Electronic Supplementary Material (ESI) for Chemical Communications.

Methylene blue phosphoramidite for DNA labelling

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Instrumentation and Chemicals

All reagents and solvents were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France), except the di(6-hydroxyhexyl)amine provided by Angene International Limited (Hong Kong), and were used without further purification.

DNA synthesis. Oligonucleotides were synthesized using an Applied Biosystems 394 RNA/DNA synthesizer (Applied Biosystems, Foster City, USA) using standard phosphoramidite protocol (1 µmole scale coupling program).

Nucleoside CE-phosphoramidite synthons were used for ODN synthesis: 5'-dimethoxytrityl-2'-deoxythymidine (dT), 5'-dimethoxytrityl-N-phenoxyacetyl-2'-deoxyadenosine,3'-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (dA), 3-[(3-(4,4'-dimethoxytrityloxy)propyl)(3-[(2-cyanoethyl)(*N*,*N*-diisopropyl)phosphoramidityl] propyl)amino]-7-dibutylamino-phenothiazin-5-ium chloride **6b** and all the oligonucleotide synthesis reagents (*i.e.*, activator solution (0.45 M tetrazole in acetonitrile), cap mix A (5% phenoxyacetic anhydride (Pac_2O) in tetrahydrofuran (THF)/pyridine), cap mix B (16% methylimidazole/THF), oxidizing solution (iodine (I_2) 0.02 M in water/pyridine/THF), deblocking mix (3% trichloroacetic acid (TCA) in dichloromethane (CH_2Cl_2)), deprotection solution (0.05 M potassium carbonate (K_2CO_3) in methanol (MeOH)), triethylammonium acetate 2M (TEAAc), were purchased from Glen Research (Sterling, Virginia). Acetonitrile (CH_3CN, DNA synthesis grade) and dichloromethane (DNA synthesis grade) were purchased from Biosolve (Valkenswaard, Netherlands).

NMR spectroscopy. All NMR experiments were recorded on a Bruker 300 MHz or 400 MHz spectrometer.

Electrospray mass spectrometry. Analyses were performed on a Bruker MicrOTOFQ-II (ICBMS, Lyon, France).

UV-Visible spectroscopy. Absorbance measurements of dimethoxytrityl cation (DMT) and oligonucleotides were performed on a Varian Cary 100 Bio UV-Visible spectrophotometer (Agilent technologies, Santa Clara, CA) using a quartz cell of 1 cm path length. Stability in basic condition of each compounds (in K_2CO_3 0.05 M in MeOH) was followed by scanning the solution between 200 to 800 nm, each hour during 16 h. Absorbance at 498 nm and 260 nm was measured for DMT group (ε =70mLμmol^{-1}cm^{-1}) and oligonucleotide quantifications, respectively.

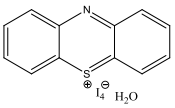
HPLC. Analysis of oligonucleotide purity was performed with an Agilent 1200 series high performance liquid chromatography (HPLC) using a Lichrospher RP18, 5 µm, WP300 column with 1 mLmin^{-1} flow rate of mobile phase. Gradient elution was from 4.5 to 12.5% of acetonitrile in 0.05 M triethylammonium acetate buffer (TEAAc), pH 7, over 30 or 40 min.

MALDI-ToF mass spectrometry. MALDI-ToF analyses were performed using a Voyager DE-PRO Applied Biosystems instrument with 3-hydroxypicolinic acid and ammonium citrate as a matrix (IBCP, Lyon, France).

Electrochemical characterization. All measurements have been done in a standard three electrodes electrochemical setup in a faraday cage. The counter electrode was a platinum plate and the reference electrodes were Ag/Ag^+ in acetonitrile containing 0.01 M $AgNO_3$ or Ag/AgCl in NaCl 3 M. The working electrode was a planar gold electrode (surface area 0.07 cm^2), consisting in successive deposit of a 10 nm thick Ti layer first (adhesive layer) and a 300 nm thick Au layer on a p-doped Si/SiO_2 wafer. The working solutions were: phosphate-buffered electrolyte (PBE): phosphate (20 mM), potassium chloride (250 mM), pH 6.4 or pH 8 in water or in a mix water/acetonitrile (50/50). All electrochemical experiments were carried out at room temperature. Solutions were deoxygenated under argon before use and blanked under argon during the experiments.
Measurements were performed using a Bio-Logic multichannel potentiostat VMP2 (Bio-Logic Science Instruments, Pont de Claix, France). Electrochemical results were recorded and analysed using EC-Lab software from BioLogic Science Instruments.

Experimental Procedures

Synthesis of phenothiazinium tetraiodide hydrate 1



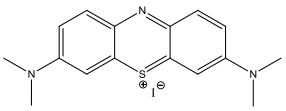
A solution of iodine (15.2 g, 60 mmol) in chloroform (450 mL) was added dropwise in a solution of phenothiazine (4.0 g, 20 mmol) in chloroform (120 mL) in a 1 L round-bottom flask placed in an ice bath. The mixture was stirred for 16 hours at 0°C. The solution was filtered over fritted glass, and the solid was washed with a large amount of chloroform to remove the excess of iodine. After drying, a dark grey powder (14.5 g, 20 mmol, 100%) was obtained. **^{1}H NMR** (300 MHz, DMSO): δ (ppm)= 8.08-7.91 (m, 4H), 7.73-7.61 (m, 4H). **MS** (ESI+): calcd. for $C_{12}H_8NS^+$ m/z=198.0, found 198.0.

Synthesis of symmetrical compounds

General protocol for the synthesis of 3,7-bis(dialkylamino)phenothiazin-5-ium iodide compounds 2

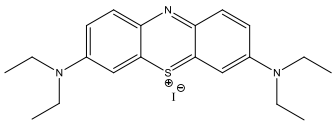
To a solution of phenothiazinium tetraiodide hydrate **1** (20 mmol, 1 eq) in methanol (300 mL) was added dropwise a secondary amine (R-H) (200 mmol, 10 eq). The reaction was carried out under stirring at room temperature. The solution turned blue while the 3,7-bis(dialkylamino)phenothiazin-5-ium **2(a-h)** was formed. When the reaction was complete (1-5h), the solution was evaporated to dryness under vacuum and purified by flash column chromatography. After collect and evaporation, the compound was obtained as a dark purple powder (33-90%).

Synthesis of 3,7-bis(dimethylamino)-phenothiazin-5-ium iodide 2a



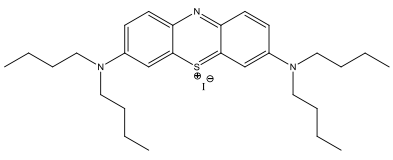
Compound **2a** was obtained following the protocol described previously. A solution of dimethylamine (2 M in methanol) was added to a solution of **1**. The reaction was carried out during 4 hours. Compound **2a** was purified by flash chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) to yield to **2a** as a dark purple solid (47%). **^{1}H NMR** (300 MHz, DMSO) δ (ppm)= 7.93 (m, 2H), 7.53 (m, 4H), 3.30 (s, 12H). **UV/Vis** (*Methanol*) : λ_{max}=653 nm. **MS** (ESI+): calcd. for $C_{16}H_{18}N_3S$+ m/z = 284.1, found m/z = 284.1.

Synthesis of 3,7-bis(diethylamino)-phenothiazin-5-ium iodide 2b



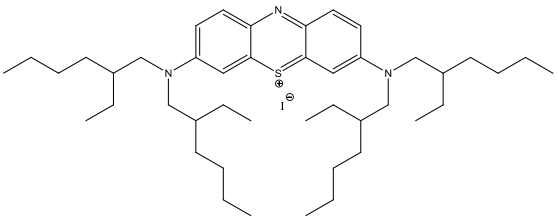
This compound was prepared by addition of diethylamine to compound **1** in 2h30 and further purification by flash column chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) yielded to dark purple solid (58%).**^{1}H NMR** (300 MHz, DMSO) δ (ppm)= 7.80-7.75 (m, 2H), 7.62-7.49 (m, 4H) 3.81-3.70, (q, 8H), 1.30-1.22, (t, 12H). **UV/Vis** (*Methanol*): λ_{max}=657 nm. **MS** (ESI+): calcd. for $C_{20}H_{26}N_3S$+ m/z = 340.2, found m/z = 340.3.

Synthesis of 3,7-bis(dibutylamino)-phenothiazin-5-ium iodide 2c



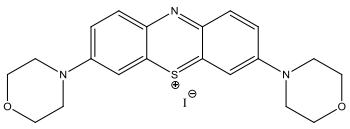
This compound was prepared by addition of dibutylamine to compound **1** in 2h and further purification by flash column chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) yielded to dark purple solid (45%). **^{1}H NMR** (300 MHz, DMSO) δ(ppm)= 7.93-7.90 (d, 2H), 7.50-7.47 (m, 4H), 3.68 (t, 8H), 2.99-2.75 (m, 8H), 1.7-1.45 (m, 8H), 1.4-1.2 (m, 8H), 0.8-1.0 (m, 12H). **UV/Vis** (*Methanol*): λ_{max}=653 nm. **MS** (ESI+): calcd. for $C_{28}H_{42}N_3S^+$ m/z = 452.3, found m/z = 452.3.

Synthesis of 3,7-bis(di(2-ethylhexyl)amino)-phenothiazin-5-ium iodide 2d



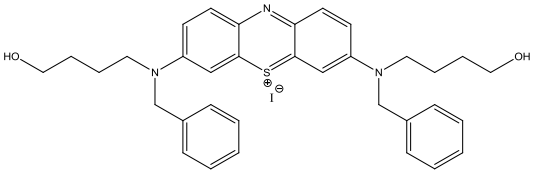
This compound was prepared by addition of di(2-ethylhexyl)amine to compound **1** in 2h and further purification by flash column chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) yielded to dark purple solid (33%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 7.52 (m, 6H), 3.44-3.42 (m, 12H), 1.66-1.14 (m, 12H), 0.90 (m, 12H). **UV/Vis** (*Methanol*): λ_{max}=673 nm. **MS** (ESI+): calcd. for $C_{44}H_{74}N_3S^+$ m/z = 676.6, found m/z = 676.7.

Synthesis of 3,7-dimorpholino-phenothiazin-5-ium iodide 2e



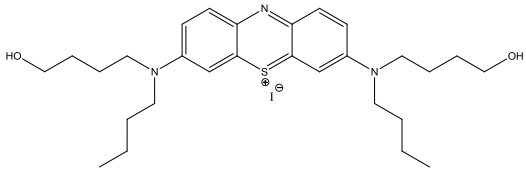
Compound **2e** was obtained following the protocol described previously. A solution of morpholine (2 M in methanol) was added to a solution of **1**. The reaction was carried out during 2 hours. The crude product was dried under vacuum, **2e** was a dark purple solid (42%). **^{1}H NMR** (300 MHz, DMSO) δ (ppm) = 8.65 (m, 4H), 8.03-7.78 (m, 2H), 3.76 (t, *J*= 4.1 Hz, 12H), 3.11 (t, *J* = 4.0 Hz, 12H). **UV/Vis** (*Methanol*): λ_{max}=658 nm. **MS** (ESI+): calcd. for $C_{20}H_{22}N_3O_2S^+$ m/z = 368.1, found m/z = 368.1.

