

Electronic supplementary information

Synthesis and DNA-binding properties of water-soluble
cationic pyropheophorbides derived from chlorophyll
a/b

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Experimental

Chemicals and instruments

Pivaloyl chloride, 4-dimethylaminopyridine and iodomethane were supplied by Tokyo Kasei Kogyo Co., Ltd. Eschenmoser's salt (*N,N*-dimethylmethyleammonium iodide), 2-dimethylaminoethanol, triethylamine, and TE saturated phenol were obtained from Aldrich. *N,N*-Diisopropylethylamine and silica gel 60 were supplied by Kanto Chemical Co., Ltd. Chloroform-*d* and DMSO-*d*₆ were purchased from Cambridge Isotope Laboratories, Inc. Ethidium bromide and alumina (basic, Brockmann Grade II–III) were purchased from Merck Ltd. (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) was supplied by Kokusan Chemical Co, Ltd. ODS (Develosil LOP ODS) was supplied by Nomura Chemical Co. Ltd. Calf thymus DNA (CT-DNA) was purchased from Sigma. Dithiothreitol (DTT) was supplied by Nacalai Tesque, Inc. Agarose, tris(hydroxymethyl)aminomethane, orange G and sodium dodecyl sulfate (SDS) were obtained from Wako Pure Chemical Industries, Ltd. 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) and wheat germ topoisomerase I were supplied by Dojindo Laboratories and Promega, respectively. Plasmid DNA (pBR322 DNA in Form I) was supplied by Nippon Gene Co. Ltd. Pyropheophorbide *a*, pyropheophorbide *b* and their methylesters were synthesized by the modified method reported previously.¹ Buffer solutions were prepared using ultrapure water treated by an ultrapure Millipore distillation apparatus (Milli-Q Labo, Nippon Millipore Ltd.). Other reagents and chemicals were used as received without further purification and all solvents were of analytical reagent grade unless specified. The ¹H NMR spectra were measured at 300 MHz with a JNM-LA300 spectrometer. The chemical shifts were expressed in ppm relative to tetramethylsilane and the coupling constants (*J* values) were given in Hz. The FAB mass spectra were recorded on a JEOL GCmate mass spectrometer. Elemental analysis was performed at the Central Laboratory of the Faculty of Science and Technology, Keio University.

Pyropheophorbide *a* 2-dimethylaminoethyl ester (8)

A solution of **7** (53.4 mg, 9.66×10^{-2} mmol) in anhydrous tetrahydrofuran (5.5 mL) and anhydrous pyridine (225 μ L) was stirred under a nitrogen atmosphere. To the solution was added fresh pivaloyl chloride (100 μ L, 0.81 mmol) and the solution was stirred for 7 min at room temperature. Then 2-dimethylaminoethanol (4.5 mL) was added to the dark-brown solution at room temperature, and the reaction mixture was stirred for 15 h at 45 °C. To the reaction mixture was added a small amount of water and it was evaporated to dryness. The residue was chromatographed on basic alumina (Merck: Brockmann Grade II–III) using dichloromethane/methanol (100/2, v/v) as eluent. The main band was collected and evaporated to dryness. The crystalline powder obtained was dissolved in a small amount of acetone and precipitated by pouring distilled water into the acetone solution. The precipitate collected by centrifugation was dried over diphosphorus pentoxide in a vacuum desiccator to give **8** (59.5 mg, 9.8×10^{-2} mmol, 91.8%) as dark-violet crystals (Found: C, 73.55; H, 7.73; N 10.88. Calc. for $C_{37}H_{43}N_5O_3$: C, 73.36; H, 7.15; N, 11.56); δ_H (300 MHz; $CDCl_3$; Me_4Si) 9.35 (1 H, s, β -H), 9.25 (1 H, s, α -H), 8.52 (1 H, s, δ -H), 7.93 (1 H, dd, J 1.4 and 11, 2a-H), 6.23 (1 H, dd, J 1.2 and 17, 2b-H), 6.12 (1 H, dd, J 1.5 and 10, 2b'-H), 5.09 and 5.25 (2 H, ABq, J_{AB} 20, 10-H and 10-H'), 4.46 (1 H, m, 8-H), 4.29 (1 H, m, 7-H), 4.07 (2 H, m, 7e- CH_2), 3.57 (2 H, m, 4a- CH_2), 3.60 (3 H, s, 5-Me), 3.38 (3 H, s, 1-Me), 3.14 (3 H, s, 3-Me), 2.38 (2 H, t, J 5.6, 7d- CH_2), 2.70–2.55 (2 H, m, 7b- CH_2), 2.28 (2 H, m, 7a- CH_2), 2.15 (6 H, s, $2 \times 7e-CH_2NMe$), 1.80 (3 H, d, J 7.3, 8-Me), 1.63 (3 H, t, J 7.6, 4b-Me), 0.32 (1 H, s, NH), -1.81 (1 H, s, NH); m/z (FAB, 3-nitrobenzyl alcohol) 606 ($M^+ + H$).

Pyropheophorbide *a* 2-trimethylammonioethyl ester iodide (**1**)

Iodomethane (1.5 mL) was added to **8** (9.7 mg, 1.6×10^{-2} mmol) in anhydrous acetone (0.5 mL) and the reaction mixture was stirred for 6 h under a nitrogen atmosphere. The precipitate obtained was collected by filtration, washed with a small amount of anhydrous acetone and dried over diphosphorus pentoxide under vacuum to give **1** (9.7 mg, 1.25×10^{-2} mmol, 78%) as dark-violet crystals (Found: C, 59.15; H, 6.25; N 8.75. Calc. for $C_{38}H_{46}IN_8O_6 \cdot 1.5H_2O$: C, 58.91; H, 6.38; N, 9.04); δ_H (300MHz; DMSO- d_6 ;

Me₄Si) 9.75 (1 H, s, β-H), 9.72 (1 H, s, α-H), 9.48 (1 H, s, δ-H), 8.25 (1 H, dd, *J* 1.4 and 11, 2a-H), 6.41 (1 H, d, *J* 18, 2b-H), 6.23 (1 H, d, *J* 12, 2b'-H), 5.16 and 5.24 (2 H, ABq, *J*_{AB} 20, 10-H and 10'-H), 4.58 (1 H, m, 8-H), 4.38 (1 H, m, 7-H), 4.38 (2 H, m, 7e-CH₂), 3.71 (2 H, m, 4a-CH₂), 3.63 (3 H, s, 5-Me), 3.50 (2 H, m, 7d-CH₂), 3.45 (3 H, s, 1-Me), 3.23 (3 H, s, 3-Me), 2.97 (9 H, s, 3 × 7e-CH₂NMe), 2.50–2.75 (2 H, m, 7b-CH₂), 2.20–2.50 (2 H, m, 7a-CH₂), 1.90 (3 H, d, *J* 7.1, 8-Me), 1.63 (3 H, t, *J* 7.8, 4b-Me), 0.30 (1 H, s, NH), –1.94 (1 H, s, NH).

2a-Devinyl-2-(3-dimethylaminoprop-1-enyl) pyropheophorbide *a* 2-dimethylaminoethyl ester (9)

By the previous method², pyropheophorbide **7** was treated with Eschenmoser's salt in dichloromethane to give **9** (Found: C, 72.04; H, 7.66; N, 12.18. Calc. for C₄₀H₅₀N₆O₃·0.5H₂O: C, 71.51; H, 7.65; N, 12.51.); δ_H(300MHz, CDCl₃; Me₄Si) 9.47 (1 H, s, β-H), 9.32 (1 H, s, α-H), 8.53 (1 H, s, δ-H), 7.78 (1 H, d, *J* 16, 2a-H), 6.78 (1 H, m, 2b-H), 5.10 and 5.26 (2 H, ABq, *J*_{AB} 20, 10-H and 10-H'), 4.47 (1 H, m, *J* 5.6, 8-H), 4.30 (1 H, m, 7-H), 4.07 (2 H, m, *J* 2.9 and 5.6, 7e-CH₂), 3.67 (2 H, q, *J* 7.8, 4a-CH₂), 3.65 (3 H, s, 5-Me), 3.56 (2 H, d, *J* 6.6, 2c-CH₂), 3.39 (3 H, s, 1-Me), 3.23 (3 H, s, 3-Me), 2.55 (6 H, s, 2 × 2c-CH₂NMe), 2.79–2.52 (2 H, m, 7b-CH₂), 2.38 (2 H, t, *J* 5.6, 7d-CH₂), 2.33–2.19 (2 H, m, 7a-CH₂), 2.15 (6 H, s, 2 × 7e-CH₂NMe), 1.80 (3 H, d, *J* 7.3, 8-Me), 1.68 (3 H, t, *J* 7.5, 4b-Me), 0.45 (1 H, s, NH), –1.70 (1 H, s, NH); *m/z* (FAB, triethanolamine) 663 (M⁺ + H).

