Chemical synthesis of lipophilic methylene blue analogues which increase mitochondrial biogenesis and frataxin levels

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ABSTRACT

As part of an ongoing program to develop potential therapeutic agents for the treatment of the neurodegenerative disease Friedreich’s ataxia (FRDA), we have prepared a number of lipophilic methylene blue analogues. Some of these compounds significantly increase mitochondrial biogenesis and frataxin levels in cultured Friedreich’s ataxia cells [1]. This data article describes the chemical synthesis and full physicochemical characterization of the new analogues.

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Several lipophilic methylene blue analogues were prepared by chemical synthesis, starting from 2-cyanophenothiazine.

N-protected 2-cyanophenothiazine was converted to the respective aldehyde, enabling introduction of the lipophilic substituents via a Wittig reaction and of the dialkylamines at positions 3 and 7 by treatment with the amines in the presence of iodine.

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Data is with this article.

The data enable the preparation of lipophilic methylene blue derivatives for evaluation in FRDA models.

Characterization of the methylene blue analogues permitted verification of structure.

The methods outlined should be extensible to additional new compounds of this type.

### 1. Data

The synthetic routes employed for the preparation of lipophilic methylene blue analogues are outlined in Schemes 1 and 2. Analogues 1–7, each having a long alkyl substituent on a phenothiazine nucleus (Scheme 1), were prepared starting from commercially available 2-cyanophenothiazine. Initially, the N atom at position 10 was protected by treatment with NaH (60% in mineral oil) at 0 °C, followed by di-tert-butyl dicarbonate, affording N-Boc derivative 8 in 72% yield. Reductive hydrolysis of protected cyanophenothiazine 8 by DIBAL-H and 2N HCl afforded aldehyde 9 in 81% yield [2]. By treating 9 with each of six alkyltriphenylphosphonium bromides in the presence of 1 M NaHMDS, according to a usual protocol for Wittig reactions, the corresponding intermediate alkenes (10–15) were obtained as cis-trans mixtures. The latter were then reduced by catalytic hydrogenation over palladium-on-carbon to afford the corresponding alkanes (16–21) in good yields. In the final step, the Boc protecting group was removed with 10 equivalents of CF₃COOH, then the intermediate was oxidized with iodine in CH₂Cl₂ followed by the subsequent addition of dimethylamine to afford analogues 1–6 (Scheme 1) [3].

Analogue 7 was obtained by treating intermediate 21 with morpholine to provide bis-morpholino derivative 7 in 28% yield (Scheme 2).

### 2. Experimental design, materials and methods

#### 2.1. General experimental procedures

Reagent grade chemicals and solvents were purchased from Sigma-Aldrich Chemicals and were used without further purification. All reactions were performed under an argon atmosphere, unless otherwise specified. Thin layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used for analytical TLC and were visualized by UV irradiation (254 nm). Flash chromatography was carried out using Silicycle 200–400 mesh silica gel. ¹H and ¹³C NMR spectra were obtained using a Varian 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to residual CHCl₃ (δ 7.26 ppm for ¹H NMR and δ 77.16 for ¹³C NMR) as the internal standard. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. High resolution mass spectra were obtained at the Arizona State University CLAS High Resolution Mass Spectrometry Facility.
Scheme 1. Synthetic routes employed for compounds 1-6.

Scheme 2. Synthetic route employed for compound 7.
2.2. Synthesis of the methylene blue analogues

2.2.1. tert-Butyl 2-Cyano-10H-phenothiazine-10-carboxylate (8)
A sample of 2.00 g (8.90 mmol) of 2-cyanophenothiazine was dissolved in 25 mL of anhydrous DMF. The reaction mixture was cooled to 0 °C and 0.53 g (13.3 mmol) of 60% NaH was added. The dark reaction mixture was stirred at 0 °C for an additional 15 min and 2.33 g (10.6 mmol) of di-tert-butyl dicarbonate was added. The reaction mixture was stirred at room temperature for 18 h and was then diluted with 60 mL of brine. The aqueous layer was extracted with three 25-mL portions of dichloromethane. The combined organic extract was dried over anhydrous MgSO4 and concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 9:1 hexanes-ethyl acetate gave 8 as a pale yellow solid: yield 2.10 g (72%); silica gel TLC Rf 0.26 (9:1 hexanes-ethyl acetate); 1H NMR (CDCl3) δ 1.49 (s, 9H), 7.16–7.20 (m, 1H), 7.28–7.33 (m, 2H), 7.40 (s, 2H), 7.49 (d, 1H, J = 8.4 Hz) and 7.80 (s, 1H); 13C NMR δ 28.1, 83.1, 110.2, 118.2, 126.6, 127.2, 127.3, 127.5, 128.1, 130.3, 130.6, 137.7, 139.0, 139.1 and 151.8; mass spectrum (APCI), m/z 325.1017 (M+H)+ (C18H17N2O2S requires m/z 325.1011).

