Electronic Supplementary Information

Synthesis and DNA interactions of a bis-phenothiazinium photosensitizer

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S1
Experimentals

Phenothiazin-5-i um tetraiodide hydrate (1). Preparation of known compound 1\(^1\) afforded a dark-blue solid product (1.63 g, 80%), mp 170 \(^\circ\)C (from CHCl\(_3\), decomp.); \(R_f = 0.09\) (CHCl\(_3\)); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 2967, 1558, 1467, 1440, 1311, 1233, 1131, 1067, 1023, 841, and 705; \(\delta_H\) (300 MHz; Acetone-\(d_6\); Me\(_4\)Si) 8.01 (2H, m), 7.92 (2H, m), and 7.64 (4H, m); \(\delta_C\) (75 MHz; acetone-\(d_6\); Me\(_4\)Si) 153.6, 130.7, 129.5, 128.6, 125.5, and 123.5; \(m/z\) (LR-ESI) 199.0 (M\(^+\) - C\(_{12}\)H\(_8\)NS requires 198.04).

3-(Dimethylamino)phenothiazin-5-ium triiodide (2). Known compound 2 was prepared by making a minor modification to a published literature procedure.\(^1\) To a solution of phenothiazin-5-ium tetraiodide hydrate (0.400 g, 0.553 mmol) in 20 mL of chloroform was added a 2 M solution of dimethylamine in methanol (0.553 mL, 1.106 mmol) drop-wise over 4 h. The reaction progress was monitored by silica gel TLC (3:7 10% aqueous ammonium acetate/methanol). The resultant precipitate was filtered, washed with chloroform and allowed to air dry. Product 2 (189 mg, 55%) was obtained as a dark-blue solid, mp 144 – 145 \(^\circ\)C (from MeOH); \(R_f = 0.28\) (3:7 10% aqueous ammonium acetate / methanol); (Found: C, 27.1; H, 1.9; N, 4.4; S, 5.2; I, 60.9. C\(_{14}\)H\(_{13}\)N\(_2\)I\(_3\) requires C, 27.0; H, 2.1; N, 4.5; S, 5.15; I, 61.2%); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 2800, 1617, 1559, 1489, 1429, 1411, 1252, 1118, 1411, 1078, 887, 835, and 772; \(\delta_H\) (300 MHz; DMSO-\(d_6\); Me\(_4\)Si) 8.22 (1H, dd, \(J = 8.0\ and \ 1.6\), H-9), 8.17 (1H, dd, \(J = 8.0\ and \ 1.6\), H-6), 8.10 (1H, d, \(J = 10\), H-1), 8.04 (1H, dd, \(J = 10\ and \ 2.4\), H-2), 8.00 (1H, d, \(J = 2.4\), H-4), 7.85 (2H, m, H-7, H-8), 3.64 and 3.60 (6H, s, 2 x N(CH\(_3\))\(_2\)); \(\delta_C\) (75 MHz, DMSO-\(d_6\); Me\(_4\)Si) 156.1, 144.1, 139.8, 139.6, 138.0, 134.6, 133.2, 129.8, 126.3, 126.1, 125.8, 109.7, 43.3, and 42.9; \(m/z\) (LR-ESI) 241.1 (M\(^+\) - C\(_{14}\)H\(_{13}\)N\(_2\)S requires 240.08).
Fig. S1  UV-visible spectra recorded at 22 °C in 10 mM sodium phosphate buffer pH 7.0 of: a) 1 µM compound 3 (●, $\lambda_{\text{max}} = 620$ nm) in the presence of 38 µM bp CT DNA (○, $\lambda_{\text{max}} = 680$ nm) or 1% SDS (w/v) (solid line, $\lambda_{\text{max}} = 676$ nm); b) 1 µM MB (□, $\lambda_{\text{max}} = 664$ nm) in the presence of 38 µM bp CT DNA (○, $\lambda_{\text{max}} = 671$ nm) or 1% SDS (w/v) (solid line, $\lambda_{\text{max}} = 661$ nm). Line markers (●,○,□) are placed at every 50th data point. Prior to data acquisition, the samples containing DNA were pre-equilibrated for 12 h in the dark at 22 °C.
Fig. S2  $^1$H NMR spectrum of compound 3: $\delta_H$(300 MHz, 4:6 CDCl$_3$/CD$_3$OD; Me$_4$Si) 7.95 (2H, d, $J$ 9.6, H-1), 7.94 (2H, d, $J$ 9.6, H-9), 7.50-7.46 (4H, m, H-6, H-8), 7.33 (2H, dd, $J$ 9.6 and 2.7, H-2), 7.26 (2H, d, $J$ 2.7, H-4), 4.42 (4H, d, $J$ 13.5, 2 x CH$_2$-α), 3.43 (12H, s, 2 x N(CH$_3$)$_2$), 3.38 (4H, m, overlap with CH$_3$OH, 2 x CH$_2$-α), 2.06 (4H, d, $J$ 11.7, 2 x CH$_2$-β), 1.81 (2H, broad, 2 x CH), and 1.44-1.31 (8H, m, CH$_2$CH$_2$, 2 x CH$_2$-β).
Fig. S3  Aromatic region with integration, enlarged from Fig. S2: $\delta_H = 7.95$ (2H, d, $J$ 9.6, H-1), 7.94 (2H, d, $J$ 9.6, H-9), 7.50-7.46 (4H, m, H-6, H-8), 7.33 (2H, dd, $J$ 9.6 and 2.7, H-2), 7.26 (2H, d, $J$ 2.7, H-4). Note: resonance at 7.43 ppm is a residual solvent peak from CHCl$_3$. 