2a-Devinyl-2-(3-trimethylammonioprop-1-enyl) pyropheophorbide *a* 2-trimethylammonioethyl ester diiodide (2)

By the similar method to that for **1**, pyropheophorbide **9** was converted to **2** (Found: C, 51.61; H, 6.04; N, 8.41. Calc. for C₄₂H₅₆I₂N₆O₃·2H₂O: C, 51.33; H, 6.15; N, 8.55.); δ_H(300MHz, DMSO-d₆; Me₄Si) 9.86 (1 H, s, β-H), 9.53 (1 H, s, α-H), 9.01 (1 H, s, δ-H), 8.48 (1 H, d, *J* 16, 2a-H), 7.03 (1 H, m, 2b-H), 5.19 and 5.28 (2 H, ABq, *J*_{AB} 20, 10-H and 10-H'), 4.62 (2 H, d, *J* 7.1, 2c-CH₂), 4.62 (1 H, m, 8-H), 4.42 (2 H, m, 7e-CH₂), 4.39 (1 H, m, 7-H), 3.77 (2 H, q, *J* 7.6, 4a-CH₂), 3.67 (3 H, s, 5-Me), 3.58 (2 H, m, 7d-CH₂), 3.54 (3 H, s, 1-Me), 3.36 (9 H, s, 3 × 2c-CH₂NMe), 3.26 (3 H, s, 3-Me), 3.03 (9 H, s, 3 ×

7e-CH₂NMe), 2.70 (2 H, m, 7b-CH₂), 2.53–2.13 (2 H, m, 7a-CH₂), 1.81 (3 H, d, *J* 7.3, 8-Me), 1.68 (3 H, t, *J* 7.8, 4b-Me), 0.21 (1H, s, NH), –1.98 (1 H, s, NH); *m/z* (FAB, triethanolamine) 693 (M⁺ + H), 819 (M⁺ + I).

Pyropheophorbide *a* 1,1-bis(dimethylaminomethyl)-2-ethyl ester (**10**)

A solution of **7** (160 mg, 0.290 mmol) in anhydrous tetrahydrofuran (8 mL) and anhydrous pyridine (150 μL) was stirred under a nitrogen atmosphere. To the solution was added fresh pivaloyl chloride (100 μL) and it was stirred for 10 min at room temperature. Then a solution mixture containing anhydrous tetrahydrofuran (1.5 mL), triethylamine (0.67 mL), 1,1-bis(dimethylaminomethyl)-2-ethanol³ (0.4 mL, 2.5 mmol) and 4-dimethylaminopyridine (110 mg) was added to the dark-brown solution, and the reaction mixture was stirred for 2 days at room temperature. To the reaction mixture was added a small amount of water and it was evaporated to dryness. The residue was chromatographed on silica gel deactivated with water (silica gel/water = 100/6, w/w) using dichloromethane/methanol (100/9, v/v) as eluent. The top and main band adsorbed on silica gel was eluted with dichloromethane/methanol (100/9, v/v) containing 3% triethylamine, and evaporated to dryness. The desired compound was dissolved in a small amount of benzene and freeze-dried to give **10** (177 mg, 0.257 mmol, 88.6%) as powder (Found: C, 71.89; H, 7.62; N, 11.70. Calc. for C₄₁H₅₂N₆O₃·2/3H₂O: C, 71.48; H, 7.95; N, 12.20); δ_H(300MHz, CDCl₃; Me₄Si) 9.51 (1 H, s, β-H), 9.36 (1 H s, α-H), 8.57 (1 H, s, δ-H), 7.97 (1 H, dd, *J* 6.4 and 11, 2a-H), 6.28 (1 H, dd, *J* 1.4 and 16, 2b-H), 6.17 (1 H, dd, *J* 1.2 and 10, 2b'-H), 5.07 and 5.21 (2 H, ABq, *J*_{AB} 20, 10-H and 10-H'), 4.45 (1 H, m, 8-H), 4.34 (1 H, m, 7-H), 4.03–3.82 (2 H, m, 7d-CH₂), 3.66 (2 H, q, *J* 7.5, 4a-CH₂), 3.62 (3 H, s, 5-Me), 3.41 (3 H, s, 1-Me), 3.22 (3 H, s, 3-Me), 1.95–2.70 (9 H, m, 7b-CH₂, 7e-H, 7f-CH₂, 7f'-CH₂ and 7a-CH₂), 2.34 (6 H, s, 2 × 7f-CH₂NMe), 2.29 (6 H, s, 2 × 7f'-CH₂NMe) 1.81 (3 H, d, *J* 7.3, 8-Me), 1.68 (3 H, t, *J* 7.6, 4b-Me), 0.4 (1H, s, NH), –1.73 (1H, s, NH); *m/z* (FAB, 3-nitrobenzyl alcohol) 678 (M⁺ + H).

Pyropheophorbide *a* 1,1-bis(trimethylammoniomethyl)-2-ethyl ester diiodide (**3**)

By the similar method to that for **1**, pyropheophorbide **10** was converted to **3** (Found: C, 49.96; H, 6.09; N, 7.83. Calc. for $C_{43}H_{58}I_2N_6O_3 \cdot 4H_2O$: C, 50.01; H, 6.44; N, 8.13); δ_H (300MHz, DMSO- d_6 ; Me₄Si) 9.79 (1 H, s, β -H), 9.50 (1 H, s, α -H), 8.91 (1 H, s, δ -H), 8.26 (1 H, dd, J 6.0 and 12, 2a-H), 6.42 (1 H, dd, J 1.5 and 18, 2b-H), 6.24 (1 H, dd, J 1.2 and 10, 2b'-H), 5.21 (2 H, s, 10-H₂), 4.59 (1 H, m, 8-H), 4.41 (1 H, m, 7-H), 4.12 (2 H, m, 7d-CH₂), 3.74 (2 H, q, J 7.8, 4a-CH₂), 3.64 (3 H, s, 5-Me), 3.46 (3 H, s, 1-Me), 3.25 (3 H, s, 3-Me), 3.09 (9 H, s, 3 \times 7f-CH₂NMe), 3.06 (9 H, s, 3 \times 7f'-CH₂NMe), 2.95–3.50 (7 H, m, 7b-CH₂, 7e-H, 7f-CH₂ and 7f'-CH₂), 2.55–2.65 (2 H, m, 7a-CH₂), 1.81 (3 H, d, J 7.3, 8-Me), 1.64 (3 H, t, J 7.8, 4b-Me), 0.35 (1 H, s, NH), -1.90 (1 H, s, NH); m/z (FAB, thioglycerol) 833 ($M^+ + I$).

2a-Devinyl-2a-carboxypyropheophorbide a 2a,7c-bis[1,1-bis(dimethylaminomethyl)-2-ethyl] ester (14)

Pyropheophorbide **12** was synthesized from **11** by the method reported previously.⁴ Pyropheophorbide **12** was dissolved in THF/water containing sulfuric acid and converted to **13** quantitatively. A solution of **13** (10.1 mg) in anhydrous tetrahydrofuran (4 mL) and anhydrous pyridine (76 μ L) was stirred under a nitrogen atmosphere. To the solution was added fresh pivaloyl chloride (34 μ L) and it was stirred for 10 min at room temperature. Then a solution mixture containing anhydrous tetrahydrofuran (1.0 mL), triethylamine (0.2 mL), 1,1-bis(dimethylaminomethyl)-2-ethanol³ (0.12 mL, 0.75 mmol) and 4-dimethylaminopyridine (16.7 mg) was added to the dark-brown solution, and the solution was stirred overnight at 45 °C. The reaction was stopped by the addition of water, and then the desired product was extracted from the water layer by dichloromethane. The dichloromethane layer was washed twice with distilled water and evaporated to dryness. The residue was chromatographed on silica gel deactivated with water (silica gel/water = 100/6, w/w) using dichloromethane/methanol (100/4, v/v) as eluent. The top and main band adsorbed on silica gel was eluted with dichloromethane/methanol (100:4) containing 2% triethylamine, and evaporated to dryness to give **14** (8.2 mg); δ_H (300MHz, CDCl₃; Me₄Si) 10.46 (1 H, s, β -H), 9.61 (1 H, s, α -H), 8.82 (1 H, s, δ -H), 5.18 and 5.33 (2 H, ABq, J_{AB} 20, 10-H and 10-H'), 4.89 (2 H, d, J 4.1, 2b-CH₂), 4.57 (1 H, m, 8-H), 4.39 (1 H, m, 7-H), 4.11 (2 H, m, 7d-CH₂), 3.74 (3 H, S6

s, 5-Me), 3.73 (2 H, m, 4a-CH₂), 3.72 (3 H, s, 1-Me), 3.33 (3 H, s, 3-Me), 2.35 (12 H, s, 2 × 2d-CH₂NMe and 2 × 2d'-CH₂NMe), 2.11 (12 H, s, 2 × 7f-CH₂NMe and 2 × 7f'-CH₂NMe), 1.80–2.80 (14 H, m, 2d-CH₂, 2d'-CH₂, 2c-H, 7b-CH₂, 7f-CH₂, 7f'-CH₂, 7e-H and 7a-CH₂), 1.72 (3 H, m, 8-Me), 1.69 (3 H, t, *J* 7.8, 4b-Me), 0.19 (1 H, s, NH), -2.05 (1 H, s, NH); *m/z* (FAB, triethanolamine) 838 (M⁺ + H).