2.2.2. tert-Butyl 2-Formyl-10H-phenothiazine-10-carboxylate (9)
To a solution of 2.30 g (7.10 mmol) of tert-butyltriphenylphosphonium bromide was dissolved in 25 mL of anhydrous CH2Cl2 was added dropwise at −78 °C 8.50 mL (8.50 mmol) of 1 M DIBAL–H in toluene. The reaction mixture was stirred at −78 °C for 3 h and was then diluted with 30 mL of brine. The aqueous layer was extracted with three 30-mL portions of CH2Cl2. The combined organic extract was dried over anhydrous MgSO4 and then concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 9:1 hexane-ethyl acetate afforded 9 as a yellow solid: yield 1.88 g (81%); silica gel TLC Rf 0.17 (9:1 hexane-ethyl acetate); 1H NMR (CDCl3) δ 1.48 (s, 9H), 7.14–7.18 (m, 1H), 7.26–7.32 (m, 2H), 7.44 (d, 1H, J = 8.0 Hz), 7.52 (d, 1H, J = 7.4 Hz), 7.64 (d, 1H, J = 8.0 Hz), 7.99 (s, 1H) and 9.96 (s, 1H); 13C NMR δ 28.2, 82.9, 126.5, 126.7, 127.2, 127.3, 127.5, 127.9, 128.4, 130.6, 135.1, 138.0, 139.2, 140.3, 152.1 and 190.9; mass spectrum (ESI), m/z 328.1003 (M+H)+ (C18H18NO3S requires m/z 328.1007).

2.2.3. tert-Butyl (E)-2-((Pent-1- enyl)-10H-phenothiazine-10-carboxylate (10)
A sample containing 2.30 g (5.81 mmol) of (1-butyl)triphenylphosphonium bromide was dissolved in 25 mL of anhydrous tetrahydrofuran. The cooled (−40 °C) reaction mixture was treated dropwise with 5.81 mL (5.81 mmol) of sodium bis(trimethylsilyl) amide. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was cooled to −78 °C and 1.90 g (5.81 mmol) of 9, dissolved in 15 mL of anhydrous tetrahydrofuran, was added. The combined reaction mixture was stirred at 0 °C for 3 h and was then diluted with 30 mL of brine. The aqueous layer was extracted with three 30-mL portions of dichloromethane. The combined organic phase was washed with 20 mL of brine, dried over anhydrous Na2SO4 and concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 4:1 hexane-dichloromethane afforded 10 as a yellow solid: yield 1.3 g (62%); silica gel TLC Rf 0.66 (9:1 hexane-dichloromethane); 1H NMR (CDCl3) δ 1.03 (t, 3H, J = 7.2 Hz), 1.57 (s, 11H), 2.42 (q, 2H, J = 7 Hz), 5.75 (m, 1H), 6.48 (d, 1H, J = 2 Hz), 7.15 (m, 2H), 7.32 (m, 3H), 7.58 (s, 1H) and 7.63 (d, 1H, J = 8 Hz); 13C NMR (CDCl3) δ 13.6, 22.8, 27.8, 30.3, 81.5, 125.7, 126.1, 126.3, 126.7, 127.0, 127.2, 127.7, 127.8, 131.8, 133.2, 136.2, 138.2, 138.5 and 152.0; mass spectrum (APCI), m/z 368.1680 (M+H)+ (C12H18NO3S requires m/z 368.1684).

2.2.4. tert-Butyl 2-Pentyl-10H-phenothiazine-10-carboxylate (16)
A sample containing 1.90 g (5.31 mmol) of 10 was dissolved in 20 mL of 7:3 ethanol-dichloromethane and purged with argon for 20 min. To the resulting solution was added 110 mg of 10% palladium-on-carbon. The suspension was stirred at room temperature under an atmosphere of H2 (40 psi) for 2 h. The reaction mixture was then filtered through a Celite pad. The filtrate was concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 4:1 hexane-dichloromethane afforded 16 as a colorless oil: yield 1.7 g (87%); silica gel TLC Rf 0.62 (9:1 hexane-dichloromethane); 1H NMR (CD3OD) δ 0.76 (s, 3H), 1.16 (s, 4H), 1.32 (s, 9H), 1.45 (s, 2H), 2.41 (s, 2H), 6.77 (s, 1H), 6.94 (s, 1H), 7.05 (m, 2H), 7.14 (s, 1H), 7.23 (s, 1H) and 7.38 (s, 1H); 13C NMR (CD3OD) δ 14.6, 23.4, 28.5, 32.1, 32.3, 36.3, 82.8, 127.0, 127.46, 127.47, 128.0,
2.2.5. N-(7-(Dimethylamino)-2-pentyl-3H-phenothiazin-3-ylidene)-N-methylmethanaminium Iodide (1)

A sample containing 1.70 g (4.60 mmol) of 16 was dissolved in 20 mL of anhydrous dichloromethane. To the resulting solution was added dropwise 2.81 mL (36.8 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with 20 mL of saturated sodium bicarbonate solution, extracted with two 30-mL portions of dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The crude residue was utilized for the next step without further purification.

