Supporting Information

Porphyrin-Peptoid Conjugates: Face-to-Face Display of Porphyrins on Peptoid Helices

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1. General methods.

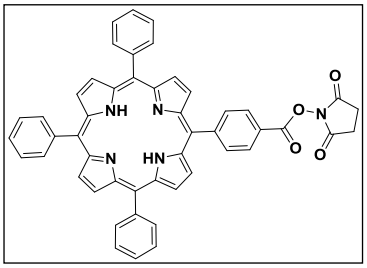
Flash column chromatography was performed with silica gel 60 (230-400 mesh, Merck, Darmstadt, Germany). Thin-layer chromatography was carried out on precoated aluminum-backed TLC sheets (silica gel 60 F_{254}, EMD Millipore, Billerica, MA, USA). Compounds were visualized with a UV/VIS lamp or a ninhydrin spray reagent (ninhydrin 2% in ethanol(w/v)). All reagents and solvents were purchased from Sigma-Aldrich, Novabiochem, and Acros Organics. They were used without further purification unless stated otherwise. Peptide synthesis grade DMF and anhydrous DCM (> 99.8%) were used for peptoid synthesis and conjugation reactions, respectively. *N*,*N*'-Diisopropylcarbodiimide was purchased from Advanced ChemTech, KY, USA. Solvents were evaporated with standard rotary evaporator under reduced pressure, and peptoid final products were lyophilized twice to eliminate any residual TFA.

Circular dichroism spectra were recorded at 25 °C on a Jasco model 810 spectropolarimeter (Jasco, Inc., Easton, MD, USA). Samples were dissolved in CH_3CN with a concentration of 50 μM immediately before the measurement. CD spectra were obtained in an 1 mm pathlength quartz cuvette and recorded from 190 ~ 260 nm for peptoids (**6** – **9**) or 190 ~ 500 nm for porphyrin-peptoid conjugates (**1** – **5**) in 1 nm increments with a scanning speed of 20 nm/min and a response time of 2 second. For each sample, scan was performed three times and averaged. Data are expressed in terms of per-residue molar ellipticity (deg cm^2/dmol) calculated per number of main chain amides in a peptoid.

UV/Vis spectra were observed on an Ultrospec 2100 pro UV/Vis Spectrophotometer (GE healthcare, Buckinghamshire, UK). Quarts cuvette with pathlength 1 mm was used. Samples were dissolved in CH_3CN, and the absorbance of CH_3CN was used as a blank. Various concentrations of samples were prepared from serial dilution of 1.0 mM stock solution. Lower concentration stock solution (0.4 mM) was used for **4** and **5**. The range of absorbance measurement was 300 ~ 700 nm.

2. Synthesis of porphyrin-peptoid conjugates.

Synthesis of 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin *N*-hydroxysuccinimide ester (or TPP-NHS ester).



Initially, synthesis of 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin was attempted using Adler's protocol.[1] However, as Fungo et al. noted, the reaction work up was cumbersome due to the difficulty of removing propionic acid and the insoluble tar present, which resulted in poor yield.[2] Hence, we employed Lindsey's method,[3] which we found to be more efficient. A dried round bottom flask was charged with 4-formylbenzoic acid (1 g, 6.66 mmol), benzaldehyde (2.03 ml, 19.98 mmol) and pyrrole (1.85 ml, 26.64 mmol), and then $CHCl_3$ (with 0.75% ethanol, purchased from SigmaAldrich, 280 ml) was added. To this mixture was added $BF_3O(Et)_2$ (0.818 ml, 6.66 mmol) dropwise. The flask was sealed with rubber septum, and the mixture was stirred for 1 h at room temperature under N_2 atmosphere. After 1 h, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 4.54 g, 19.98 mmol) was added, and the oxidation reaction was kept for 1 h. The reaction was quenched by slow addition of triethylamine (1.39 ml, 9.99 mmol), and the reaction mixture was stirred for another 30 minutes. The mixture was concentrated under reduced pressure, and the resulting dark purple residue was purified by short silica gel plug to afford 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin (DCM: MeOH = 20 : 1, R_f = 0.3). The mono-carboxylate form of tetraphenylporphyrin was difficult to be obtained in a completely pure form by single silica gel chromatography. Instead of repeated purifications, we proceeded to the next step to provide an ester which was readily purified by silica gel chromatography.

To a dry round bottom flask was added *N*-hydroxysuccinimide (56.7 mg, 0.493 mmol), *N*,*N*'-dicyclohexylcarbodiimide (101.7 mg, 0.493 mmol), and purified 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin (approximately 130 mg), followed by dichloromethane (6 mL). Then, pyridine (0.040 ml, 0.493 mmol) and a few pieces of 4-dimethylaminopyridine were added into the flask. The mixture was stirred overnight at room temperature. The mixture was then evaporated by rotary evaporator, and the residue was purified by flash column chromatography (DCM 100%, R_f = 0.35). TPP-NHS ester was obtained as purple film with roughly 15% yield over 2 steps. Spectral data matched with the previously reported.[4]

General procedure of peptoid synthesis.