2a-Devinyl-2a-carboxypyropheophorbide a 2a,7c-bis[1,1-bis(trimethylammoniomethyl)-2-ethyl] ester tetraiodide (4)

By the similar method to that for **1**, pyropheophorbide **14** was converted to **4** (Found: C, 42.43; H, 6.13; N, 7.34. Calc. for C₅₂H₈₀I₄N₈O₅·4H₂O: C, 42.29; H, 6.01; N, 7.58.); δ_H (300MHz, DMSO-d₆; Me₄Si) 10.43 (1 H, s, β-H), 9.96 (1 H, s, α-H), 9.25 (1 H, s, δ-H), 5.31 (2 H, m, 10-H₂), 4.91 (2 H, m, 2b-CH₂), 4.70 (1 H, m, 8-H), 4.45 (1 H, m, 7-H), 4.16 (2 H, m, 7d-CH₂), 3.81 (2 H, m, 4a-CH₂), 3.74 (3 H, s, 5-Me), 3.70 (3 H, s, 1-Me), 3.51 (3 H, s, 3-Me), 3.30 (18 H, s, 3 × 2d-CH₂NMe and 3 × 2d'-CH₂NMe), 3.16 (9 H, s, 3 × 7f-CH₂NMe), 3.14 (9 H, s, 3 × 7f'-CH₂NMe), 2.95–3.60 (10 H, m, 2d-CH₂, 2d'-CH₂, 2c-H, 7f-CH₂, 7f'-CH₂ and 7e-H), 2.55–2.85 (2 H, m, 7b-CH₂), 2.05–3.35 (2 H, m, 7a-CH₂), 1.81 (3 H, m, 8-Me), 1.67 (3 H, t, *J* 7.6, 4b-Me), -0.20 (1 H, m, NH), -2.24 (1 H, s, NH).

3a-Deformyl-3a-carboxypyropheophorbide b 3a,7c-bis[1,1-bis(dimethylaminomethyl)-2-ethyl] ester (18)

Pyropheophorbide **16**⁴ was dissolved in THF/water containing sulfuric acid and converted to **17** quantitatively. The esterification was performed according to the literature.⁵ A solution of **17** (41 mg) in anhydrous dichloromethane (3.5 mL), *N,N*-diisopropylethylamine (0.52 mL) and 1,1-bis(dimethylaminomethyl)-2-ethanol³ (0.78 mL) was stirred under a nitrogen atmosphere at -20°C (carbon tetrachloride/dry ice). To the solution was added BOP (121 mg) in anhydrous dichloromethane (1.5 mL) and the reaction mixture was stirred overnight with gradual warming to room temperature. The reaction was stopped by the addition of water, and the desired product was extracted from the water layer by dichloromethane. The dichloromethane layer was washed twice with water saturated with

potassium hydrogen phthalate, with water saturated with sodium hydrogen carbonate and twice with distilled water, and evaporated to dryness. The residue was chromatographed on ODS using acetone/water (100/4, v/v) containing 2% (v/v) triethylamine as eluent and at a flow rate of 8 mL/min. The main band was collected and evaporated to dryness to give **18** (6.2 mg); δ_{H} (300MHz, CDCl_3 ; Me_4Si) 10.54 (1 H, s, β -H), 9.66 (1 H, s, α -H), 8.54 (1 H, s, δ -H), 8.05 (1 H, dd, J 6.3 and 12, 2a-H), 6.36 (1 H, dd, J 1.4 and 16, 2b-H), 6.20 (1 H, dd, J 1.5 and 10, 2b'-H), 5.09 and 5.25 (2 H, ABq, J_{AB} 20, 10-H and 10-H'), 4.80 (2 H, d, J 4.4, 3b- CH_2), 4.50 (1 H, m, 8-H), 4.30 (1 H, m, 7-H), 4.10 (2 H, m, 7d- CH_2), 4.10 (2 H, m, 4a- CH_2), 3.67 (3 H, s, 5-Me), 3.40 (3 H, s, 1-Me), 2.36 (12 H, s, $2 \times 3\text{d-CH}_2\text{NMe}$ and $2 \times 3\text{d}'\text{-CH}_2\text{NMe}$), 2.13 (12 H, s, $2 \times 7\text{f-CH}_2\text{NMe}$ and $2 \times 7\text{f}'\text{-CH}_2\text{NMe}$), 1.85–2.80 (14 H, m, 3d- CH_2 , 3d'- CH_2 , 3c-H, 7b- CH_2 , 7f- CH_2 , 7f'- CH_2 , 7e-H and 7a- CH_2), 1.83 (3 H, d, J 7.3, 8-Me), 1.79 (3 H, t, J 7.6, 4b-Me), 0.38 (1 H, s, NH), -1.61 (1 H, s, NH); m/z (FAB, triethanolamine) 850 ($\text{M}^+ + \text{H}$).

3a-Deformyl-3a-carboxypyropheophorbide b 3a,7c-bis[1,1-bis(trimethylammoniomethyl)-2-ethyl] ester tetraiodide (5)

By the similar method to that for **1**, pyropheophorbide **18** was converted to **5** (Found: C, 44.94; H, 5.99; N, 7.75. Calc. for $\text{C}_{53}\text{H}_{80}\text{I}_4\text{N}_8\text{O}_5$: C, 44.93; H, 5.69; N, 7.91.); δ_{H} (300MHz, DMSO-d_6 ; Me_4Si) 10.57 (1 H, s, β -H), 10.11 (1 H, s, α -H), 8.93 (1 H, s, δ -H), 8.05 (1 H, dd, J 6.3 and 11, 2a-H), 6.47 (1 H, d, J 18, 2b-H), 6.30 (1 H, m, 2b'-H), 5.23 (2 H, m, 10- H_2), 4.82 (2 H, m, 3b- CH_2), 4.61 (1 H, m, 8-H), 4.36 (1 H, m, 7-H), 4.14 (4 H, m, 4a- CH_2 and 7d- CH_2), 3.70 (3 H, s, 5-Me), 3.44 (3 H, s, 1-Me), 3.05–3.55 (10 H, m, 3d- CH_2 , 3d'- CH_2 , 3c-H, 7e-H, 7f- CH_2 and 7f'- CH_2), 2.51 (36 H, s, $3 \times 3\text{d-CH}_2\text{NMe}$, $3 \times 3\text{d}'\text{-CH}_2\text{NMe}$, $3 \times 7\text{f-CH}_2\text{NMe}$ and $3 \times 7\text{f}'\text{-CH}_2\text{NMe}$), 2.05–2.90 (4 H, m, 7b- CH_2 and 7a- CH_2), 1.78 (3 H, t, J 8.8, 4b-Me), 1.76 (3 H, d, J 7.3, 8-Me), 0.31 (1 H, br s, NH), -1.74 (1 H, br s, NH).

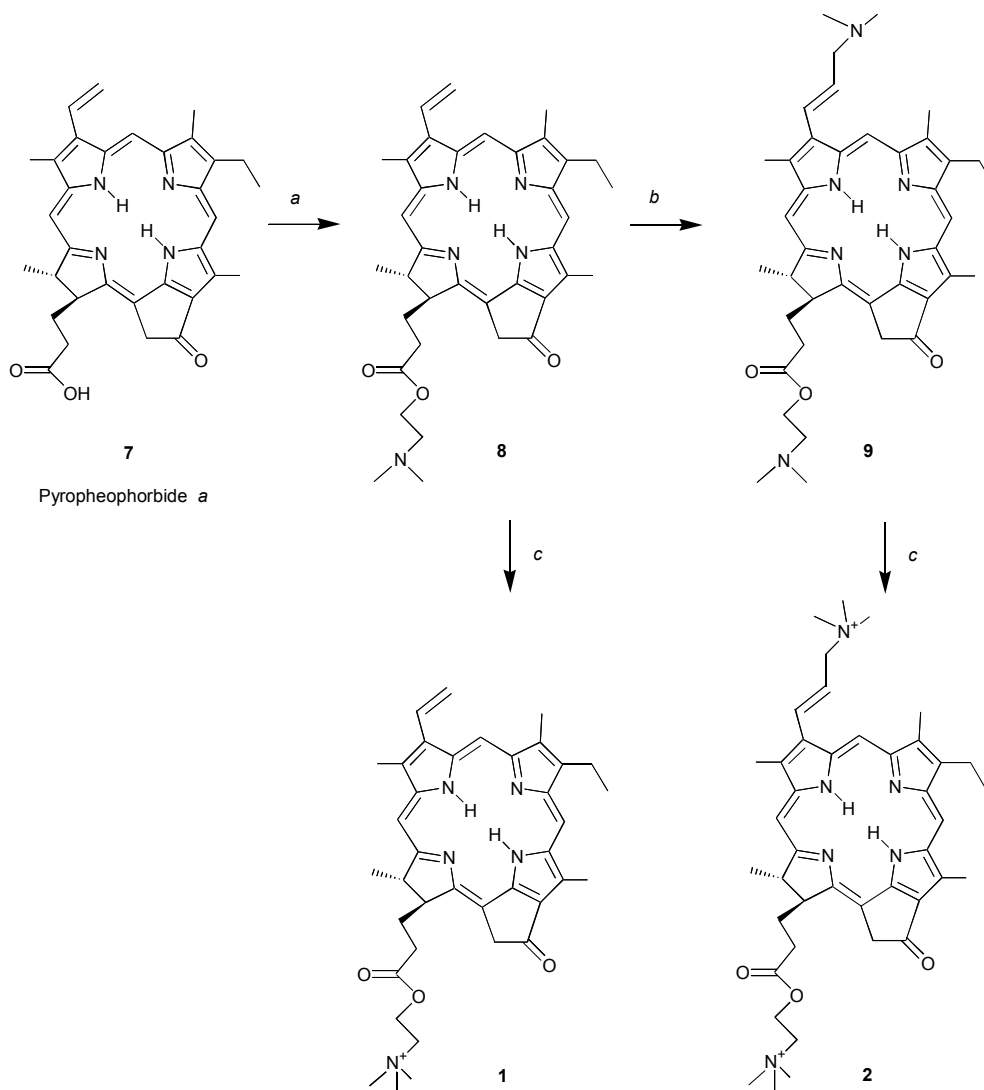
2a-Devinyl-3a-deformyl-2a,3a-bis(carboxy)pyropheophorbide b 2a,3a,7c-tris[1,1-bis(trimethylaminomethyl)-2-ethyl] ester (21)