Note: Supp. Material (ESI) for Organic & Biomolecular Chemistry. This journal is (c) The Royal Society of Chemistry 2008.
Fig. S4  Aliphatic region with integration, enlarged from Fig. S2: $\delta_\text{H} = 4.42$ (4H, d, $J$ 13.5, 2 x CH$_2$-$\alpha$), 3.43 (12H, s, 2 x N(CH$_3$)$_2$), 3.38 (4H, m, overlap with CH$_3$OH, 2 x CH$_2$-$\alpha$), 2.06 (4H, d, $J$ 11.7, 2 x CH$_2$-$\beta$), 1.81 (2H, broad, 2 x CH), and 1.44-1.31 (8H, m, CH$_2$-CH$_2$, 2 x CH$_2$-$\beta$).
Fig. S5 $^{13}$C NMR spectrum of compound 3: $\delta$ (75 MHz, 4:6 CDCl$_3$/CD$_3$OD; Me$_4$Si) 154.3 and 153.3 (C-3, C-7), 139.2 and 138.7 (C-1, C-9), 136.4, 135.9, 135.8, and 134.8 (C$_4$a, C$_5$a, C$_9$a and C$_{10}$a), 119.3 and 118.6 (C-2, C-8), 107.1 and 106.4 (C-4, C-6), 49.3 (C-$\alpha$), 41.6 (NCH$_3$), 35.8 (CH), 33.0 and 32.9 (C-$\beta$, CH$_2$-CH$_2$).
Fig. S6  HMQC NMR spectrum of compound 3 in DMSO-$d_6$ at 25 °C recorded using a Varian Unity Plus 500 MHz instrument. The $^1$H - $^{13}$C correlations in this spectrum were utilized to assign the proton and carbon resonances in the spectra shown in Figs. S2 –S5.
Fig. S7  Viscometric measurements conducted at 25 ± 0.1 °C in 10 mM sodium phosphate buffer pH 7.0 of 50 µM bp alternating poly[(dA-dT)]$_2$ and 50 µM bp poly(dA)•poly(dT) DNA pre-equilibrated for 12 h in the dark at 22 °C with 0.0 to 3 µM of the phenothiazines a) compound 3 and b) MB.

- □: compound 3: alternating poly[(dA-dT)]$_2$ slope = 1.11  R = 0.9518
- ○: compound 3: poly(dA)•poly(dT) slope = 0.22  R = 0.9202
- ■: MB: alternating poly[(dA-dT)]$_2$ slope = 1.18  R = 0.9066
- □: MB: poly(dA)•poly(dT) slope = 0.17  R = 0.7111
Table

Table S1 UV-visible absorbance at 1 μM of phenothiazine

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Compound 3</th>
<th>MB</th>
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<tr>
<td>676</td>
<td>0.0707</td>
<td>0.0424</td>
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<tr>
<td>700</td>
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<td>0.0107</td>
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<tr>
<td>710</td>
<td>0.0204</td>
<td>0.0034</td>
</tr>
</tbody>
</table>

a 1 μM of each phenothiazine was pre-equilibrated with 38 μM bp of CT DNA in 10 mM sodium phosphate buffer pH 7.0 for 12 h at 22 °C.

Reference