To a solution containing 180 mg (0.66 mmol) of the crude residue in 5 mL of dichloromethane was added 543 mg (2.14 mmol) of iodine and the reaction mixture was stirred in the dark for 15 min. To the resulting solution was added dropwise 1.70 mL (3.34 mmol) of 2 M dimethylamine in THF, and the reaction mixture was stirred at room temperature for 4 h. The greenish blue mixture was purified on a silica gel column (20 × 3 cm). Elution with at 1:1 methanol-acetonitrile afforded 1 as a blue solid: yield 145 mg (20%); silica gel TLC Rf 0.23 (CH3CN); 1H NMR (CDCl3) δ 0.93 (t, 3 H, J = 6.4 Hz), 1.31 (s, 13H), 1.53 (s, 10H), 2.38 (q, 2H, J = 7 Hz), 5.71 (m, 1H), 6.40 (d, 1H, J = 11.2 Hz), 7.13 (m, 2H), 7.28 (q, 2H, J = 7.4 Hz), 7.35 (d, 1H, J = 7.6 Hz), 7.49 (s, 1H) and 7.57 (d, 1H, J = 7.6 Hz); 13C NMR (CDCl3) δ 14.2, 22.8, 27.3, 28.3, 29.4, 29.7, 29.8, 29.9, 30.0, 32.0, 82.1, 126.1, 126.6, 127.3, 127.7, 127.8, 127.9, 129.9, 130.0, 131.0, 132.3, 134.0, 136.7, 138.6, 138.9 and 152.5; mass spectrum (ESI), m/z 354.1997 (M⁺) (C23H28N3S requires 354.2004); ultraviolet/visible spectrum λmax 665 nm (CH2Cl2), λmax 665 nm (MeOH).

2.2.6. tert-Butyl (E)-2-(Undec-1-enyl)-10H-phenothiazine-10-carboxylate (11)

A sample containing 792 mg (1.64 mmol) of (1-decyl)triphenylphosphonium bromide was dissolved in 12 mL of anhydrous dichloromethane. To the resulting solution was added dropwise 1.64 mL (1.64 mmol) of sodium bis(trimethylsilyl) amide. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was cooled to −78 °C, and 537 mg (1.64 mmol) of 9, dissolved in 8 mL of anhydrous tetrahydrofuran, was added. The reaction mixture was stirred at −78 °C and 357 mg (1.64 mmol) of 16, dissolved in 20 mL of anhydrous dichloromethane, was added. The reaction mixture was stirred at −78 °C for 18 h. The reaction mixture was extracted with two 15-mL portions of dichloromethane. The organic phase was washed with 15 mL of brine, dried over anhydrous Na2SO4 and concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 1 cm). Elution with 4:1 hexane-dichloromethane afforded 11 as a yellow solid: yield 145 mg (20%); silica gel TLC Rf 0.68 (1:1 hexane-dichloromethane); 1H NMR (CDCl3) δ 0.93 (t, 3 H, J = 6.4 Hz), 1.31 (s, 13H), 1.53 (s, 10H), 2.38 (q, 2H, J = 7 Hz), 5.71 (m, 1H), 6.40 (d, 1H, J = 11.2 Hz), 7.13 (m, 2H), 7.28 (q, 2H, J = 7.4 Hz), 7.35 (d, 1H, J = 7.6 Hz), 7.49 (s, 1H) and 7.57 (d, 1H, J = 7.6 Hz); 13C NMR (CDCl3) δ 14.2, 22.8, 27.3, 28.3, 29.4, 29.7, 29.8, 29.9, 30.0, 32.0, 82.1, 126.1, 126.6, 127.3, 127.7, 127.8, 127.9, 129.9, 130.0, 131.0, 132.3, 134.0, 136.7, 138.6, 138.9 and 152.5; mass spectrum (APCI), m/z 452.2617 (M⁺) (C28H38NO2S requires 452.2623).