Pyropheophorbide **19**⁴ was dissolved in THF/water containing sulfuric acid and converted to **20** quantitatively. A solution of **20** (6.65 mg) in anhydrous dichloromethane (0.75 mL), *N,N*-diisopropylethylamine (65 μ L) and 1,1-bis(dimethylaminomethyl)-2-ethanol³ (100 μ L) was stirred under a nitrogen atmosphere at -20°C (carbon tetrachloride/dry ice). To the solution was added BOP (29.9 mg) in anhydrous dichloromethane (0.75 mL) and it was stirred overnight with gradual warming to room temperature. The reaction was stopped by the addition of water, and the desired product was extracted from the water layer by dichloromethane. The dichloromethane layer was washed twice with water saturated with potassium hydrogen phthalate, with water saturated with sodium hydrogen carbonate and twice with distilled water, and evaporated to dryness. The residue was chromatographed on silica gel deactivated with water (silica gel/water = 100/6, w/w) using dichloromethane/methanol (10/1, v/v) as eluent. The top and main band adsorbed on silica gel was eluted with dichloromethane/methanol (5/1) containing 2% triethylamine, and evaporated to dryness to give **21** (6.6 mg); δ_{H} (300MHz, CDCl_3 ; Me_4Si) 11.43 (1 H, s, β -H), 9.79 (1 H, s, α -H), 8.77 (1 H, s, δ -H), 5.16 and 5.31 (2 H, ABq, J_{AB} 20, 10-H and 10-H'), 4.89 and 4.80 (4 H, each d, J 3.7 and 4.4, 2b- CH_2 and 3b- CH_2), 4.57 (1 H, m, 8-H), 4.37 (1 H, m, 7-H), 4.13 (2 H, m, 7d- CH_2), 4.13 (2 H, m, 4a- CH_2), 3.73 (3 H, s, 5-Me), 3.71 (3 H, s, 1-Me), 2.36 (24 H, s, $2 \times 3\text{d-CH}_2\text{NMe}$, $2 \times 3\text{d}'\text{-CH}_2\text{NMe}$, $2 \times 2\text{d-CH}_2\text{NMe}$ and $2 \times 2\text{d}'\text{-CH}_2\text{NMe}$), 2.12 (12 H, s, $2 \times 7\text{f-CH}_2\text{NMe}$ and $2 \times 7\text{f}'\text{-CH}_2\text{NMe}$), 1.90–2.90 (19 H, m, 2d- CH_2 , 2d'- CH_2 , 2c-H, 3d- CH_2 , 3d'- CH_2 , 3c-H, 7b- CH_2 , 7f- CH_2 , 7f'- CH_2 , 7e-H and 7a- CH_2), 1.85 (3 H, d, J 7.1, 8-Me), 1.82 (3 H, t, J 7.6, 4b-Me), 0.20 (1 H, s, NH), -1.90 (1 H, s, NH).

2a-Devinyl-3a-deformyl-2a,3a-bis(carboxy)pyropheophorbide *b* **2a,3a,7c-tris[1,1-bis(trimethylammoniomethyl)-2-ethyl] ester hexaiodide (6)**

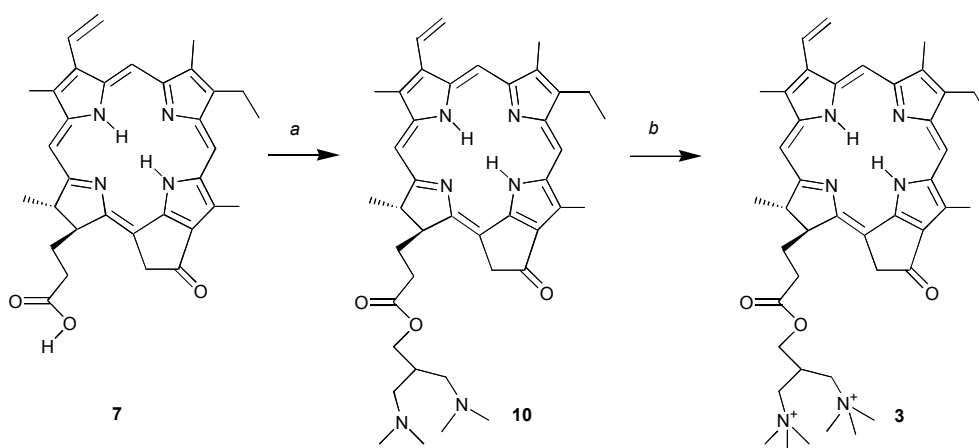
By the similar method to that for **1**, pyropheophorbide **21** was converted to **6** (Found: C, 38.03; H, 5.84; N, 6.99. Calc. for $\text{C}_{62}\text{H}_{102}\text{I}_6\text{N}_{10}\text{O}_7 \cdot 5\text{H}_2\text{O}$: C, 38.17; H, 5.79; N, 7.18.); δ_{H} (300MHz, DMSO-d_6 ; Me_4Si) 11.41 (1 H, s, β -H), 10.24 (1 H, s, α -H), 9.28 (1 H, s, δ -H), 5.32 (2 H, m, 10- H_2), 4.86 (4 H, m, 2b- CH_2 and 3b- CH_2), 4.73 (1 H, m, 8-H), 4.44 (1 H, m, 7-H), 4.16 (2 H, m, 4a- CH_2), 3.75 (3 H, s, 5-Me), 3.19

(3 H, s, 1-Me), 3.35 and 2.50 (18 H and 36 H, each s, $3 \times 3d\text{-CH}_2\text{NMe}$, $3 \times 3d'\text{-CH}_2\text{NMe}$, $3 \times 2d\text{-CH}_2\text{NMe}$, $3 \times 2d'\text{-CH}_2\text{NMe}$, $3 \times 7f\text{-CH}_2\text{NMe}$ and $3 \times 7f'\text{-CH}_2\text{NMe}$), 3.00–4.30 (17 H, m, 7d-CH₂, 2c-H, 2d-CH₂, 2d'-CH₂, 3c-H, 3d-CH₂, 3d'-CH₂, 7e-H, 7f-CH₂ and 7f'-CH₂), 2.05–2.95 (4 H, m, 7b-CH₂ and 7a-CH₂), 1.82 (3 H, d, *J* 6.8, 8-Me), 1.79 (3 H, t, *J* 7.6, 4b-Me), -0.22 (1 H, br s, NH), -2.05 (1 H, br s, NH).



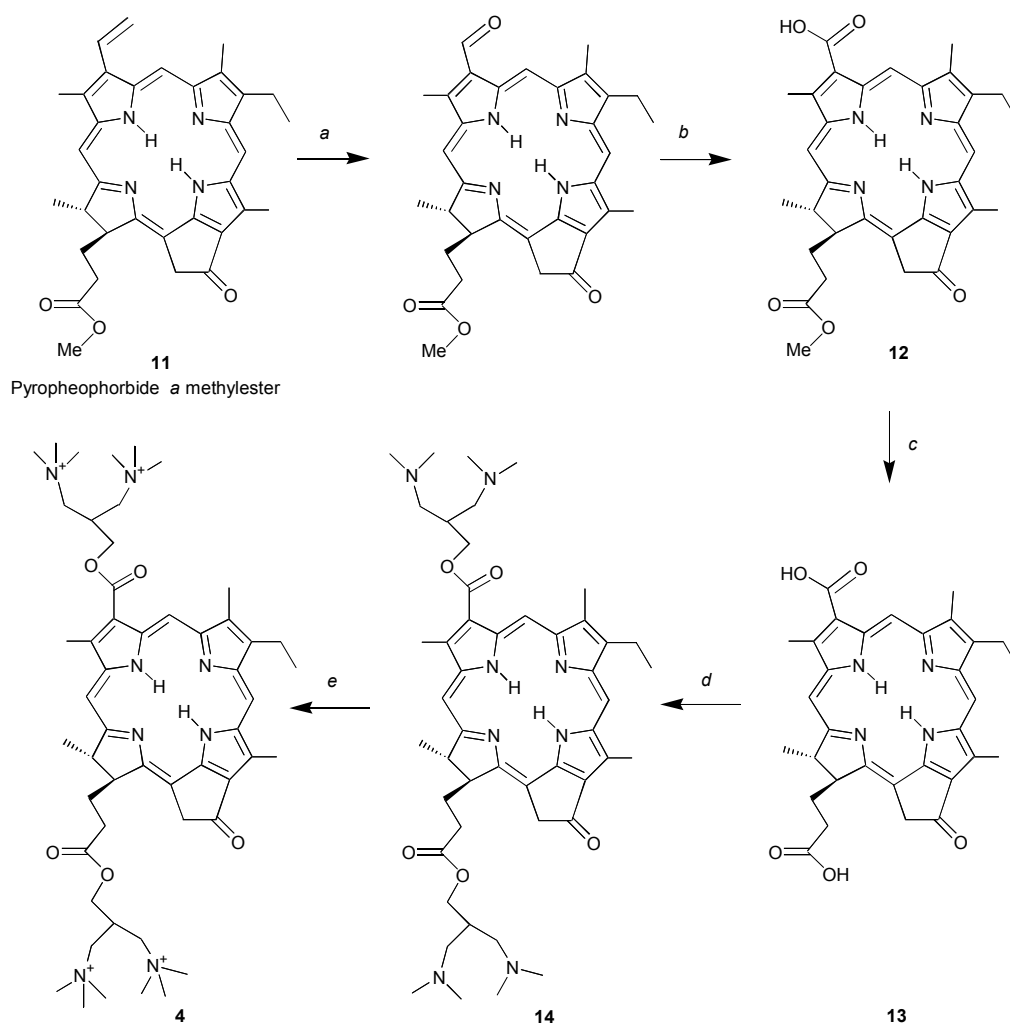
Reagents: a. $(\text{CH}_3)_2\text{NCH}_2\text{CH}_2\text{OH} / (\text{CH}_3)_3\text{CCOCl}$; b. $\text{CH}_2=\text{N}^+(\text{CH}_3)_2\text{I}^-$; c. CH_3I