Synthesis of 3,7-bis(di(4-hydroxybutylbenzyl)amino)-phenothiazin-5-ium iodide 2f



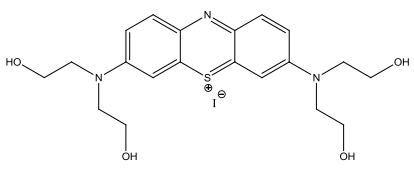
This compound was prepared by addition of 4-benzylamino-1-butanol to compound **1** in 2h30 and further purification by flash column chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) yielded to dark purple solid (90%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 7.9 (d, *J*= 9.7Hz, 1H), 7.63 (m, 4H), 7.40-7.39 (m, 9H), 7.23-7.21 (d, *J*=6.9Hz, 2H), 4.2 (s, 4H), 3.68 (t, *J*=9.7Hz, 4H), 3.03 (t, *J*=6.9Hz, 4H), 2.06-2.03 (m, 4H), 1.73-1.69 (m, 8H). **UV/Vis** (*Methanol*): λ_{max}=658 nm. **MS** (ESI+): calcd. for $C_{34}H_{38}N_3O_2S^+$ m/z = 552.3, found m/z = 552.3.

Synthesis of 3,7-bis(di(4-hydroxybutylbutyl)amino)-phenothiazin-5-ium iodide 2g



The (4-hydroxybutyl)butylamine was added to a solution of **1**. The reaction was carried out during 2 hours. Compound **2g** was purified by flash chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) to yield to **2g** as a dark purple solid (41%). **^{1}H NMR** (300 MHz, CD_3CN) δ (ppm) = 7.88 (d, *J*=9.7 Hz, 2H), 7.38 (dd, *J*=9.7 Hz, 2.8 Hz, 2H), 7.24 (d, *J*=2.7 Hz, 2H), 3.66-3.57 (m, 8H) 3.02-2.94 (m, 4H), 1.80-1.36 (m, 16H), 1.03-0.93 (m, 6H). **UV/Vis** (*Methanol*): λ_{max}=663 nm. **MS** (ESI+): calcd. for $C_{28}H_{42}N_3O_2S^+$ m/z = 484.3, found m/z = 484.3.

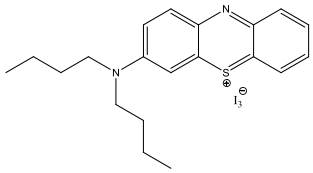
Synthesis of 3,7-bis(di(2-hydroxyethyl)amino)-phenothiazin-5-ium iodide 2h



A solution of di(2-hydroxyethyl)amine (2 M in methanol) was added to a solution of **1**. The reaction was carried out during 4 hours. Compound **2h** was purified by flash chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) to yield to **2h** as a dark purple solid (52%). **^{1}H NMR** (300 MHz, DMSO) δ (ppm) = 7.90 (d, *J*=10.0 Hz, 2H), 7.58 (m, 4H), 3.85 (m, 8H), 3.72 (m, 8H). **UV/Vis** (*Methanol*): λ_{max}=662 nm. **MS** (ESI+): calcd. for $C_{20}H_{26}N_3O_4S^+$ m/z = 404.2, found m/z = 404.2.

Synthesis of asymmetrical compounds

Synthesis of 3-dibutylamino-phenothiazin-5-ium triiodide 3

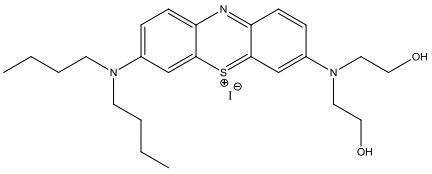


To a solution of phenothiazin-5-ium tetraiodide hydrate **1** (20 mmol, 1 eq) in methanol (300 mL) was added dropwise dibutylamine (40 mmol, 2 eq), under magnetic stirring at room temperature. The solution turned green while the 3-dibutylaminophenothiazin-5-ium **3** was formed. When the reaction was complete (2 hours), a precipitate was collected by filtration and washed with methanol (3 x 30 mL). After drying under vacuum, a dark grey powder (57%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.34-8.29 (m, 1H), 8.19-8.17 (m, 1H), 8.02-8.00 (m, 1H), 7.84-7.81 (m, 2H), 7.73-7.71 (m, 2H), 4.00-3.89 (m, 4H), 1.98-1.81 (m, 4H), 1.69-1.54 (m, 4H), 1.07 (t, *J*= 3.0 Hz, 6H). **MS** (ESI+): calcd. for $C_{20}H_{25}N_2S^+$ m/z = 325.2, found m/z = 325.2.

General protocol for the synthesis of 3-di(hydroxyalkyl)amino-7-dibutylamino-phenothiazin-5-ium iodide compounds 4

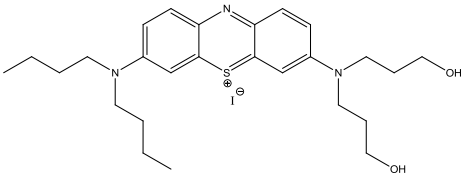
To a solution of 3-dibutylaminophenothiazin-5-ium triiodide **3** (10 mmol, 1 eq) in methanol (150 mL) was added dropwise a secondary amine R'-H (20 mmol, 2 eq), or a solution in methanol if the amine was not a liquid, under magnetic stirring at room temperature. The solution turned blue while compound **4** was formed. When the reaction was complete (2-24 h), the solution was evaporated to dryness under vacuum and purified by flash column chromatography. After collect and evaporation, the expected compounds were obtained as a dark purple powder.

Synthesis of 3-di(hydroxyethyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4a



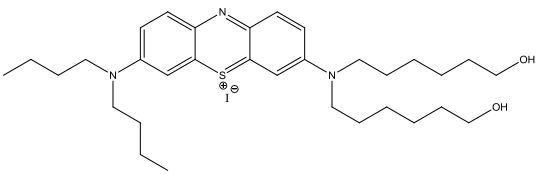
A solution of di(2-hydroxyethyl)amine (2 M in methanol) was added to a solution of **3**. The reaction was carried out during 16 hours. Compound **4a** was purified by flash chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) to yield to **4a** as a dark purple solid (51%). **^{1}H NMR** (300 MHz, CD_3CN) δ (ppm) = 7.86-7.44 (m, 6H), 3.87-3.85 (m, 4H), 3.78-3.73 (m, 4H), 3.67-3.63 (m, 4H), 1.62-1.60 (m, 4H), 1.42-1.38 (m, 4H), 0.95-0.92 (t, 6H). **^{13}C NMR** (101 MHz, DMSO) δ (ppm) = 154.51, 153.04, 138.49, 137.98, 135.48, 135.42, 134.29, 133.97, 128.85, 128.41, 120.12, 119.48, 118.93, 107.51, 106.97, 59.12, 54.46, 51.67, 19.91, 14.22. **UV/Vis** (*Methanol*): λ_{max}=663 nm. **MS** (ESI+): calcd. for $C_{24}H_{34}N_3O_2S^+$ m/z = 428.2, found m/z = 428.2.

Synthesis of 3-di(hydroxypropyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4b



This compound was prepared by addition of dipropanolamine to compound **3** and further purification by flash column chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) yielded to dark purple solid (17%). **UV/Vis** (*Methanol*): λ_{max}=663 nm. **MS** (ESI+): calcd. for $C_{26}H_{38}N_3O_2S^+$ m/z = 456.3, found m/z = 456.4. The product was obtain with too low quantities for NMR measurement.

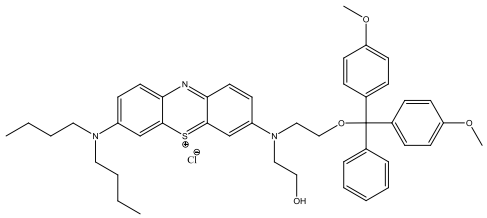
Synthesis of 3-di(hydroxyhexyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4c



This compound was prepared by addition of dihexanolamine to compound **3** and further purification by flash column chromatography (eluent CH_2Cl_2/MeOH 95/5 (v/v)) yielded to dark purple solid (34%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 7.90-7.88 (d, *J*= 1.8 Hz, 2H), 7.79 (d, *J*= 2.7 Hz, 1H), 7.60-7.59 (d, *J*= 2.7 Hz, 1H), 7.21-7.15 (m, 2H), 3.74-3.64 (m, 12H), 1.79-1.75 (m, 8H), 1.54-1.52 (m, 16H), 1.05-1.02 (t, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ (ppm) = 152.60, 138.66, 135.96, 135.45, 118.58, 118.04, 106.95, 106.59, 62.06, 52.30, 32.28, 29.88, 26.45, 25.43, 20.23, 13.88. **UV/Vis** (*Methanol*): λ_{max}=665 nm. **MS** (ESI+): calcd. for $C_{32}H_{50}N_3O_2S^+$ m/z = 540.4, found m/z = 540.5.

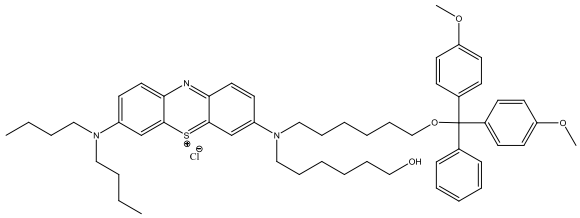
Phosphoramidite synthesis

Synthesis of 3-[(3-(4,4'-dimethoxytrityloxy)ethyl)ethylamino]-7-dibutylamino-phenothiazin-5-ium chloride 5a



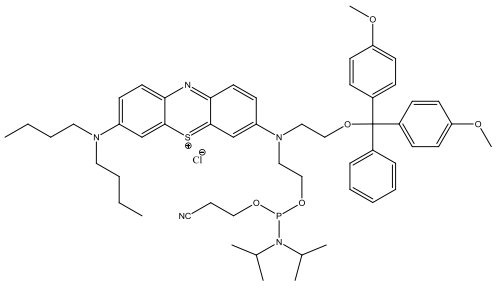
To a solution of **4a** (5.13 mmol, 1 eq) in anhydrous acetonitrile (300 mL) was added diisopropylethylamine (5.13 mmol, 1 eq), under magnetic stirring. Then, a solution of 4,4′-dimethoxytriphenylmethyl chloride (4.62 mmol, 0.9 eq) in anhydrous acetonitrile (80 mL) was added dropwise. The mixture was stirred at room temperature. When the reaction was complete (1h30), the reaction was stopped by addition of MeOH (1 mL). Then, the solution was evaporated to dryness under vacuum and purified by flash column chromatography (eluent CH_2Cl_2/MeOH/triethylamine 94/5/1 (v/v/v)). After drying under vacuum, the expected compound was obtained as a dark purple solid (52%). **^{1}H NMR** (300 MHz, CD_3CN) δ (ppm) = 7.8-7.78 (m, 1H), 7.64-7.61 (m, 1H), 7.39-7.19 (m,13H), 6.81-6.78 (m, 4H), 3.92 (m, 2H), 3.68 (s, 6H), 3.65-3.61 (m, 4H), 3.49-3.41 (m, 2H), 1.71-1.69 (m, 4H), 1.46-1.41 (m, 8H), 1.02-0.99 (t, 6H). **^{13}C NMR** (101 MHz, CD_3CN) δ (ppm) = 158.63, 153.03, 144.99, 138.41, 135.66, 129.96, 127.89, 127.83, 126.81, 119.06, 113.08, 107.30, 106.24, 60.82, 54.92, 52.75, 51.82, 45.93, 42.33, 29.47, 19.74, 13.16, 7.99, 7.03. **MS** (ESI+): calcd. for $C_{45}H_{52}N_3O_4S^+$ m/z = 730.4, found m/z = 730.7.