2.2.7. tert-Butyl 2-Undecyl-10H-phenothiazine-10-carboxylate (17)

A sample containing 850 mg (1.88 mmol) of 17 was dissolved in 10 mL of 7:3 ethanol-dichloromethane and purged with argon for 20 min. To the resulting solution was added 40 mg of 10% palladium-on-carbon. The suspension was stirred at room temperature under an atmosphere of H2 (40 psi) for 2 h. The reaction mixture was then filtered through a Celite pad. The filtrate was concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 1 cm). Elution with 4:1 hexane-dichloromethane afforded 17 as a colorless oil: yield 546 mg (80%); silica gel TLC Rf 0.68 (1:1 hexane-dichloromethane); 1H NMR (CDCl3) δ 0.96 (t, 3 H, J = 6.7 Hz), 1.35 (s, 16H), 1.56 (s, 9H), 1.69 (m, 2H), 2.67 (t, 2H, J = 7.7 Hz), 7.02 (d, 1H, J = 8 Hz), 7.16 (t, 1H, J = 7.7 Hz), 7.28 (d, 2H, J = 8 Hz), 7.37 (d, 1H, J = 8 Hz), 7.44 (s, 1H) and 7.60 (d, 1H, J = 7.5 Hz); 13C NMR (CDCl3) δ 14.1, 22.6, 28.1, 29.2, 29.3, 29.5, 29.57, 29.6, 29.7, 31.4, 31.9, 35.5, 81.7, 125.8, 126.2, 126.9, 127.0, 127.1, 127.2, 127.3, 128.7, 132.4, 138.6, 138.8, 141.6 and 152.4; mass spectrum (APCI), m/z 454.2772 (M⁺) (C28H40NO2S requires 454.2780).
2.2.8. N-(7-(Dimethylamino)-3H-phenothiazin-3-ylidene-2-undecyl)-N-methyl methanaminium iodide (2)

A sample containing 820 mg (1.81 mmol) of 17 was dissolved in 12 mL of anhydrous dichloromethane. To the resulting solution was added dropwise 1.10 mL (14.5 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with 10 mL of saturated sodium bicarbonate solution, extracted with two 20-mL portions of dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The crude residue was utilized for the next step without further purification.

To a solution containing 1.40 g (4.00 mmol) of the crude residue in 20 mL of dichloromethane was added 3.20 g (12.9 mmol) of iodine and the reaction mixture was stirred in the dark for 15 min. To the resulting solution was added dropwise 10.1 mL (20.3 mmol) of 2 M dimethylamine in THF and the reaction mixture was stirred at room temperature for 4 h. The greenish blue mixture was purified on a silica gel column (20 × 3 cm). Elution with methanol afforded 2 as a blue-green solid: yield 102 mg (25%); silica gel TLC Rf 0.07 (methanol); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.7 Hz), 1.26 (s, 18H), 1.72 (m, 2H), 2.85 (t, 2H, J = 7.7 Hz), 3.35 (s, 4H), 3.46 (s, 5H), 3.56 (s, 4H), 7.12 (d, 1H, J = 12.5 Hz), 7.37 (s, 1H), 7.39 (s, 1H), 7.45 (s, 1H) and 7.60 (d, 1H, J = 10 Hz); 13C NMR (CDCl3) δ 14.2, 22.7, 29.4, 29.5, 29.6, 29.7, 30.2, 32.0, 34.2, 42.5, 44.8, 106.8, 111.2, 119.9, 132.0, 135.7, 137.1, 137.4, 138.7, 138.8, 154.2 and 158.2; mass spectrum (ESI), m/z 438.2948 (M+)+ (C27H40N3S requires 438.2943); ultraviolet/visible spectrum λmax 665 nm (MeOH).

2.2.9. tert-Butyl (E)-2-(Tridec-1-enyl)-10H-phenothiazine-10-carboxylate (12)

A sample containing 2.65 g (5.19 mmol) of (1-dodecyl)triphenylphosphonium bromide was dissolved in 25 mL of anhydrous tetrahydrofuran. The cooled (−78 °C) reaction mixture was treated with 5.19 mL (5.19 mmol) of sodium bis(trimethylsilyl) amide. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was cooled to −78 °C and 1.70 g (5.19 mmol) of 9, dissolved in 15 mL of anhydrous tetrahydrofuran was added. The reaction mixture was stirred at room temperature for 18 h. The product was extracted with two 30-mL portions of dichloromethane. The organic phase was washed with 20 mL of brine, dried over anhydrous Na2SO4 and concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with methanol afforded 12 as a yellow solid: yield 1.60 g (64%); silica gel TLC Rf 0.68 (1:1 hexane-dichloromethane); 1H NMR (CDCl3) δ 1.01 (t, 3H, J = 7 Hz), 1.38 (s, 18H), 1.58 (s, 9H), 2.45 (q, 2H, J = 6.8 Hz), 5.76 (m, 1H), 6.45 (t, 1H, J = 10 Hz), 7.14 (m, 2H), 7.31 (m, 3H) and 7.62 (q, 2H, J = 10.3 Hz); 13C NMR (CDCl3) δ 13.9, 22.5, 27.9, 28.4, 29.1, 29.2, 29.3, 29.5, 29.7, 31.7, 81.4, 125.7, 126.1, 126.2, 126.6, 127.0, 127.1, 127.2, 127.6, 129.7, 131.9, 133.4, 136.2, 138.3, 138.5 and 152.0; mass spectrum (APCI), m/z 482.3092 (M+)+ (C30H42NO2S requires 482.3093).