Scheme S1 Synthesis of cationic pyropheophorbides **1** and **2**.



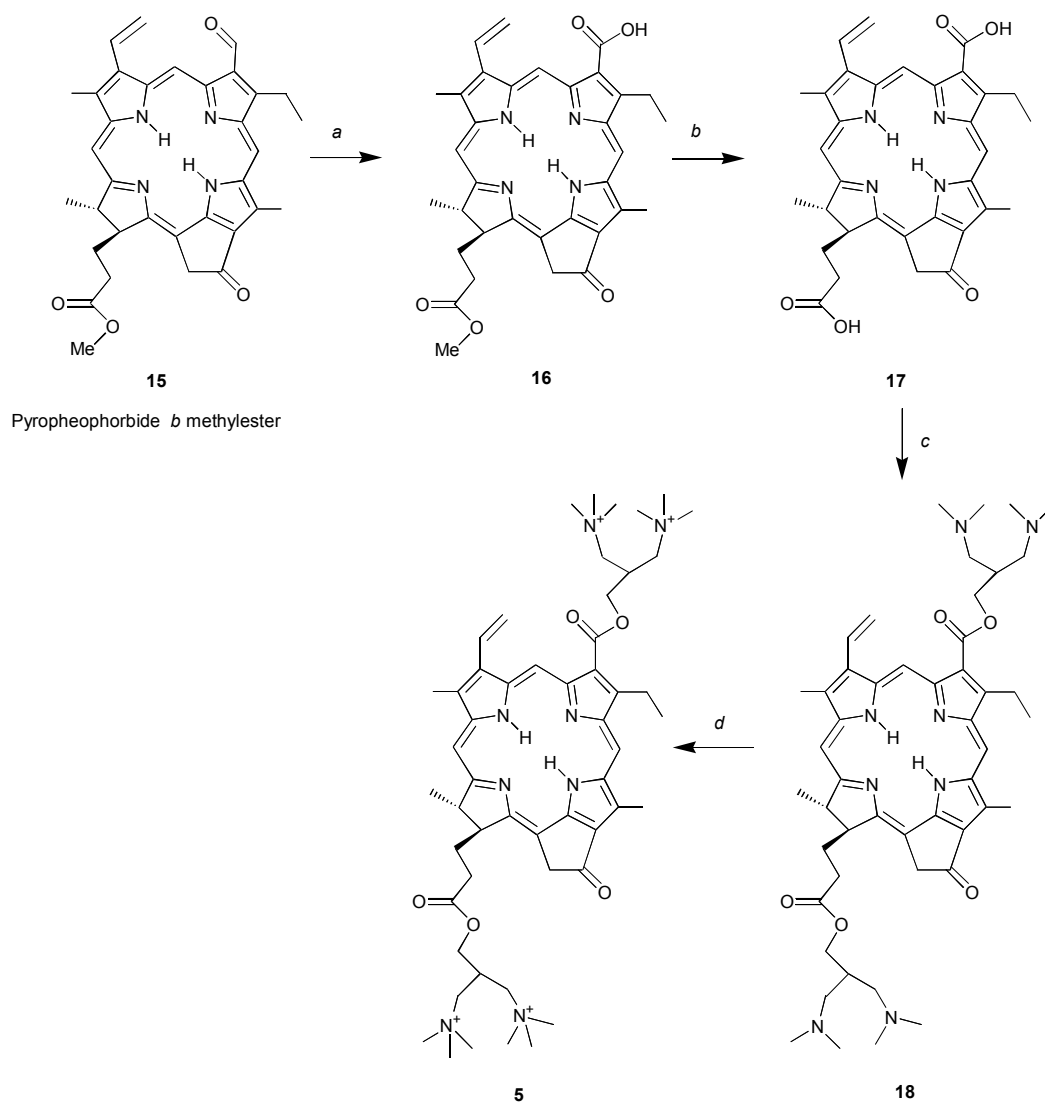
Reagents: a. 1,1-bis(dimethylaminomethyl)-2-ethanol / $(\text{CH}_3)_3\text{CCOCl}$; b. CH_3I

Scheme S2 Synthesis of cationic pyropheophorbide **3**.



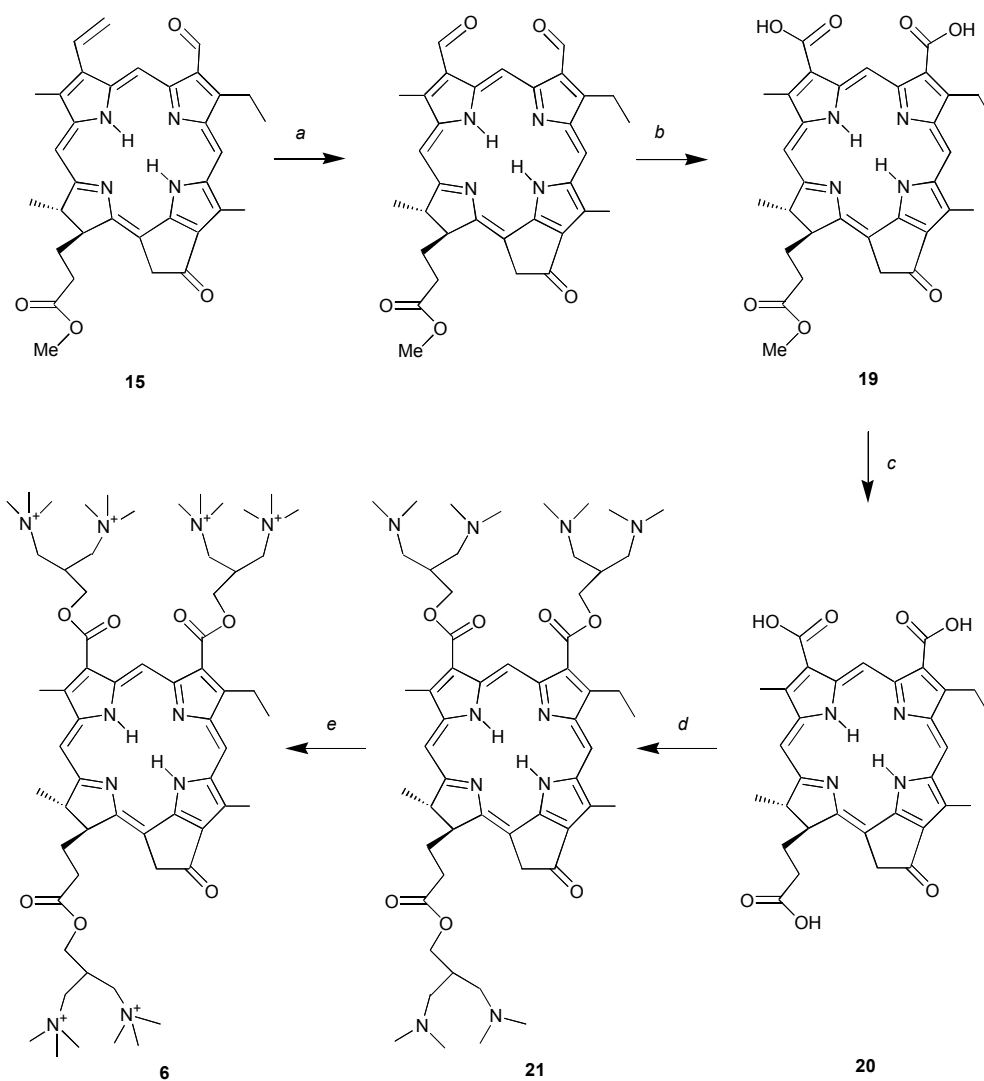
Reagents: a. $\text{OsO}_4 / \text{NaIO}_4$; b. $\text{NH}_2\text{SO}_3\text{H} / \text{NaClO}_2$; c. $\text{H}_2\text{SO}_4 / \text{H}_2\text{O}$;
d. 1,1-bis(dimethylaminomethyl)-2-ethanol / $(\text{CH}_3)_3\text{CCOCl}$; e. CH_3I

Scheme S3 Synthesis of cationic pyropheophorbide 4.