Synthesis of 3-[(3-(4,4'-dimethoxytrityloxy)hexyl)hexylamino]-7-dibutylamino-phenothiazin-5-ium chloride 5c



To a solution of **4c** (0.258 g, 0.39 mmol, 1 eq) in anhydrous pyridine (5 mL) was added dropwise a 4,4′-dimethoxytriphenylmethyl chloride solution (0.118 g, 0.35 mmol, 0.9 eq) in anhydrous pyridine (2 mL). The mixture was stirred at room temperature. The reaction was stopped after 16 hours by the addition of methanol (1 mL), the solution was evaporated to dryness under vacuum and purified by flash column chromatography (eluent CH_2Cl_2/ethyl acetate/triethylamine 94/5/1 (v/v/v)). After drying under vacuum, the expected compound was obtained as a dark purple solid (32%). **MS** (ESI+): calcd. for $C_{53}H_{68}N_3O_4S^+$ m/z = 842.5, found m/z = 842.5. The product was obtain with too low quantities for NMR measurement.

Synthesis of 3-[(3-(4,4'-dimethoxytrityloxy)ethyl)(3-[(2-cyanoethyl) (N,N-diisopropyl)phosphoramidityl]ethyl)amino]-7-dibutylamino-phenothiazin-5-ium chloride 6a



To a solution of **5a** (0.172 g, 0.23 mmol, 1 eq) in anhydrous acetonitrile (8 mL) was added diisopropylethylamine (78 µL, 0.45 mmol, 2 eq), under magnetic stirring. Then, a solution of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (60 µL, 0.27 mmol, 1.2 eq) was added dropwise. The mixture was stirred at room temperature. The reaction was stopped after 30 min, the solution was evaporated to dryness under vacuum and purified by flash column chromatography (eluent CH_2Cl_2/triethylamine 99/1, then CH_2Cl_2/acetonitrile/triethylamine 89/10/1 (v/v/v)). After drying under vacuum, the expected compound was obtained as a dark blue oil (23%). **^{1}H NMR** (300 MHz, CD_3CN) δ (ppm) = 7.40-7.23 (m, 14H), 6.86-6.77 (m, 5H), 4.14-4.06 (m, 4H), 3.68 (s, 6H), 3.56-3.46 (m, 10H), 2.80-2.76 (t, 2H), 2.64-2.60 (t, 2H), 1.71-1.69 (m, 4H), 1.50-1.40 (m, 4H), 1.20-1.05 (m, 12H), 1.00 (t, 6H). **^{13}C NMR** (75 MHz, CD_3CN) δ (ppm) = 158.50, 135.51, 129.84, 127.80, 112.94, 58.09, 58.02, 54.76, 44.92, 44.83, 23.81, 22.11, 22.07, 22.03, 22.00, 19.57, 19.48. **^{31}P NMR** δ (ppm) = 148.00. **MS** (ESI+): calcd. for $C_{54}H_{69}N_5O_5PS^+$ m/z = 930.5, found m/z = 930.5.

DNA synthesis and stability studies

Compound **6a** and **6b** were incorporated at the end of a DNA $d(T)_{10}$-**6a** $d(T)_{10}$-**6b** using a standard protocol with the phosphoramidite chemistry. The coupling time of **6a** and **6b** was 3 min.

After the first and the last nucleotide incorporations, dimethoxytrityl groups were released with deblocking mix (3% trichloroacetic acid (TCA) in dichloromethane), and titrated at 498 nm (ε=70 $mL\mu mol^{-1}cm^{-1}$) to determine the incorporation yield. Nucleobase deprotection and release from solid support were achieved in deprotection solution, 1-6 hours at 20°C under gentle stirring. After deprotection the solution was filtrated under ultra amicon YM3000 to remove all deprotection residues. HPLC analyses were performed to characterize and to purify **6a** and **6b**-ODN.

HPLC analyses were monitored at 260 nm and 677 nm for **6b**-ODN characterization (Figure S1). After HPLC purification and evaporation to dryness, the solution was analyzed by MALDI-ToF. **MS** (MALDI) **6a**-ODN ($[M-H]^-$ calcd. 3470.62; found 3472.1; **6b**-ODN ($[M-H]^-$ calcd. 3496.62; found 3495.3 (Figure S2).

At the same time **6a,b**-ODN degradation in deprotection solution were studied. HPLC analyses were performed every hour and the percentage of degradation peaks were calculated. A decrease of **6a,b**-ODN peak was observed and peak area were compared to obtain the degradation percentage against time (Figure 3).

We also succeeded in synthesizing a fully deprotected **6b**-$dT(A)_{20}$. HPLC analyses were monitored at 260 nm and 677 nm (Figure S3 A). After HPLC purification and evaporation to dryness, the solution was analyzed by MALDI-ToF. **MS** (MALDI) $[M-H]^-$ calcd. 7024.02; found 7021.9; (Figure S3 B).

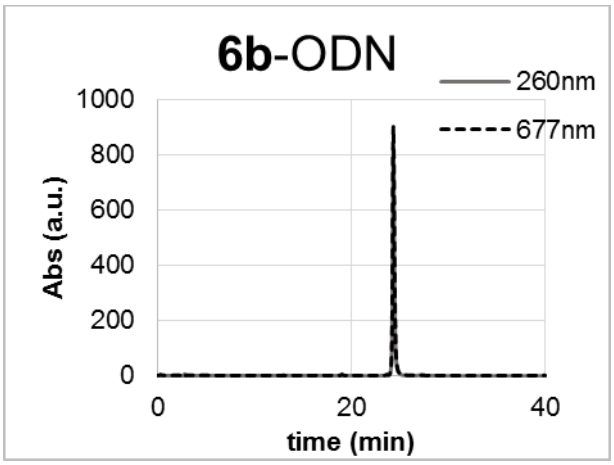


Figure S1: HPLC analysis of poly-$(dT)_{10}$ modified with **6b** at the 5'-end; gradient elution from 4.5% to 12.5% of acetonitrile in 0.05 M TEAAc in 40 min. Superposition of two chromatograms at a different wavelength: λ=260 nm (oligonucleotide maximum absorbance), and λ=677 nm (maximum absorbance for methylene blue covalently bound to oligonucleotide).

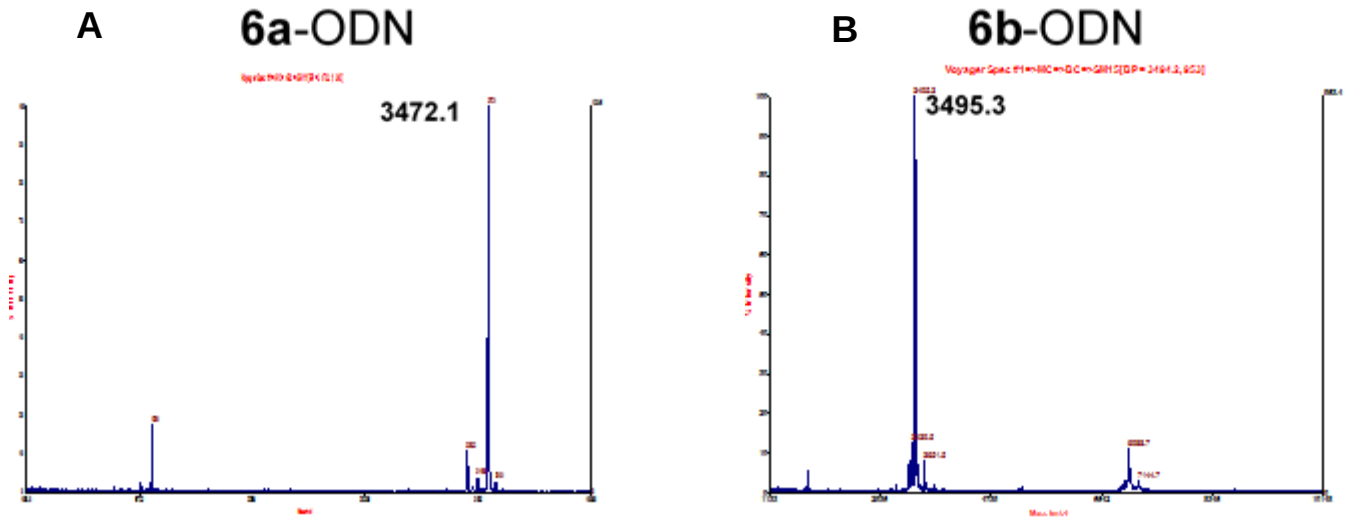


Figure S2: MALDI-ToF spectrometry with 2-hydroxypiccolinic acid, ammonium citrate as matrix A) **6a**-ODN $[M-H]^-$ calcd. 3470.62; found 3472.1; B) **6b**-ODN $[M-H]^-$ calcd. 3496.62; found 3495.3.

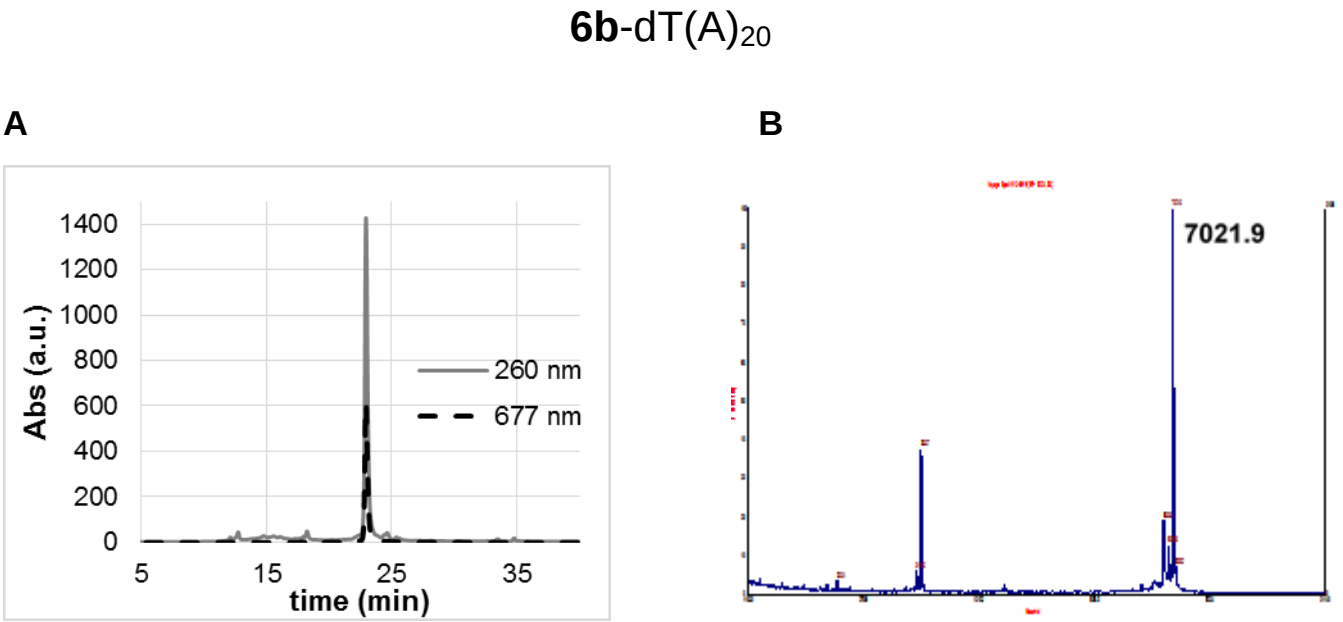


Figure S3 : ODN **6b**-$dT(A)_{20}$ characterizations: A) HPLC analysis of $dT(A)_{20}$ modified with **6b** at the 5'-end; gradient elution from 4.5% to 12.5% of acetonitrile in 0.05 M TEAAc in 30 min. Superposition of two chromatograms at a different wavelength: λ=260 nm (oligonucleotide maximum absorbance), and λ=677 nm (maximum absorbance for methylene blue covalently bound to oligonucleotide); B) MALDI-ToF spectrometry with 2-hydroxypiccolinic acid, ammonium citrate as matrix.