2.2.10. tert-Butyl-2-tridecyl-10H-phenothiazine-10-carboxylate (18)

A sample containing 1.60 g (3.34 mmol) of 12 was dissolved in 20 mL of 7:3 ethanol-dichloromethane and purged with argon for 20 min. To the resulting solution was added 70 mg of 10% of palladium-on-carbon. The suspension was stirred at room temperature under an atmosphere of H2 (40 psi) for 2 h. The reaction mixture was filtered through a Celite pad and the filtrate was then concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 4:1 hexane-dichloromethane afforded 18 as a colorless oil: yield 1.50 g (94%); silica gel TLC Rf 0.68 (1:1 hexane-dichloromethane); 1H NMR (CDCl3) δ 0.97 (s, 3H), 1.35 (s, 20H), 1.56 (s, 9H), 1.69 (s, 2H), 2.67 (s, 2H), 7.02 (d, 1H, J = 7 Hz), 7.17 (d, 1H, J = 6.5 Hz), 7.28 (d, 2H, J = 7.5 Hz), 7.37 (d, 1H, J = 7 Hz), 7.45 (s, 1H) and 7.60 (d, 1H, J = 7 Hz); 13C NMR (CDCl3) δ 14.4, 22.7, 28.1, 29.31, 29.39, 29.5, 29.6, 29.7, 31.5, 31.9, 35.6, 81.7, 125.9, 126.2, 127.0, 127.1, 127.2, 127.3, 127.4, 128.8, 132.4, 138.6, 138.8, 141.6 and 152.4; mass spectrum (APCI), m/z 482.3092 (M+)+ (C30H42NO2S requires 482.3093).

2.2.11. N-(7-(Dimethylamino)-3H-phenothiazin-3-ylidene-2-tridecyl)-N-methylmethanaminium iodide (3)

A sample containing 1.50 g (3.11 mmol) of 18 was dissolved in 20 mL of anhydrous dichloromethane. To the resulting solution was added dropwise 1.90 mL (24.9 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 18 h. The reaction was then quenched with 20 mL of saturated sodium bicarbonate solution, extracted with two 30-mL portions of
dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The crude residue was utilized for the next step without further purification.

To a solution containing 1.40 g (3.67 mmol) of the crude residue in 20 mL of dichloromethane was added 3.00 g (11.7 mmol) of iodine and the reaction mixture was stirred in the dark for 15 min. To the resulting solution was added dropwise 9.20 mL (18.3 mmol) of 2 M dimethylamine in THF and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was treated dropwise with 5.19 mL (5.19 mmol) of sodium bis(trimethylsilyl) amide. The reaction mixture was dissolved in 25 mL of anhydrous tetrahydrofuran. The cooled (2 °C) reaction mixture was stirred at room temperature for 4 h. The greenish blue mixture was filtered through a Celite pad. The resulting solution was added dropwise 9.20 mL (18.3 mmol) of 2 M dimethylamine in THF and the reaction mixture was stirred in the dark for 15 min. To the resulting solution was added 5.95 g (23.4 mmol) of iodine and the reaction mixture was stirred in the dark for 15 min. The crude residue was utilized for the next step without further purification.

A sample containing 2.80 g (5.19 mmol) of (1-tetradecyl)triphenylphosphonium bromide was dissolved in 20 mL of anhydrous tetrahydrofuran. The cooled (−78 °C) reaction mixture was treated with 20 mL of saturated sodium bicarbonate solution, extracted with two 30-mL portions of dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 4:1 hexane-dichloromethane afforded 13 as a yellow solid: yield 2.0 g (76%); silica gel TLC Rf 0.03 (methanol); 1H NMR (CDCl3) δ 0.98 (t, 3H, J = 6 Hz), 1.21 (s, 20H), 1.66 (s, 2H), 2.63 (s, 4H), 2.82 (s, 2H), 3.35 (s, 4H), 3.49 (s, 4H), 7.37 (s, 2H), 7.80 (s, 1H), 7.90 (d, 1H, J = 1.5 Hz) and 8.63 (s, 1H); 13C NMR (CDCl3) δ 14.0, 22.6, 28.1, 28.6, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 31.9, 81.7, 125.9, 126.3, 126.4, 126.8, 126.9, 127.2, 127.3, 127.8, 129.9, 132.1, 133.6, 136.5, 138.4, 138.7 and 152.3; mass spectrum (APCI), m/z 508.3258 (M+); (C32H48NO2S requires 508.3249); ultraviolet/visible spectrum λmax 663 nm (MeOH).