Reagents: a. $\text{NH}_2\text{SO}_3\text{H} / \text{NaClO}_2$; b. $\text{H}_2\text{SO}_4 / \text{H}_2\text{O}$;
c. 1,1-bis(dimethylaminomethyl)-2-ethanol / BOP; d. CH_3I

Scheme S4 Synthesis of cationic pyropheophorbide **5**.



Reagents: a. $\text{OsO}_4 / \text{NaIO}_4$; b. $\text{NH}_2\text{SO}_3\text{H} / \text{NaClO}_2$; c. $\text{H}_2\text{SO}_4 / \text{H}_2\text{O}$;
d. 1,1-bis(dimethylaminomethyl)-2-ethanol / BOP; e. CH_3I

Scheme S5 Synthesis of cationic pyropheophorbide **6**.

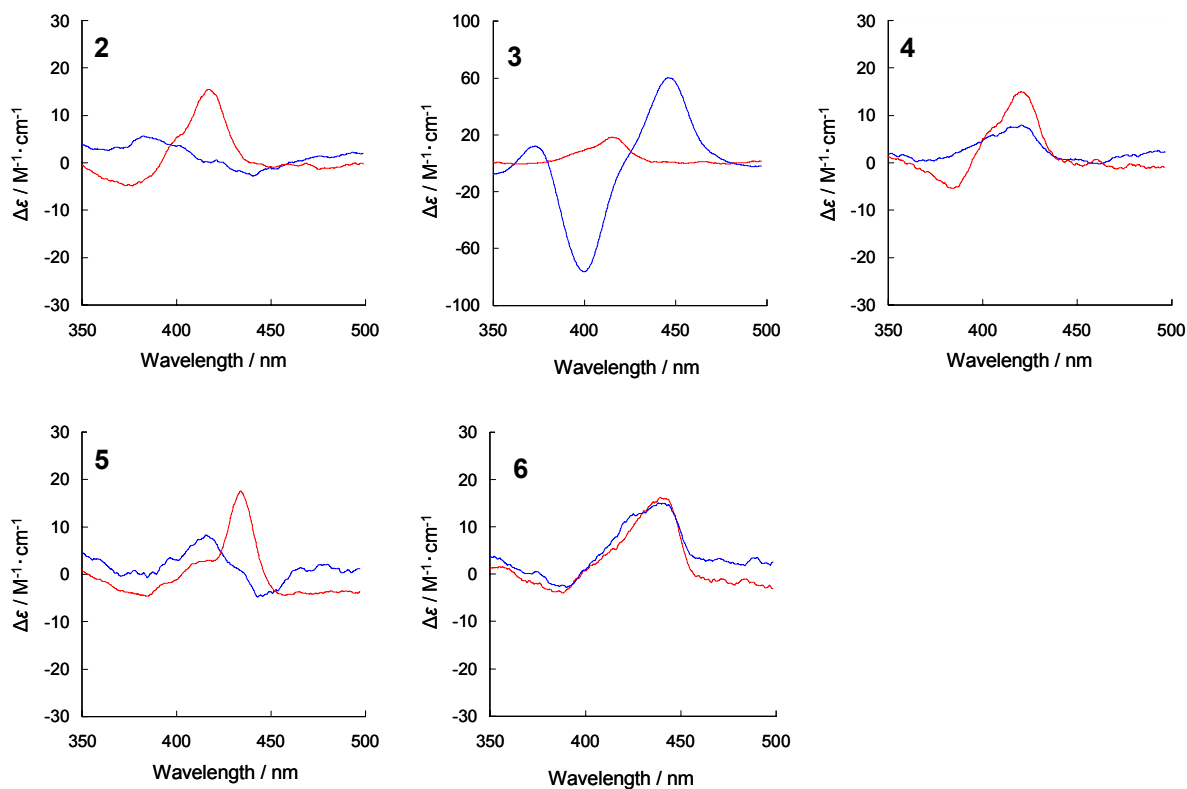


Fig. S1 CD spectra of cationic pyropheophorbides **2–6** in 5 mM HEPES buffer (blue line) and in DMSO (red line) at 25 °C. The concentration of **2**, **3**, **4**, **5** and **6** is 2.0, 4.9, 3.0, 4.9, 2.4 and 2.9 μM , respectively.

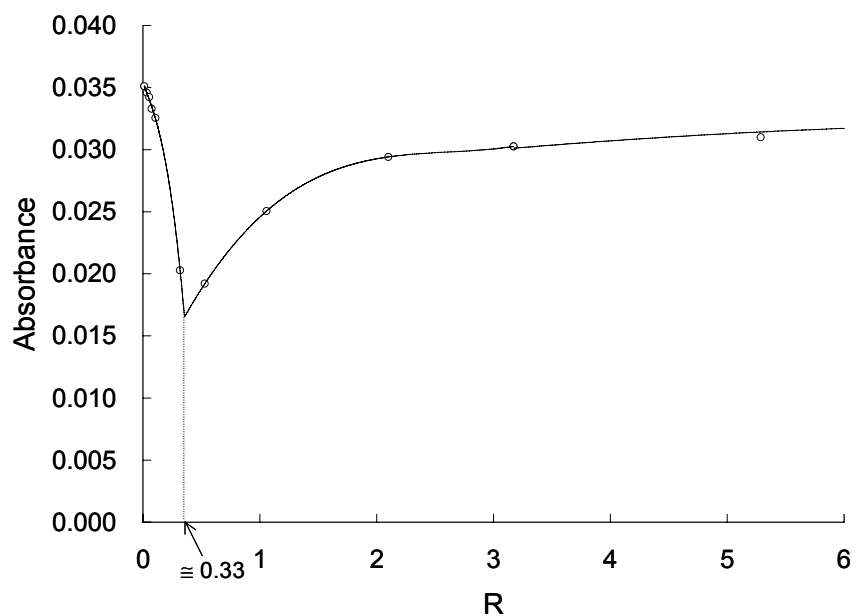


Fig. S2 Changes in absorbance of **6** at the Q band region upon increasing addition of CT-DNA in HEPES buffer (pH7.0 at 25 °C).

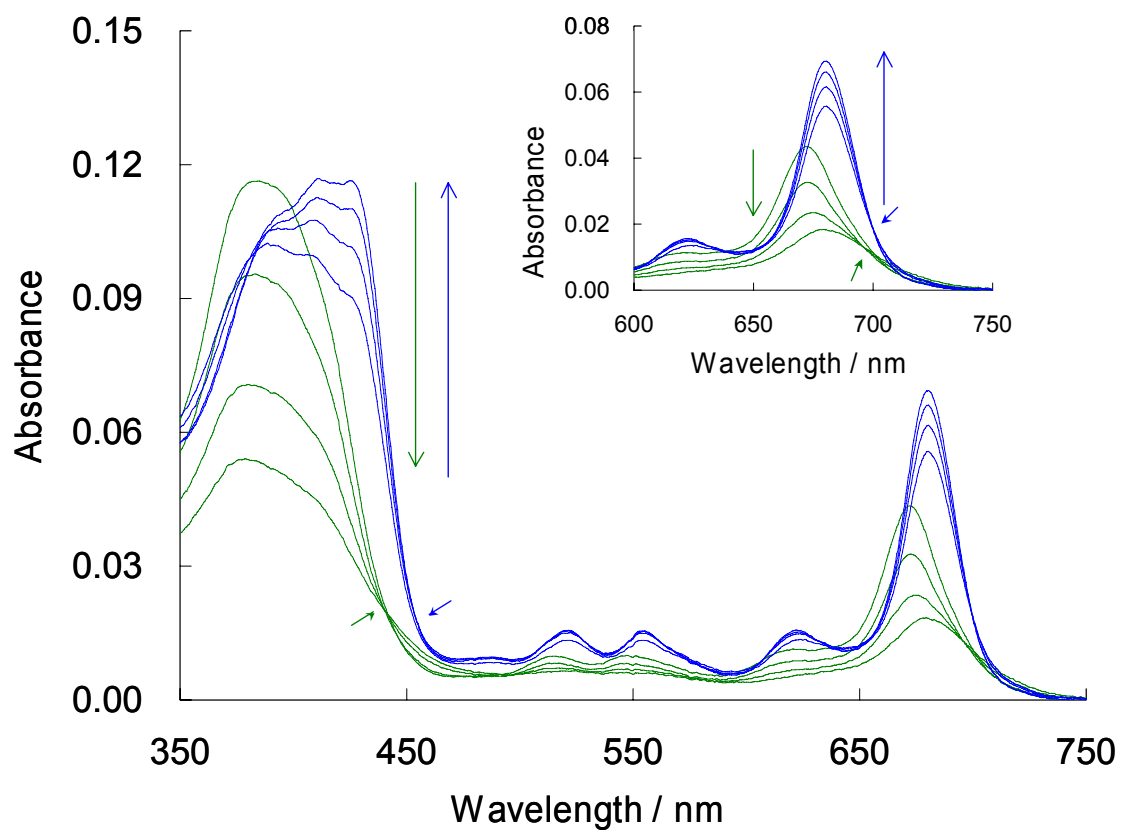


Fig. S3 Visible absorption spectral changes of **2** upon increasing addition of CT-DNA. The direction of arrows corresponds to decreasing R values (∞ , 7.3, 3.1, 1.1, 0.10, 0.073, 0.052, 0.031).