Electrochemical measurements

All compounds (**2a-2h; 4a-4c**) have been characterized by electrochemistry. 1mL of MB solutions from 0.05 to 2 mM, in PBE was introduced in the 3 electrodes cell. The signal of MB (**2a-2h; 4a-4c**) was recorded by scanning the potential between -0.7 and -0.4 V *vs* Ag/Ag^+ using cyclic voltammetry (CV) with a scan rate of 10 mVs^{-1} (see Table S1, and voltammograms S20-S24).

6b-ODN was characterized in the same system using a phosphate-buffered electrolyte phosphate (20 mM), potassium chloride (250 mM), pH 6.4 in water, with Ag/AgCl as reference electrode. Voltammogram is shown in Figure S4.

compound	λ_{max} (nm)	E_{pa} (mV)	E_{pc} (mV)	ΔE_p (mV)	$E_{1/2}$ (mV)
2a	653	-213	-258	45	-236
2b	657	-235	-281	46	-258
2c	663	-196	-262	66	-229
2d [a]	673	/	/	/	/
2e	658	-68	-112	44	-90
2f	658	-191	-232	41	-212
2g	663	-212	-245	33	-229
2h	662	-213	-253	40	-233
4a	663	-226	-255	29	-241
4b	663	-210	-235	25	-223
4c	665	-218	-245	27	-232

[a] too low solubility in aqueous buffer for electrochemical characterization.

Table S1: Optical and electrochemical characterization of MB derivatives (**2a-h**, **4a-c**). Data have been recalculated *vs* Ag/AgCl reference to be compared to literature. E_{pa}, E_{pc} are the anodic peak and cathodic peak potentials, respectively. ΔE_p is the difference between E_{pa} and E_{pc} and $E_{1/2}$ is the measured half-wave potential.

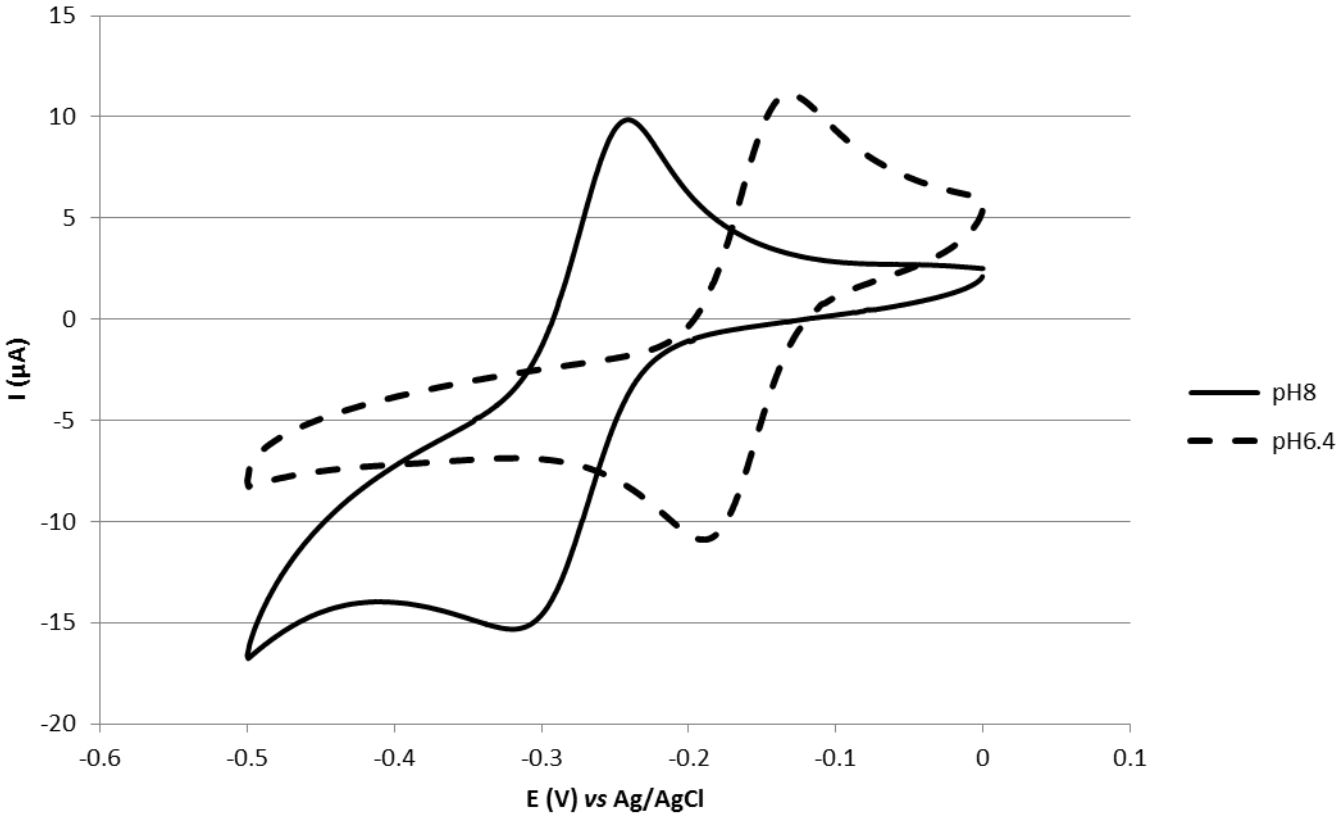
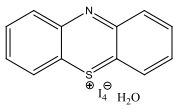


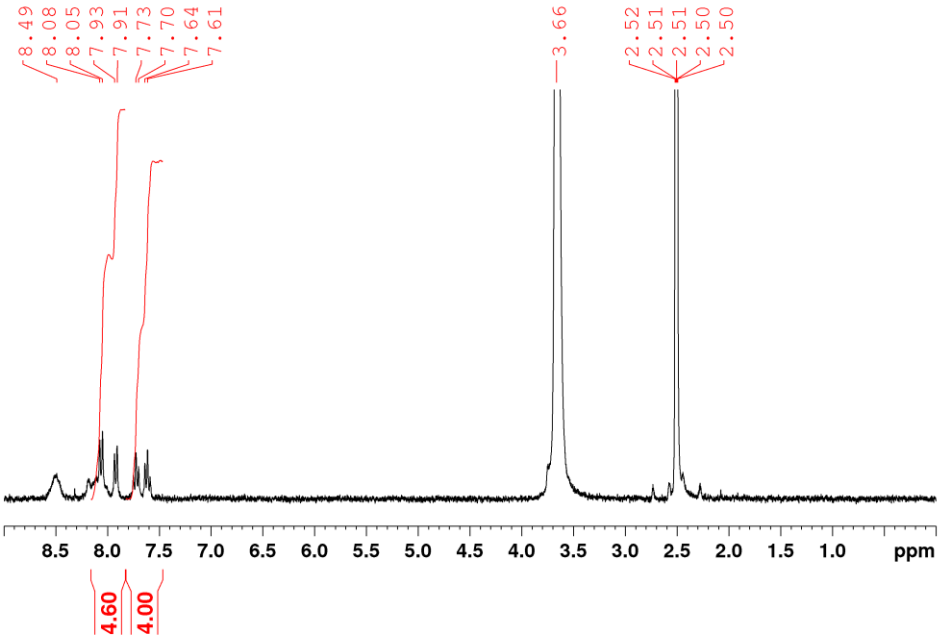
Figure S4: Electrochemical response of **6b**-ODN (1.5 mM in 20 mM phosphate, 250 mM KCl, pH 6.4 or pH 8) by cyclic voltammetry at 10 mVs^{-1}.

NMR spectra

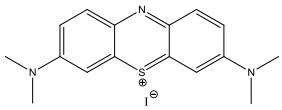
x: solvents, or residual reagents

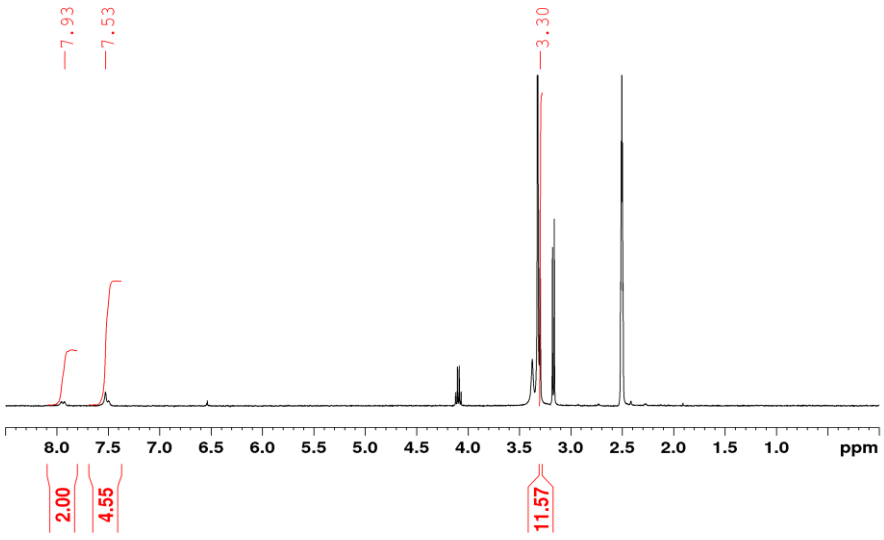
phenothiazinium tetraiodide hydrate 1



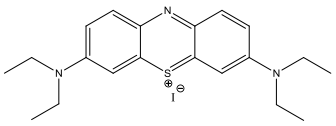


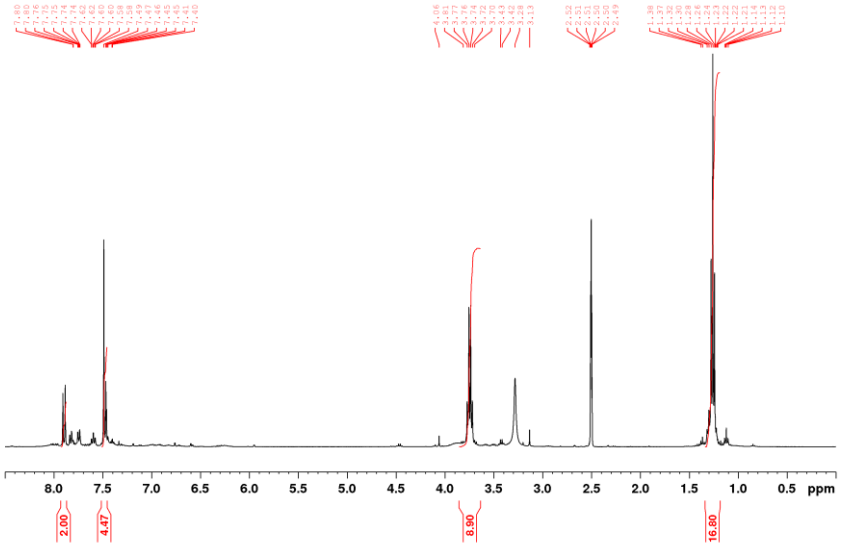
3,7-bis(dimethylamino)-phenothiazin-5-ium iodide 2a