A sample containing 1.89 g (3.71 mmol) of N-((7-(Dimethylamino)-2-pentadecyl-3H-phenothiazin-3-ylidene)-N-methylmethanaminium iodide (4)

A sample containing 1.89 g (3.71 mmol) of 19 was dissolved in 25 mL of anhydrous dichloromethane. To the resulting solution was added dropwise 2.30 mL (24.7 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 18 h. The reaction was then quenched with 20 mL of saturated bicarbonate solution, extracted with two 30-mL portions of dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The crude residue was utilized for the next step without further purification.

To a solution containing 3.00 g (7.33 mmol) of the crude residue in 25 mL of dichloromethane was added 5.95 g (23.4 mmol) of iodine and the reaction mixture was stirred in the dark for 15 min. To the...
resulting solution was added dropwise 18.3 mL (36.6 mmol) of 2 M dimethylamine in THF and the reaction mixture was stirred at room temperature for 4 h. The greenish blue mixture was purified on a silica gel column (20 × 3 cm). Elution with at 1:1 ethyl acetate-methanol afforded 4 as a blue solid: yield 0.39 g (15%); silica gel TLC Rf 0.06 (methanol); 1H NMR (CDCl3) δ 0.87 (t, 3H, J = 5 Hz), 1.25 (s, 24H), 1.71 (m, 2H), 2.79 (s, 4H), 2.84 (t, 2H, J = 7.5 Hz), 3.34 (s, 4H), 3.48 (s, 4H), 7.27 (s, 1H), 7.38 (s, 1H), 7.41 (s, 1H), 7.88 (s, 1H) and 7.99 (d, 1 H, J = 10 Hz); 13C NMR (CDCl3) δ 14.2, 22.7, 29.4, 29.5, 29.6, 29.7, 30.2, 32.0, 34.2, 35.1, 42.6, 44.7, 106.9, 111.3, 119.8, 132.0, 135.8, 137.1, 137.3, 137.4, 138.5, 138.7 and 152.3; mass spectrum (APCI), m/z 494.3576 (M+ (C31H48N3S requires 494.3563)); ultraviolet/visible spectrum λmax 664 nm (CH2Cl2), λmax 665 nm (MeOH).

2.2.15. tert-Butyl (E)-2-(Hexadec-1-enyl)-10H-phenothiazine-10-carboxylate (14)
A sample containing 1.82 g (3.30 mmol) of (1-pentadecyl)triphenylphosphonium bromide was dissolved in 20 mL of anhydrous tetrahydrofuran. The cooled (−78 °C) reaction mixture was treated dropwise with 3.30 mL (3.30 mmol) of sodium bis(trimethylsilyl) amide. The reaction mixture was stirred at 0 °C for 3 h. The mixture was cooled to −78 °C and 1.08 g (3.30 mmol) of 9, dissolved in 15 mL of anhydrous tetrahydrofuran was added. The reaction mixture was stirred at 0°C for 18 h. The product was extracted with two 30-mL portions of dichloromethane. The organic phase was washed with 20 mL of brine, dried over anhydrous Na2SO4 and concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 3 cm). Elution with 5:1 hexane-dichloromethane afforded 14 as a colorless solid: yield 1.27 g (74%); silica gel TLC Rf 0.68 (1:1 hexane-dichloromethane); 1H NMR (CDCl3) δ 0.97 (t, 3H, J = 5 Hz), 1.35 (s, 22H), 1.53 (m, 11H), 2.41 (q, 2H, J = 8.3 Hz), 5.74 (m, 1H), 6.42 (t, 1H, J = 10 Hz), 7.15 (m, 2H), 7.30 (m, 2H), 7.38 (d, 1H, J = 5 Hz), 7.53 (s, 1H) and 7.60 (d, 1H, J = 10 Hz); 13C NMR (CDCl3) δ 14.1, 22.7, 28.1, 28.6, 29.3, 29.4, 29.5, 29.6, 29.71, 29.74, 29.9, 31.9, 318.1, 125.9, 126.4, 126.5, 126.8, 127.2, 127.3, 127.4, 127.7, 129.9, 132.1, 133.7, 136.5, 138.5, 138.7 and 152.3; mass spectrum (APCI), m/z 522.3400 (M+H)+ (C33H48NO2S requires 522.3406).

2.2.16. tert-Butyl-2-hexadecyl-10H-phenothiazine-10-carboxylate (20)
A sample containing 1.27 g (3.30 mmol) of (1-pentadecyl)triphenylphosphonium bromide was dissolved in 20 mL of anhydrous dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The residue was utilized for the next step without further purification.

2.2.17. N-(7-(Dimethylamino)-2-hexadecyl-3H-phenothiazin-3-ylidene)-N-methylmethanaminium Iodide (5)
A sample containing 1.23 g (2.35 mmol) of 20 was dissolved in 20 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature for 18 h. The reaction was then quenched with 20 mL of saturated sodium bicarbonate solution, extracted with two 30-mL portions of dichloromethane, dried over anhydrous Na2SO4 and then concentrated under diminished pressure. The crude residue was utilized for the next step without further purification.

