937	118751	35.7	64.3	97974	140493	41.1	58.9	38.4	61.6	3.8	3.8
8	40351	130613	23.6	76.4	19609	166285	10.5	89.5	17.1	82.9	9.2	9.2
9	118619	0	100.0	0.0	374249	0	100	0	100	0	0	0

Fig. a

	Sum intensity				Sum intensity							
	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	135986	10053	93.1	6.9	80858	0	100	0	96.6	3.4	4.87	4.87
2	113579	20520	84.7	15.3	35142	35424	49.8	50.2	67.2	32.8	24.68	24.68
3	129394	0	100.0	0.0	93217	0	100.0	0.0	100.0	0.0	0.00	0.00
4	125688	25608	83.1	16.9	33404	46735	41.7	58.3	62.4	37.6	29.27	29.27
5	113337	4332	96.3	3.7	88295	0	100.0	0.0	98.2	1.8	2.60	2.60
6	64548	33348	65.9	34.1	28518	37958	42.9	57.1	54.4	45.6	16.29	16.29
7	78943	0	100.0	0.0	97602	0	100.0	0.0	100.0	0.0	0.00	0.00
8	30514	30445	50.1	49.9	40834	63367	39.2	60.8	44.6	55.4	7.69	7.69
9	93515	0	100.0	0.0	103124	0	100.0	0.0	100.0	0.0	0.00	0.00
10	28355	29734	48.8	51.2	33233	53280	38.4	61.6	43.6	56.4	7.35	7.35

Fig. b

	Sum intensity				Sum intensity							
	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	163532	0	100	0	113394	0	100	0	100.0	0.0	0.00	0.00
2	62503	0	100.0	0.0	104938	0	100.0	0.0	100.0	0.0	0.00	0.00
3	108855	0	100.0	0.0	58072	0	100.0	0.0	100.0	0.0	0.00	0.00
4	162707	0	100.0	0.0	62278	8809	87.6	12.4	93.8	6.2	8.76	8.76
5	147879	23650	86.2	13.8	68718	46950	59.4	40.6	72.8	27.2	18.95	18.95
6	55494	25456	68.6	31.4	35986	69861	34.0	66.0	51.3	48.7	24.43	24.43
7	38632	60707	38.9	61.1	15421	37356	29.2	70.8	34.1	65.9	6.84	6.84
8	27417	150350	15.4	84.6	10833	45351	19.3	80.7	17.4	82.6	2.73	2.73

Fig. 2

	Sum intensity				Sum intensity							
	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	391198	0	100	0	95813	0	100	0	100.0	0.0	0.00	0.00
2	352192	0	100	0	149121	0	100	0	100.0	0.0	0.00	0.00
3	269758	135568	66.6	33.4	150320	55233	73.1	26.9	69.8	30.2	4.65	4.65
4	158716	176404	47.4	52.6	94991	109390	46.5	53.5	46.9	53.1	0.62	0.62
5	57741	149861	27.8	72.2	45617	149348	23.4	76.6	25.6	74.4	3.12	3.12
6	123395	0	100	0	106179	0	100	0	100.0	0.0	0.00	0.00
7	196990	122179	61.7	38.3	118073	109762	51.8	48.2	56.8	43.2	7.00	7.00
8	142584	154397	48.0	52.0	68102	149350	31.3	68.7	39.7	60.3	11.80	11.80
9	44269	172340	20.4	79.6	48113	150828	24.2	75.8	22.3	77.7	2.65	2.65