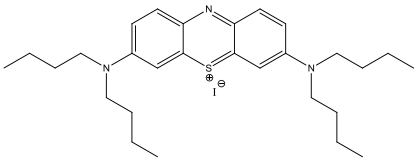


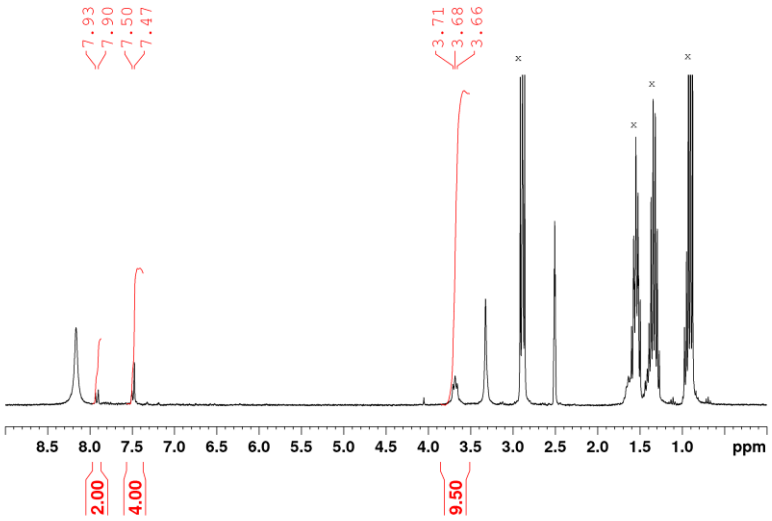
3,7-bis(diethylamino)-phenothiazin-5-ium iodide 2b



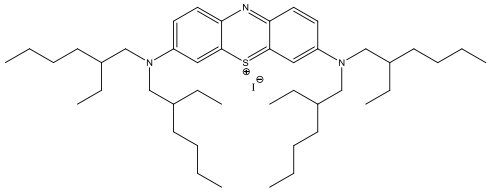


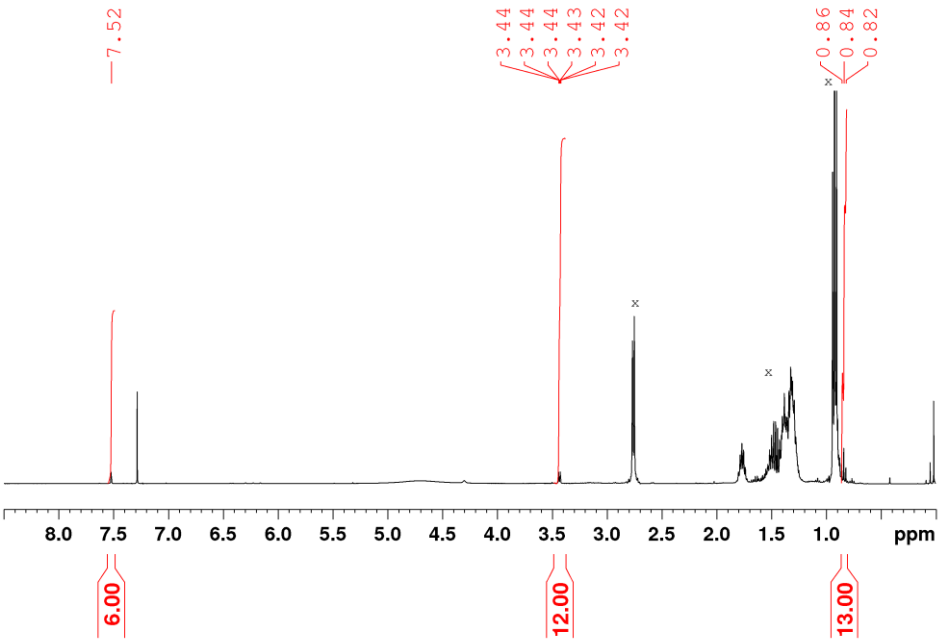
3,7-bis(dibutylamino)-phenothiazin-5-ium iodide 2c



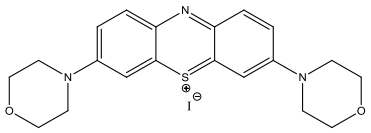


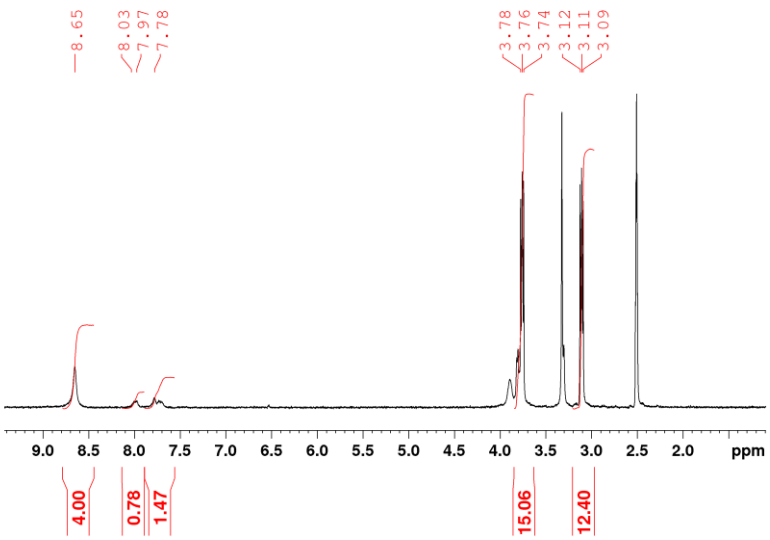
3,7-bis(di(2-ethylhexyl)amino)-phenothiazin-5-ium iodide 2d



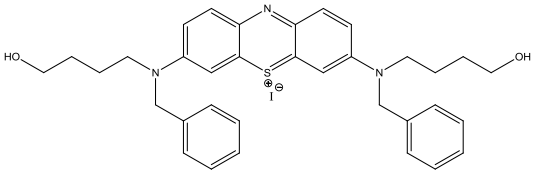


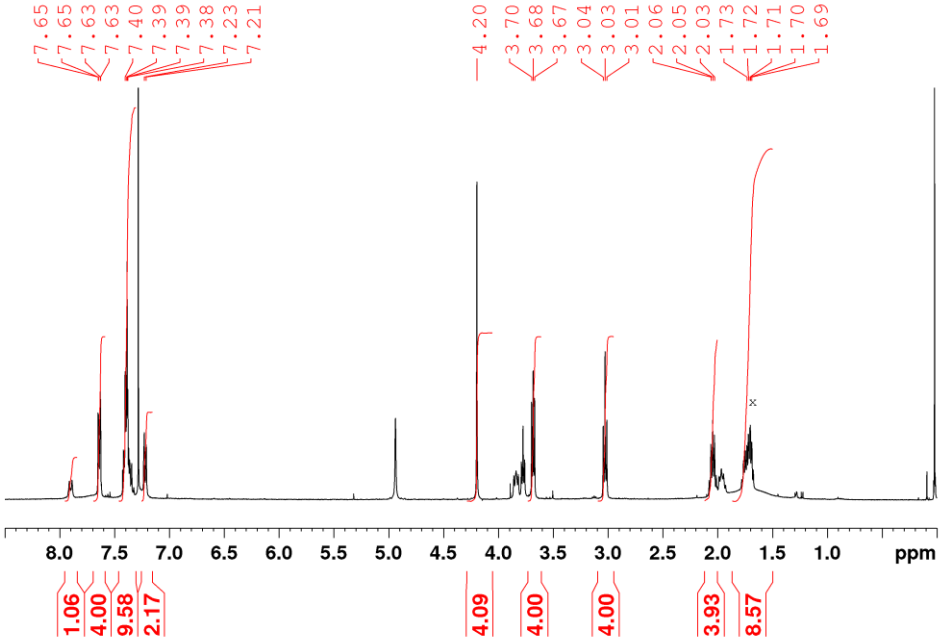
3,7-dimorpholino-phenothiazin-5-ium iodide 2e



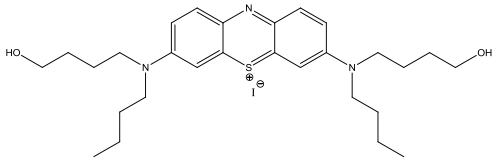


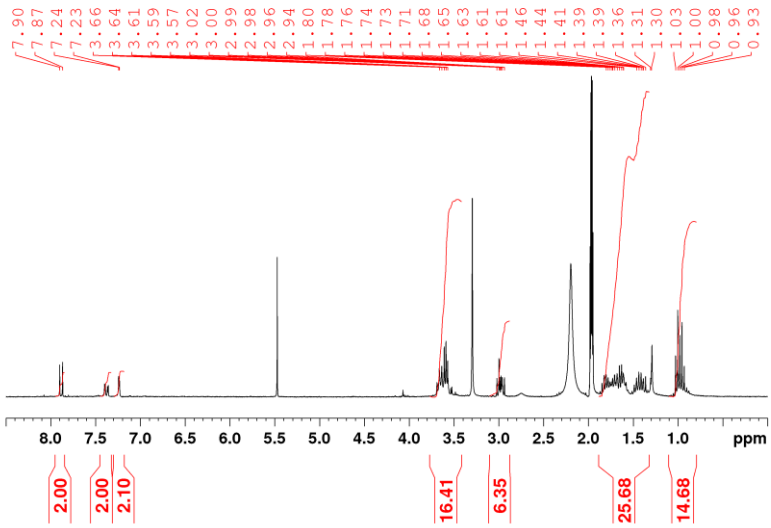
3,7-bis(di(4-hydroxybutylbenzyl)amino)-phenothiazin-5-ium iodide 2f