To a solution containing 1.15 g (2.71 mmol) of the crude residue in 20 mL of dichloromethane was dissolved 1.55 g of iodine and the reaction mixture was stirred in the dark for 15 min. To the resulting solution was added dropwise 6.70 mL (13.5 mmol) of 2 M dimethylamine in THF and the reaction mixture was stirred at room temperature for 4 h. The greenish blue mixture was purified on a silica gel column (20 × 3 cm). Elution with 1:1 ethyl acetate-methanol afforded 5 as a blue solid:
yield 0.36 g (24%); silica gel TLC R<sub>f</sub> 0.06 (methanol); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.84 (t, J = 7.5 Hz), 1.22 (s, 26H), 1.67 (s, 2H), 2.67 (s, 4H), 2.83 (s, 2H), 3.36 (s, 4H), 3.52 (d, J = 4H), δ 7.37 (s, 2H), 7.83 (s, 1H), 7.93 (s, 1H) and 8.58 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.1, 22.7, 28.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.5, 31.9, 34.1, 35.0, 42.6, 44.6, 107.0, 111.2, 119.9, 131.8, 135.5, 136.9, 137.4, 138.6, 138.9, 154.1 and 158.1; mass spectrum (ESI), m/z 508.3734 (M<sup>+</sup>); <sup>1</sup>C<sub>32</sub>H<sub>50</sub>N<sub>3</sub>S requires 508.3720); ultraviolet/visible spectrum λ<sub>max</sub> 664 nm (MeOH).

2.2.18. tert-Butyl (E)-2-(Heptadec-1-enyl)-10H-phenothiazine-10-carboxylate (15)

A sample containing 174 mg (0.30 mmol) of (1-hexadecyl)triphenylphosphonium bromide was dissolved in 10 mL of anhydrous tetrahydrofuran. The cooled (−78 °C) reaction mixture was treated dropwise with 0.30 mL (0.30 mmol) of sodium bis(trimethylsilyl) amide. The reaction mixture was stirred at 0 °C for 3 h. The mixture was cooled to −78 °C and 100 mg (0.30 mmol) of 9, dissolved in 7 mL of anhydrous tetrahydrofuran was added. The reaction mixture was stirred at 0 °C for 18 h. The product was extracted with two 10-mL portions of dichloromethane. The organic phase was washed with 10 mL of brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 1 cm). Elution with 4:1 hexane-dichloromethane afforded 15 as a yellow solid: yield 117 mg (72%); silica gel TLC R<sub>f</sub> 0.27 (9:1 hexane-dichloromethane); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.93 (t, J = 6.6 Hz), 1.31 (s, 26H), 1.52 (s, 9H), 2.38 (q, J = 6.9 Hz), 5.70 (m, 1H), 6.40 (d, J = 11.6 Hz), 7.11 (m, 2H), 7.26 (m, 2H), 7.33 (d, J = 7.6 Hz), 7.50 (s, 1H) and 7.56 (d, J = 8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.2, 22.8, 28.2, 28.7, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 29.8, 30.0, 32.0, 82.0, 126.1, 126.5, 126.6, 127.0, 127.3, 127.5, 127.6, 127.8, 130.0, 132.3, 133.9, 136.7, 138.6, 138.8 and 152.5; mass spectrum (APCI), m/z 536.3556 (M<sup>+</sup>); (C<sub>32</sub>H<sub>50</sub>N<sub>3</sub>S requires 536.3562).

2.2.19. tert-Butyl-2-heptadecyl-10H-phenothiazine-10-carboxylate (21)

A sample containing 134 mg (0.25 mmol) of 15 was dissolved in 10 mL of 7:3 ethanol-dichloromethane and purged with argon for 20 min. To the resulting solution was added 5 mg of 10% of palladium-on-carbon. The suspension was stirred at room temperature under an atmosphere of H<sub>2</sub> (40 psi) for 2 h. The reaction mixture was filtered through a Celite pad. The filtrate was then concentrated under diminished pressure. The residue was purified on a silica gel column (20 × 1 cm). Elution with 4:1 hexane-dichloromethane afforded 21 as a colorless oil: yield 134 mg (93%); silica gel TLC R<sub>f</sub> 0.06 (methanol); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.88 (t, J = 6.8 Hz), 1.26 (s, 28H), 1.49 (s, 9H), 1.60 (m, 2H), 2.59 (t, J = 7.6 Hz), 6.96 (dd, 1H, J = 1.6 Hz), 7.12 (t, 1H, J = 7.4 Hz), 7.25 (m, 2H), 7.32 (dd, 1H, J = 1.2 Hz), 7.35 (d, 1H, J = 1.2Hz) and 7.52 (d, 1H, J = 8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.1, 22.7, 28.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 31.5, 31.9, 35.6, 81.7, 125.8, 126.2, 126.3, 127.0, 127.1, 127.19, 127.3, 128.8, 132.4, 138.6, 138.8, 141.6 and 152.4; mass spectrum (APCI), m/z 538.3732 (M<sup>+</sup>); (C<sub>34</sub>H<sub>52</sub>N<sub>3</sub>S requires 538.3719).