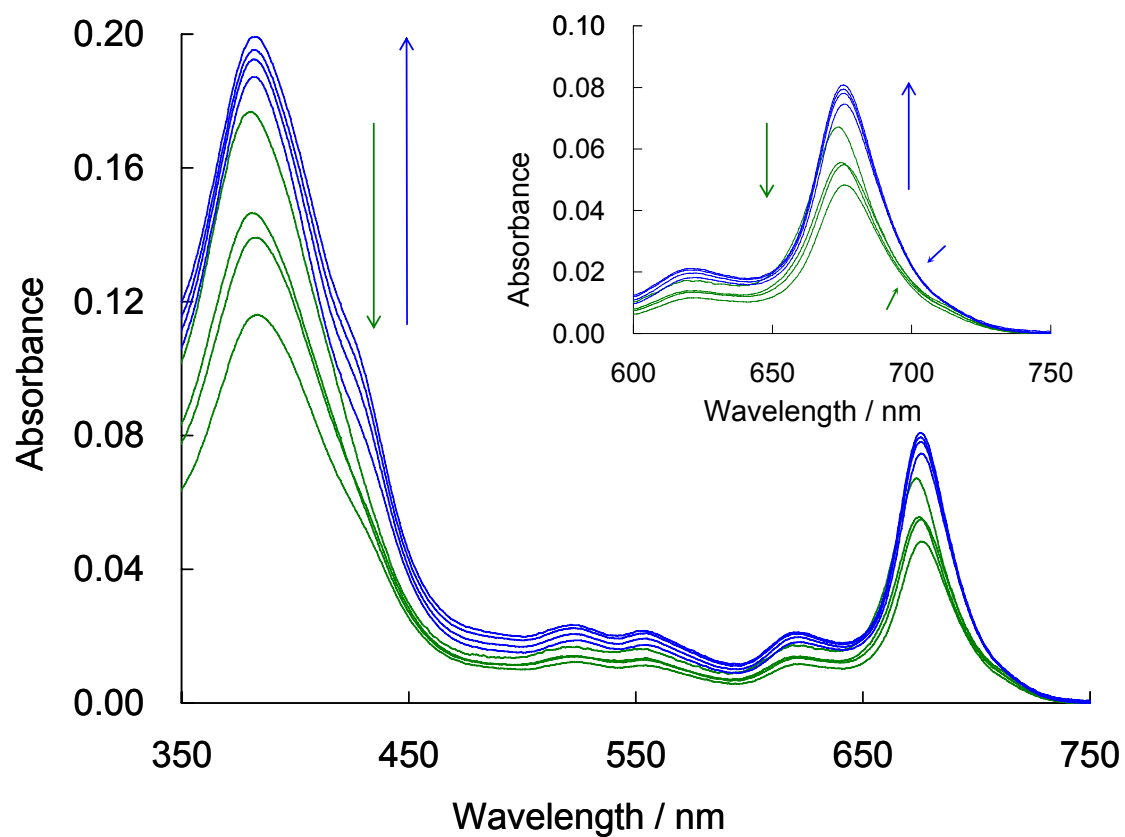


Fig. S4 Visible absorption spectral changes of **3** upon increasing addition of CT-DNA. The direction of arrows corresponds to decreasing R values (∞ , 7.3, 3.1, 1.1, 0.52, 0.10, 0.052, 0.031).

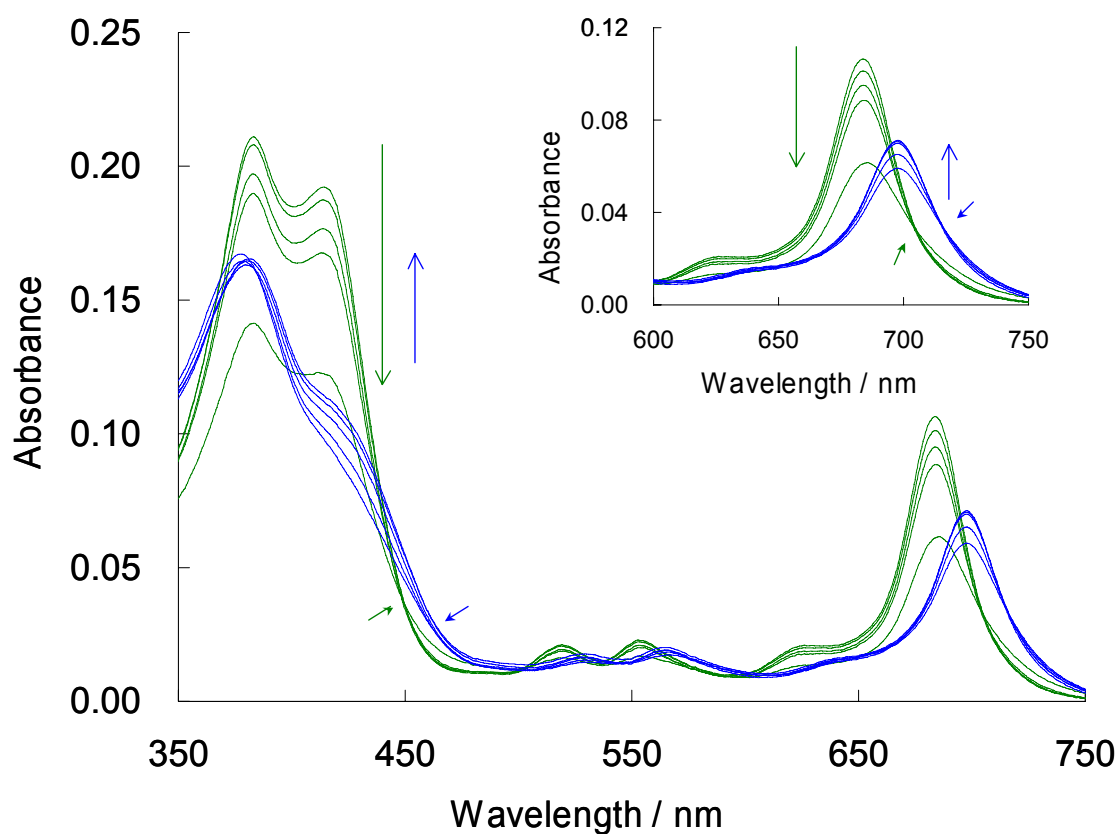


Fig. S5 Visible absorption spectral changes of **4** upon increasing addition of CT-DNA. The direction of arrows corresponds to decreasing R values (∞ , 10, 7.2, 3.1, 1.0, 0.51, 0.31, 0.10, 0.051, 0.031).

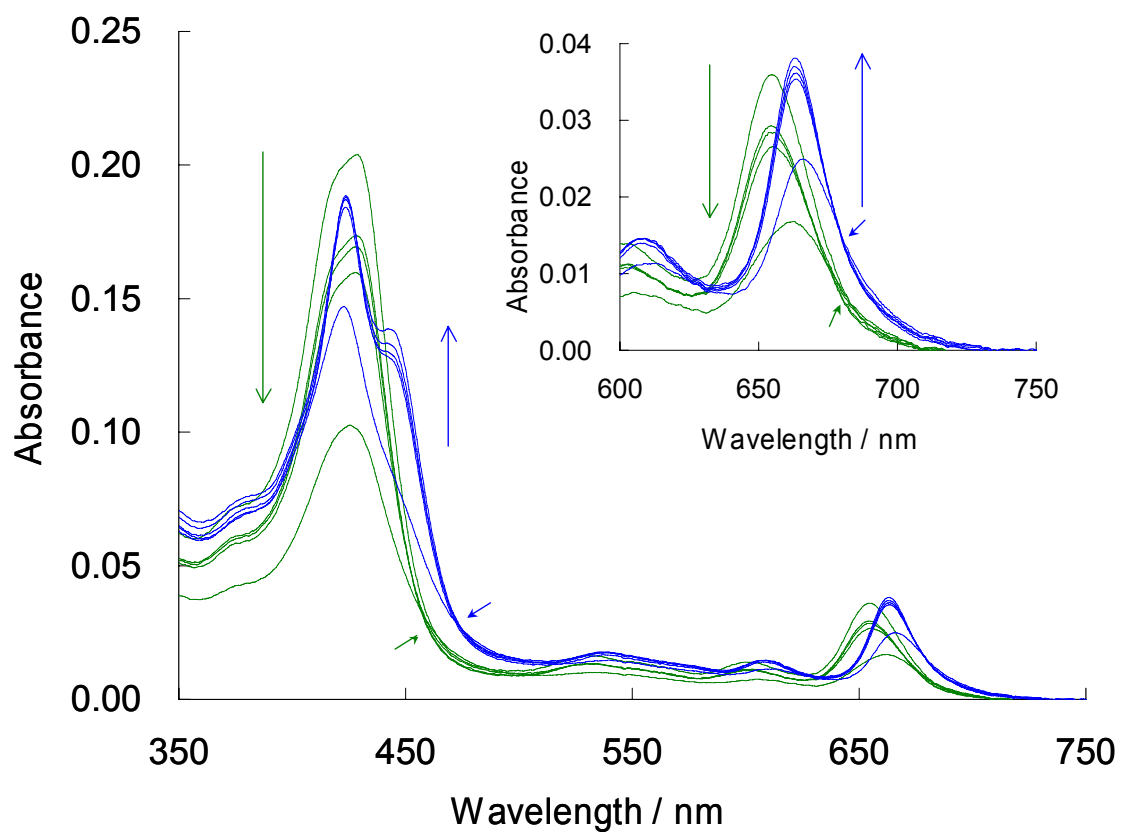


Fig. S6 Visible absorption spectral changes of **5** upon increasing addition of CT-DNA. The direction of arrows corresponds to decreasing R values (∞ , 5.3, 3.2, 1.1, 0.53, 0.32, 0.11, 0.074, 0.053, 0.032).