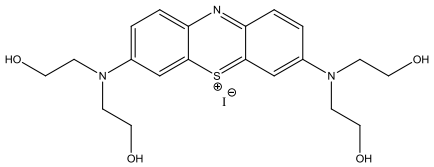


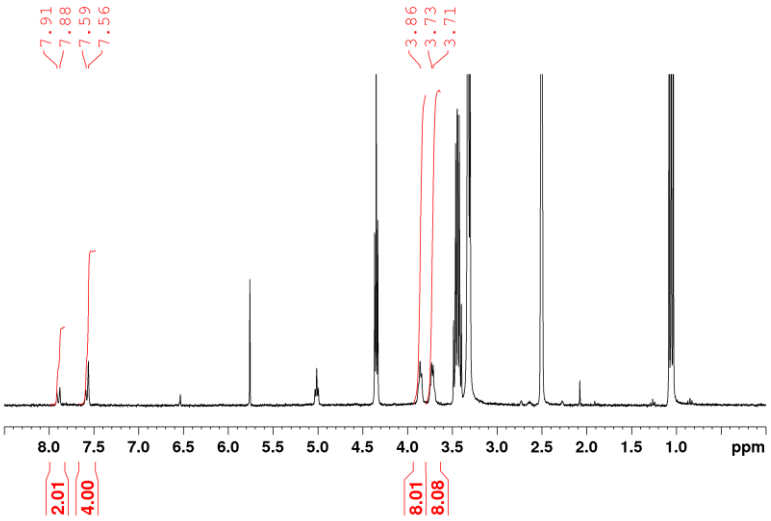
3,7-bis(di(4-hydroxybutylbutyl)amino)-phenothiazin-5-ium iodide 2g



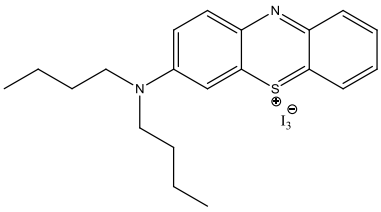


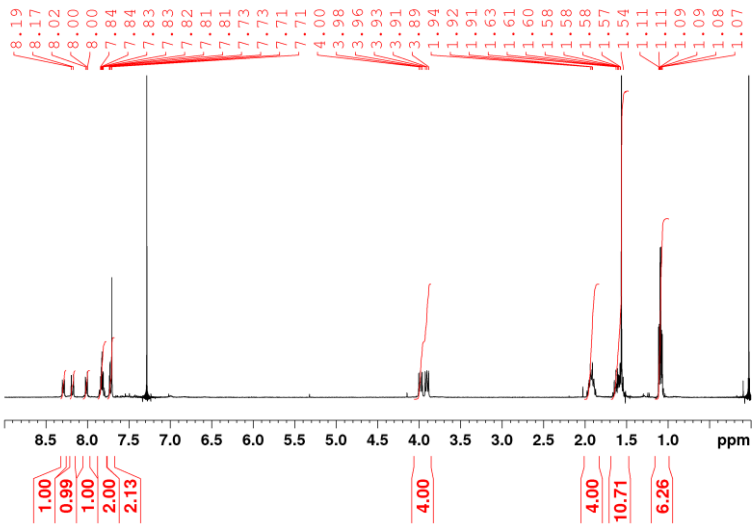
3,7-bis(di(2-hydroxyethyl)amino)-phenothiazin-5-ium iodide 2h

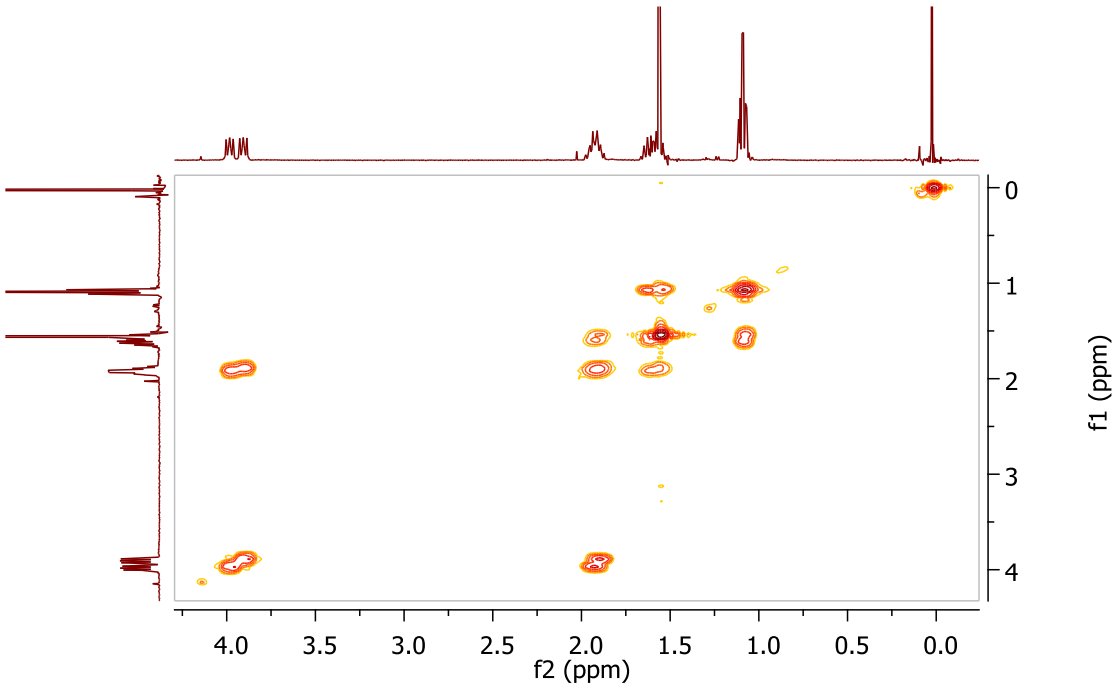




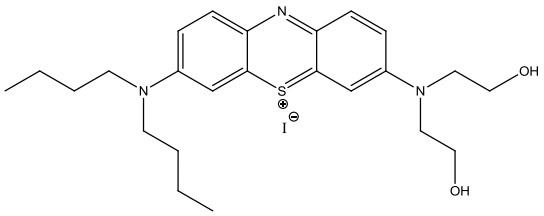
3-dibutylamino-phenothiazin-5-ium triiodide 3

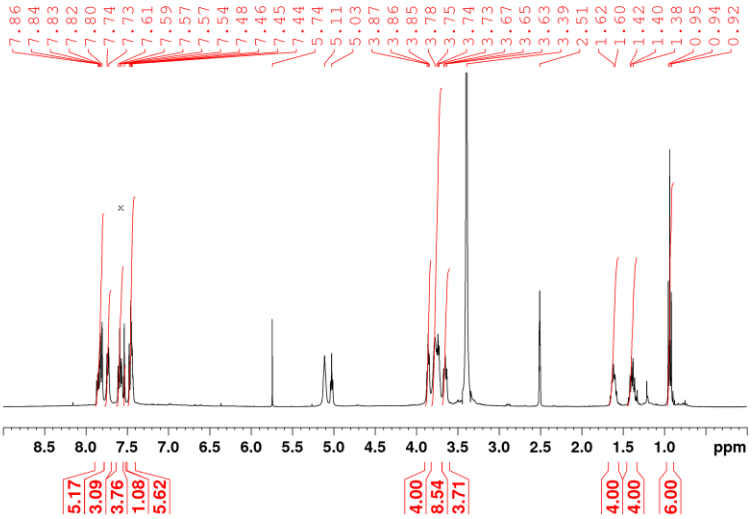


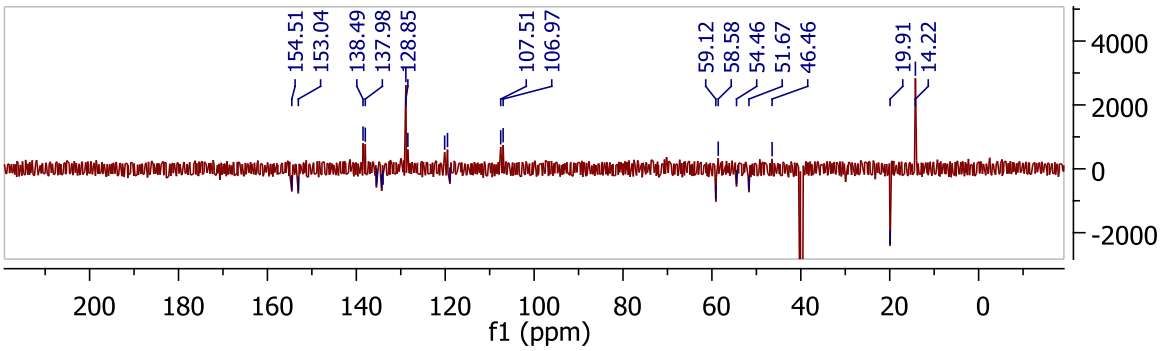




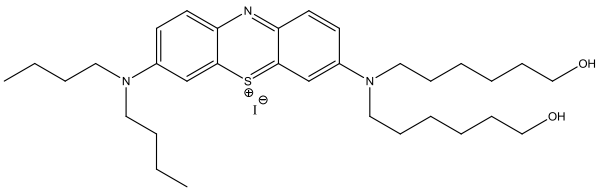
3-di(hydroxyethyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4a