2.2.20. N-(7-(Dimethylamino)-2-heptadecyl-3H-phenothiazin-3-ylidene)-N-methylmethanaminium iodide (6)

To a solution of 0.23 g (0.43 mmol) of 21 in 8 mL of anhydrous CH<sub>2</sub>Cl<sub>2</sub> was added dropwise 0.26 mL (3.44 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere and quenched with 50 mL of saturated NaHCO<sub>3</sub> solution. The aqueous layer was extracted with three 30-mL portions of CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layer was dried over anhydrous MgSO<sub>4</sub> and then concentrated under diminished pressure. The crude residue was utilized in the next step without further purification.

To a solution of 45.0 mg of the crude residue in 5 mL of CH<sub>2</sub>Cl<sub>2</sub> was added 81.0 mg (0.32 mmol) of iodine followed by 0.25 mL (0.50 mmol) of 2 M dimethylamine in THF. The reaction mixture was stirred at room temperature under an argon atmosphere for 12 h. The greenish blue mixture was purified on a silica gel column (10 × 2 cm). Elution with ethyl acetate afforded 6 as a green solid: yield 16 mg (25%); silica gel TLC R<sub>f</sub> 0.40 (ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.86 (t, 3H, J = 6.6 Hz), 1.15–1.37 (m, 28H), 1.69–1.72 (m, 2H), 2.81–2.85 (m, 2H), 3.33 (s, 6H), 3.46 (s, 6H), 7.36–7.38 (m, 1H), 7.41 (s, 1H), 7.76 (m, 1H), 7.89 (s, 1H) and 7.99 (d, 1H, J = 7.6 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.2, 22.7, 29.4, 29.6, 29.70, 29.74, 29.78, 29.79, 30.2, 32.0, 34.1, 34.8, 42.6, 44.7, 107.4, 111.5, 119.9, 132.1, 135.92, 135.94,
137.1, 137.4, 138.5, 138.9, 154.1 and 158.1; mass spectrum (APCI), m/z 522.3882 (M⁺) (C33H52N3S requires m/z 522.3882); ultraviolet/visible spectrum λ_max 670 nm (CH₂Cl₂), λ_max 665 nm (MeOH).

2.2.21. 4-(2-Heptadecyl-7-morpholino-3H-phenothiazin-3-ylidene)morpholin-4-ium Iodide (7)

To a solution of 0.23 g (0.43 mmol) of 21 in 8 mL of anhydrous CH₂Cl₂ was added dropwise 0.26 mL (3.44 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere and then neutralized with 50 mL of saturated NaHCO₃ solution. The aqueous layer was extracted with three 30-mL portions of CH₂Cl₂. The combined organic layer was dried over anhydrous MgSO₄ and concentrated under diminished pressure. The crude product was utilized in the next step without further purification.

To a solution of 82.0 mg of the crude product in 8 mL of CH₂Cl₂ was added 144 mg (0.57 mmol) of iodine followed by 61.0 μL (0.72 mmol) of morpholine. The reaction mixture was stirred at room temperature under an argon atmosphere for 3 h. The greenish blue mixture was purified on a silica gel column (20 × 1 cm). Elution with at 1:1 ethyl acetate-methanol afforded 7 as a dark green solid: yield 38 mg (28%); silica gel TLC Rf 0.13 (1:1 ethyl acetate-methanol); 1H NMR (CDCl₃) δ 0.88 (t, 3H, J = 6.8 Hz), 1.24–1.40 (m, 30H), 1.76–1.80 (m, 7H), 2.76 (t, 2H, J = 7.8 Hz), 3.43–3.45 (m, 3H), 3.92–3.98 (m, 6H), 7.56 (s, 1H), 7.61–7.64 (m, 1H), 7.69 (d, 1H, J = 2.0 Hz), 8.06 (s, 1H) and 8.13 (d, 1H, J = 9.6 Hz); 13C NMR (CDCl₃) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.6, 29.7, 30.1, 30.9, 31.9, 32.1, 48.9, 52.3, 66.6, 107.7, 113.7, 121.2, 130.9, 137.6, 137.9, 139.7, 139.8, 140.1, 153.9 and 157.9; mass spectrum (ESI), m/z 606.4113 (M⁺) (C37H56N3O2S requires m/z 606.4093); ultraviolet/visible spectrum λ_max 670 nm (CH₂Cl₂), λ_max 665 nm (MeOH).

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