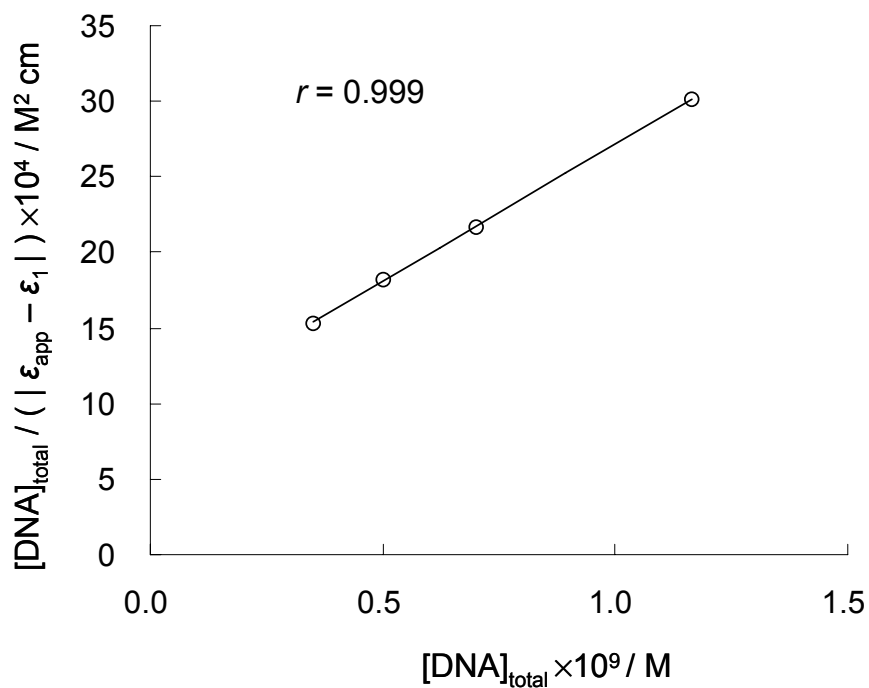


Fig. S7 Determination of the K_{app} of 4 according to eqn 3.

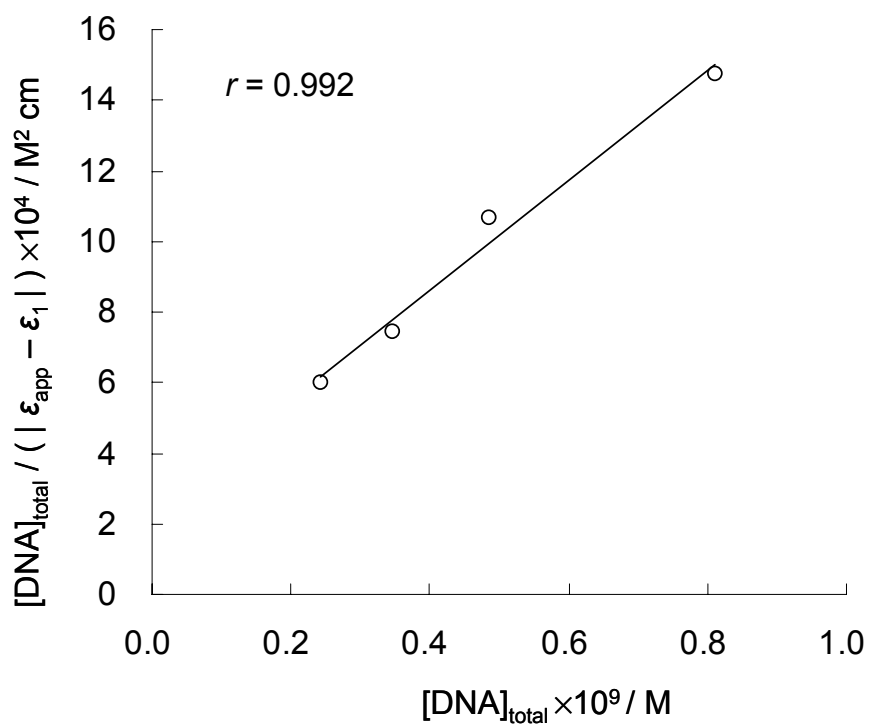


Fig. S8 Determination of the K_{app} of 5 according to eqn 3.

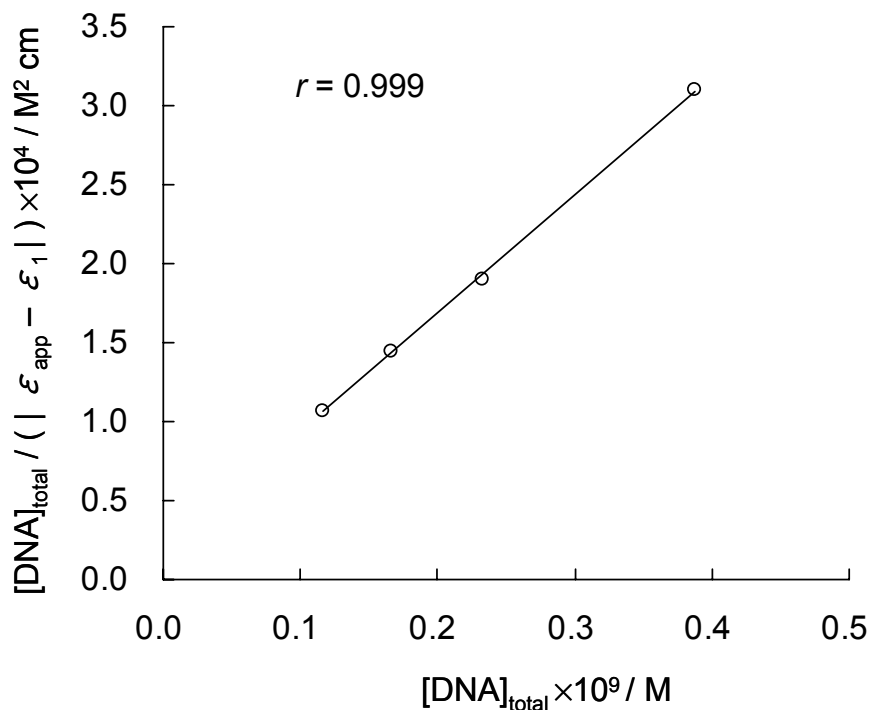


Fig. S9 Determination of the K_{app} of **6** according to eqn 3.

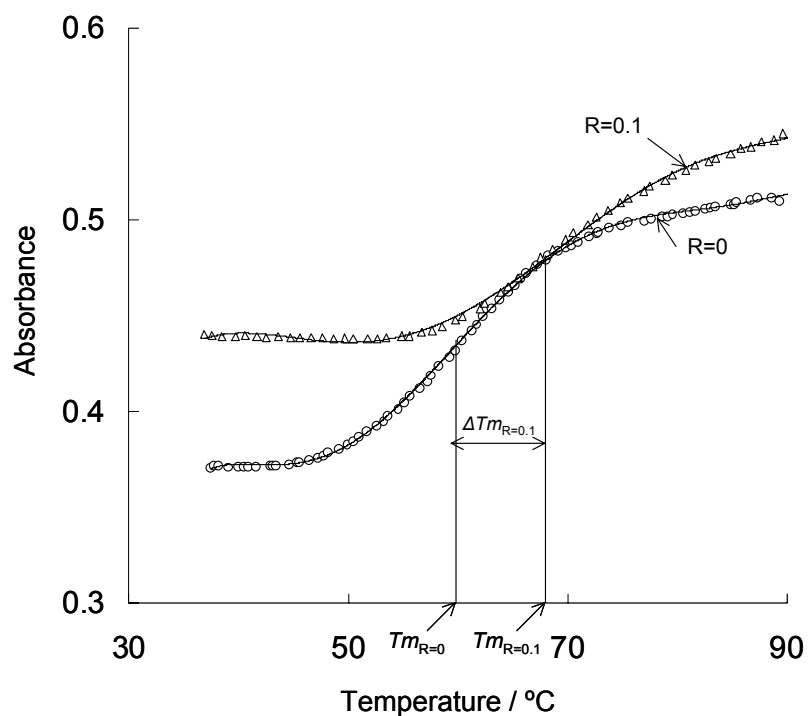


Fig. S10 Typical melting curves of CT-DNA in the absence ($R = 0$) and in the presence ($R = 0.1$) of **6**.

The similar curves were obtained in the case of **2–5**.

References

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