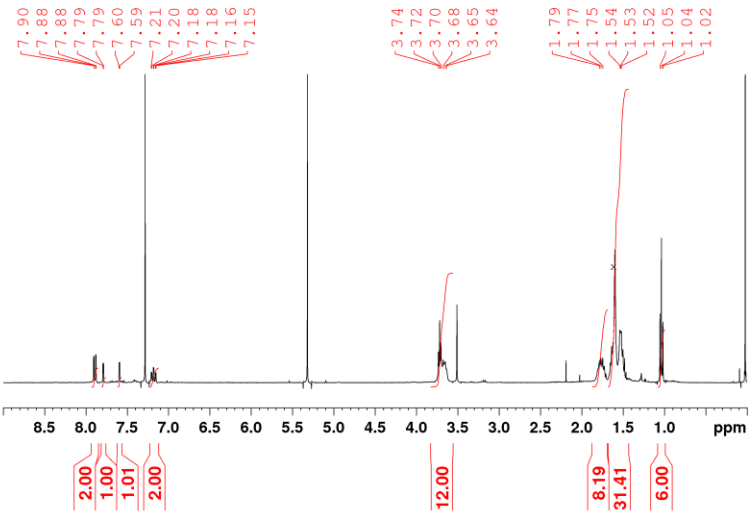


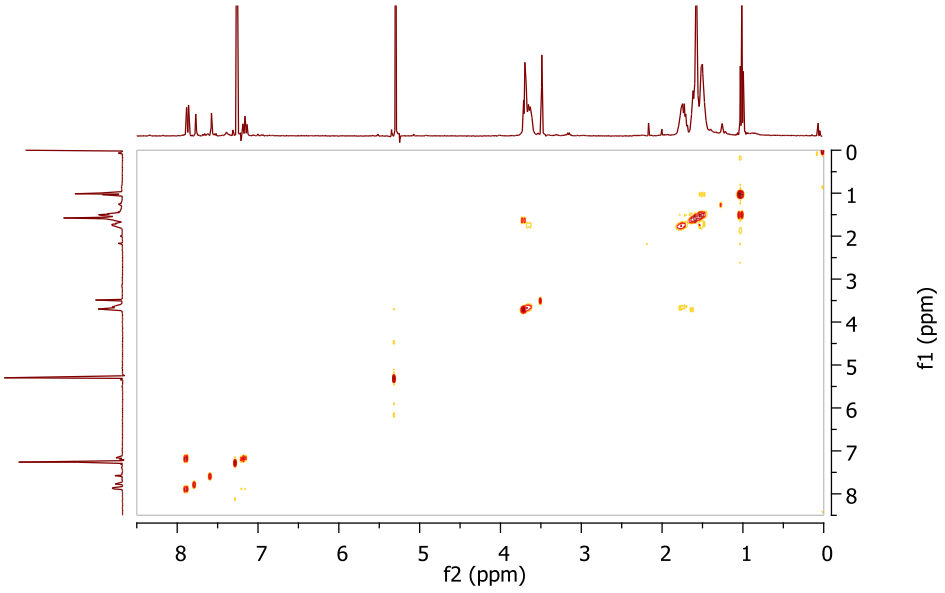


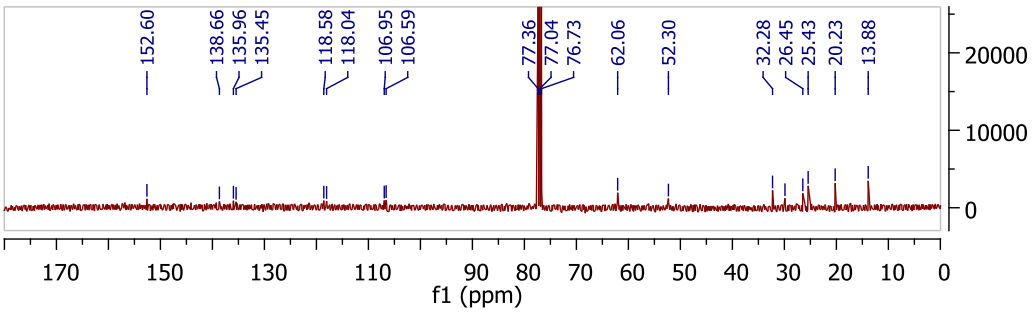


3-di(hydroxyhexyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4c

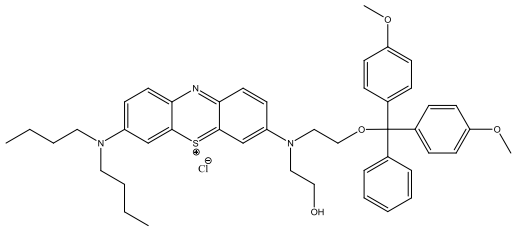


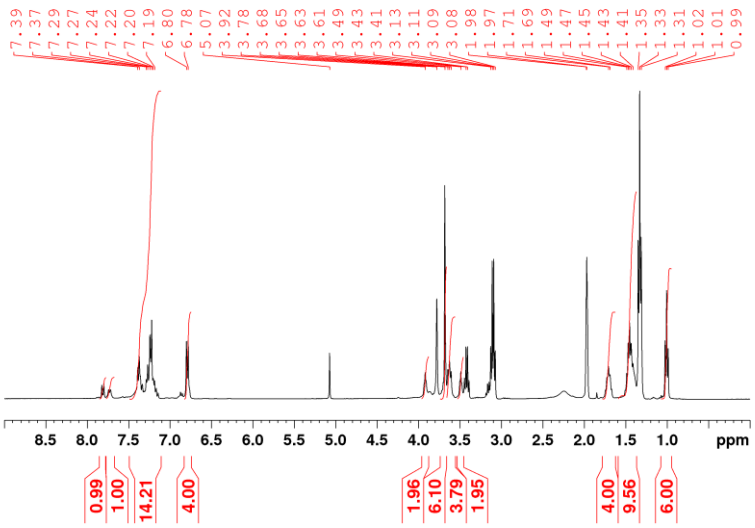


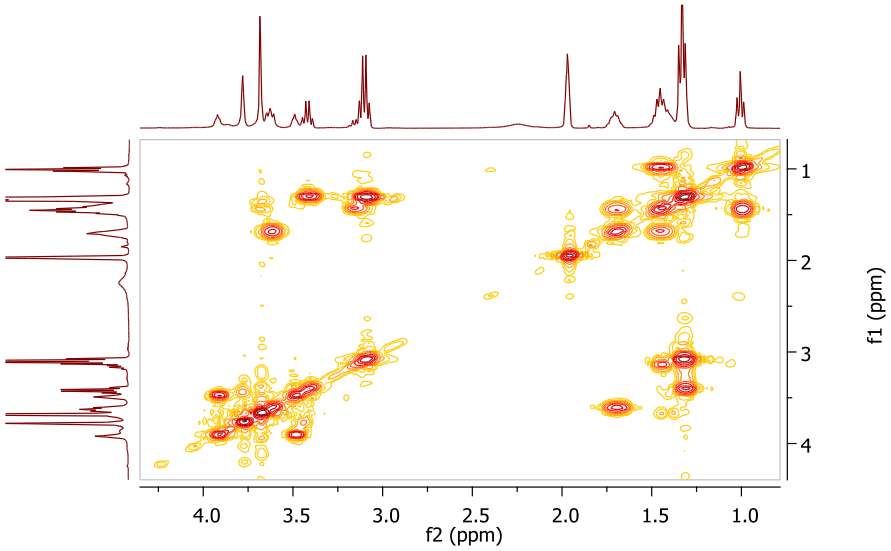


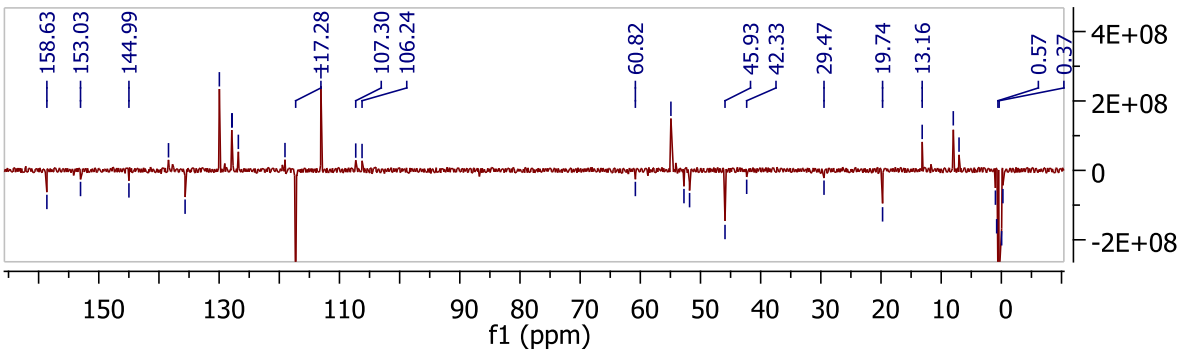


3-[(3-(4,4'-dimethoxytrityloxy)ethyl)ethylamino]-7-dibutylamino-phenothiazin-5-ium chloride 5a

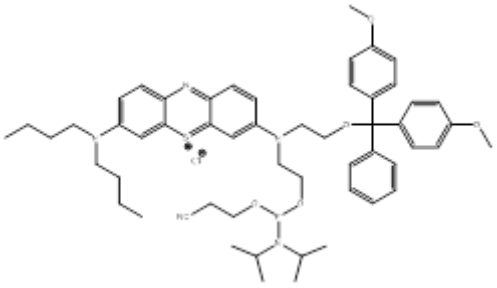


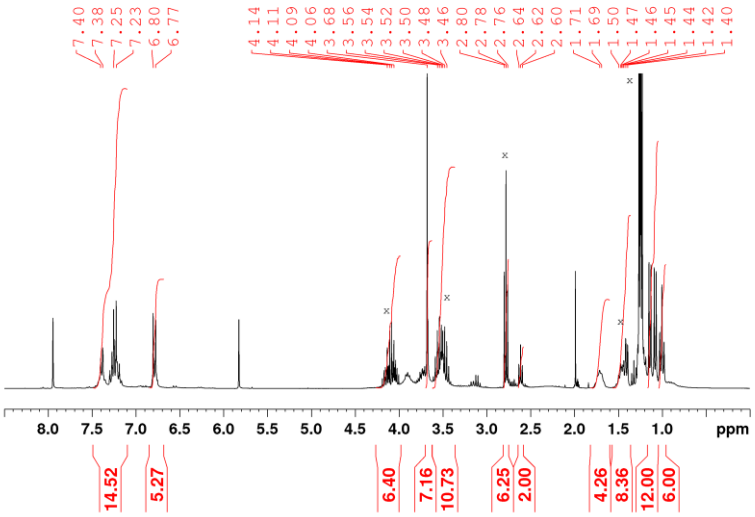


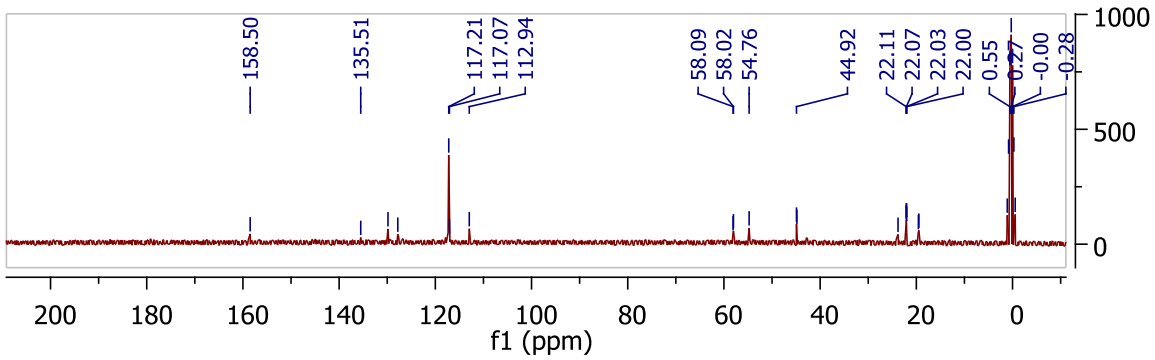


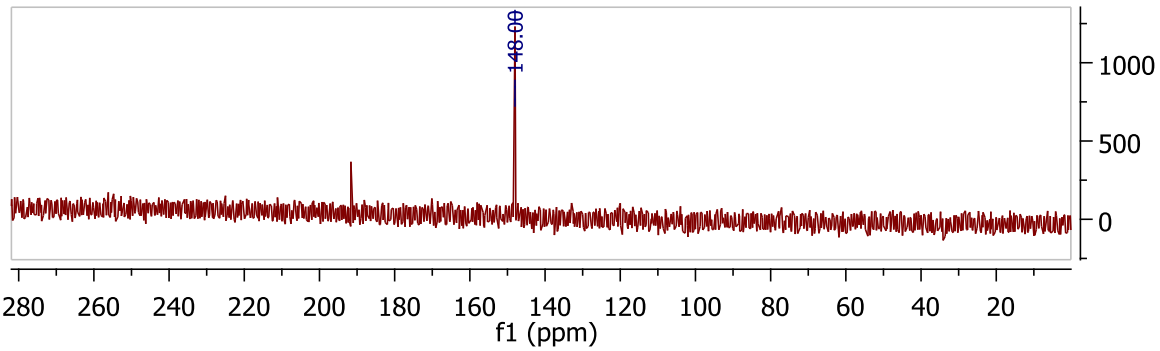


3-[(3-(4,4'-dimethoxytrityloxy)ethyl)(3-[(2-cyanoethyl) (N,N-diisopropyl)phosphoramidityl]ethyl)amino]-7-dibutylamino-phenothiazin-5-ium chloride 6a





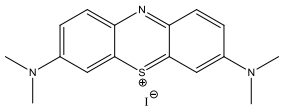


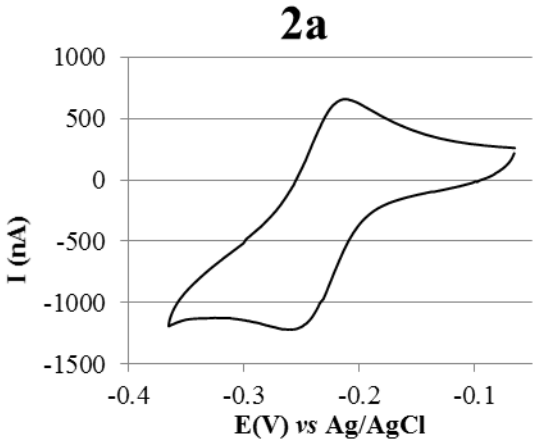


Cyclic voltammograms

Electrochemical characterization of MB derivatives (**2a-h**, **4a-c;** excepted **2d** due to its low solubility) were recorded by scanning the potential between -0.65 and -0.4 V *vs* Ag/Ag+ using cyclic voltammetry (CV) with a scan rate of 10 mVs^{-1}. The working solutions were: phosphate-buffered electrolyte (PBE): phosphate (20 mM), potassium chloride (250 mM), pH 6.4 in a mix water/acetonitrile (50/50). Data have been recalculated *versus* Ag/AgCl reference to be compared to literature.

3,7-bis(dimethylamino)-phenothiazin-5-ium iodide 2a





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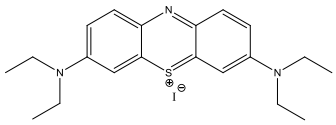

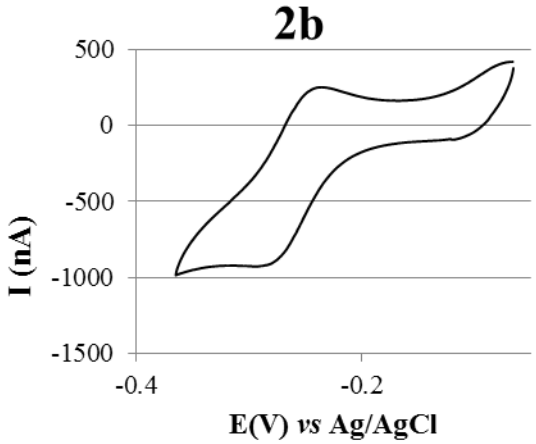

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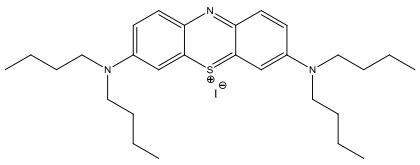
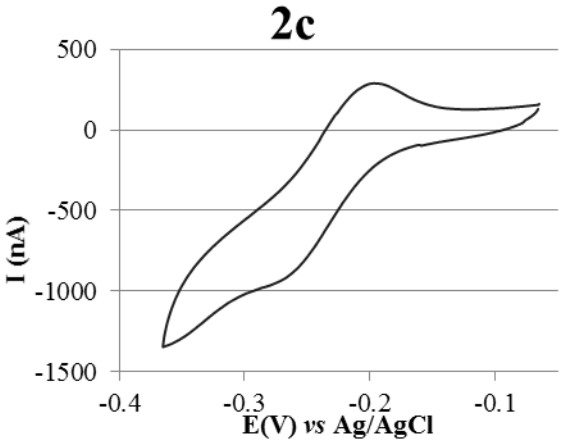
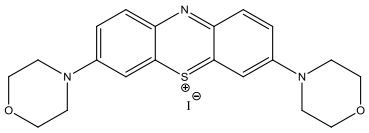
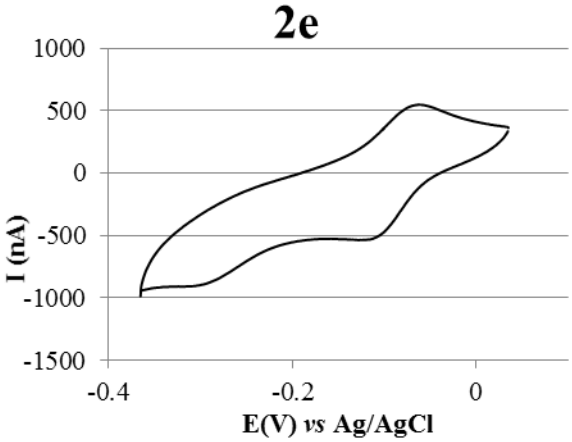


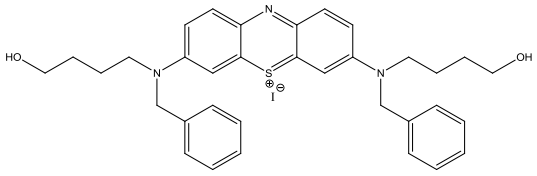


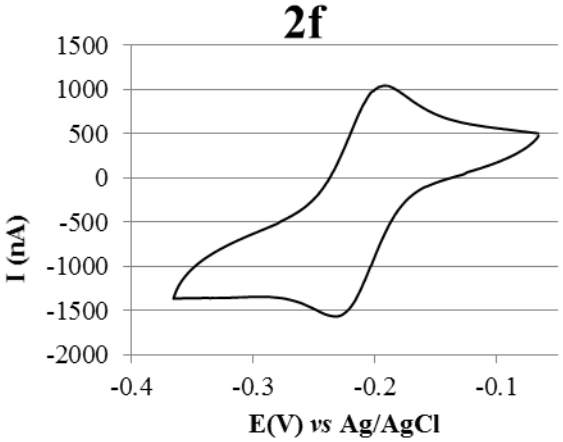
3,7-dimorpholino-phenothiazin-5-ium iodide 2e



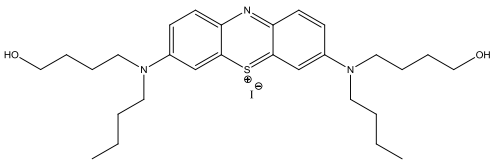


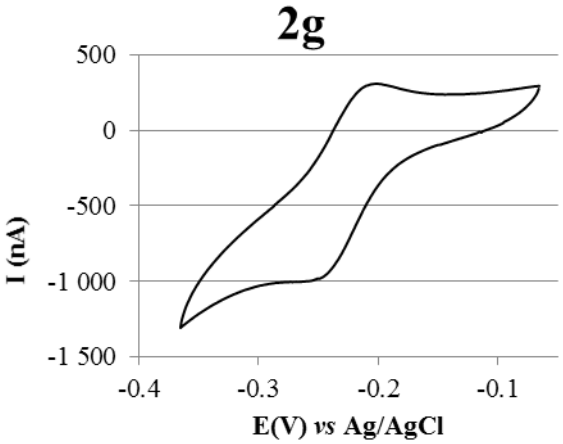
3,7-bis(di(4-hydroxybutylbenzyl)amino)-phenothiazin-5-ium iodide 2f



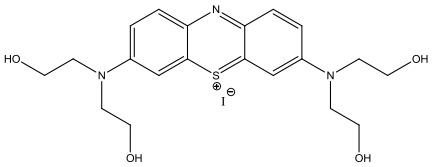


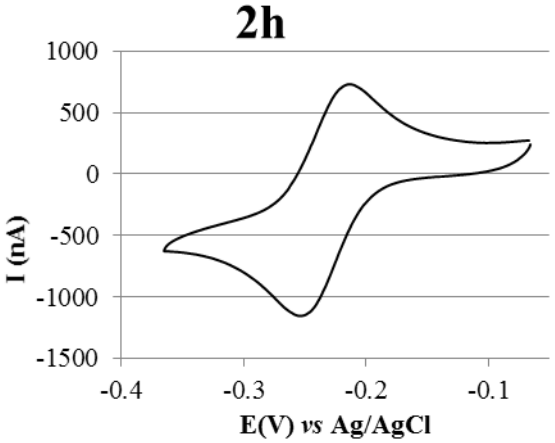
3,7-bis(di(4-hydroxybutylbutyl)amino)-phenothiazin-5-ium iodide 2g



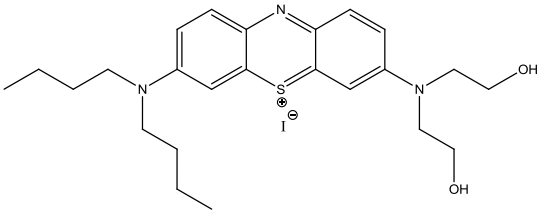


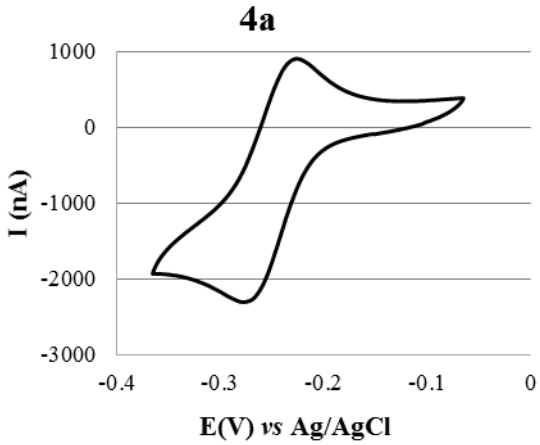
3,7-bis(di(2-hydroxyethyl)amino)-phenothiazin-5-ium iodide 2h



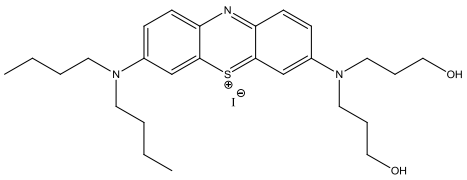


3-di(hydroxyethyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4a

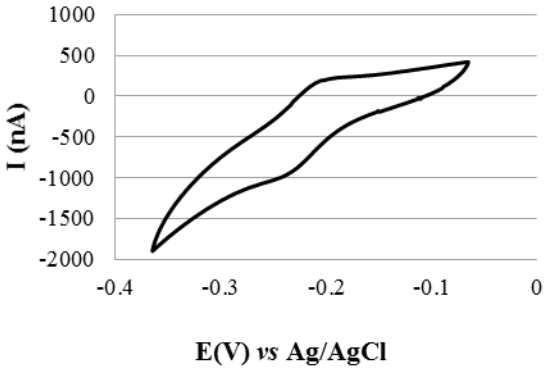




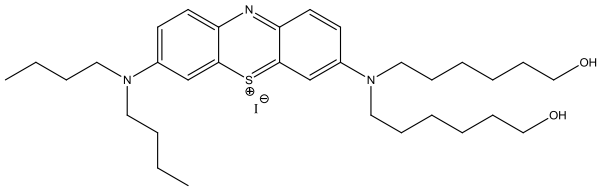
3-di(hydroxypropyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4b



4b



3-di(hydroxyhexyl)amino-7-dibutylamino-phenothiazin-5-ium iodide 4c



4c