Peptoid nonamers and dodecamers were synthesized by solid-phase submonomer method on a resin bead using a manual synthesis and microwave heating. A CEM MARS multimodal microwave reactor equipped with a fiber-optics temperature probe and magnetic stirrer was used (CEM Corp., Matthews, NC, USA). The

fiber-optics temperature probe was positioned in the reaction mixture, and the solution was stirred and irradiated at different reaction conditions as described below. All microwave reactions were carried out at atmospheric pressure. Fmoc-Rink amid MBHA resin (0.59 mmol/g, Novabiochem, San Diego, CA, USA) was used to generate C-terminal amide peptoids. After Fmoc deprotection, each monomer was added by a series of bromoacetylation and displacement of bromide by a primary amine. These two steps were iterated with appropriate primary amines until desired peptoid sequence was obtained. The N-terminal acetylation of peptoid oligomers was performed by adding excess amount of acetic anhydride (50 equivalent) and pyridine (55 equivalent) in DMF. Typically, 0.25 mmol reaction scale was used (0.42 g of resin). For Fmoc deprotection, Fmoc-Rink amide resin was treated twice with 20% (v/v) piperidine in DMF (5 mL each) at room temperature for 60 seconds and at 80 °C (microwave, 600W max power, ramp 2 minutes) for 2 minutes. For bromoacetylation, bromoacetic acid (4.18 mL, 1.2 M in DMF, 5 mmol) and *N*,*N'*-diisopropylcarbodiimide (0.78 mL, 5 mmol) were added, and the reaction mixture was stirred and irradiated at 35 °C (microwave, 400W 15% power, ramp 0.5 minutes) for 2 minutes. For the displacement step, (S)-*N*-(1-phenylethyl)glycine (*N*spe, 5 mL, 2.0 M in NMP, 10 mmol), Benzylamine (*N*pm, 5 mL, 2.0 M in NMP, 10 mmol), or mono-Mmt protected 1,4-diaminobutane (*N*Lys(Mmt), 5 mL, 1.0 M in NMP, 5 mmol) were used as primary amines according to desired peptoids sequence. The mixture was stirred and irradiated at 95 °C (microwave, 400W 75% power, ramp 2 minutes) for 1.5 minutes. Between each step, the resin was thoroughly washed with DMF and DCM. The N-terminal acetylation was performed by adding acetic anhydride (1.2 mL, 12.5 mmol) and pyridine (1.1 mL, 13.7 mmol) to the resin-bound peptoid in DMF (1.5 mL). The reaction was kept at room temperature for 2 h.

Synthesis of porphyrin-peptoid conjugates.

Prior to porphyrin conjugation reaction, Mmt deprotection was performed by treating resin-bound peptoid with 0.75% TFA (DCM : TIS : TFA = 94.25 : 5 : 0.75). To the resin was added 0.75% TFA solution (6 ml), and stirring continued for 2 minutes at room temperature. After draining orange color solution, the resin was washed with DCM. These steps were repeated for 5 times. Then, the deprotected amine was conjugated with tetraphenylporphyrin (TPP) using the previously prepared TPP-NHS ester. Typically, 1.5 equivalent of TPP-NHS ester per amine was used. Resin-bound and Mmt deprotected peptoid (0.0625 mmol) was washed first with the solution of DCM (4 mL) and DIEA (0.15 mL) for 1 minute to remove any residual TFA. To the resin was added TPP-NHS ester (150 mg, 0.20 mmol) in DCM (7 mL), followed by DIEA (0.07 mL, 0.40

mmol). The cartridge was sealed, and the reaction was stirred overnight under N_2 atmosphere. The reaction mixture was drained, and the resin was washed thoroughly with DMF and DCM. Cleavage from resin was performed with cleavage solution (DCM : TFA : triisopropylsilane : H_2O = 50 : 45 : 2.5 : 2.5) for 10 minutes at room temperature. After cleavage reaction, the solution was filtered by solid-phase extraction (SPE) cartridge with 20μ hydrophobic polyethylene frits (Applied separations, Allentown, PA, USA). The filtrate solution was lyophilized, and the crude peptoid was dissolved in CH_3CN and analyzed by analytical HPLC and ESI-MS.

HPLC conditions.

Analytical HPLC was performed on a Waters HPLC system (Waters 2489 UV/Visble Detector, Waters 1525 Binary HPLC Pump, Waters 2707 Autosampler, and Waters 5CH column oven) with a C18 column (SunFire C18, 4.6 x 250 mm, 5 μm). The column oven temperature was set at 40 °C. The mobile phase was used as follows: (A, water + 0.1% TFA; B, CH_3CN + 0.1% TFA) 5 min using 30% of B, a linear gradient to 100% B over 20 min, and then holding 100% B over 15 min. The flow rate was 1 mL/min. The purity of sample was monitored by absorbance at 220 nm. Peptoids were purified by preparative HPLC system (Waters prepLC system, Waters 2489 UV/Visible Detector, Waters fraction collector III) with a C18 column (SunFire C18, 19 x 150 mm, 5 μm) at a flow rate of 14 mL/min. Sample elution was monitored at 220 and 254 nm by absorbance. The purity of the product fractions were confirmed by analytical HPLC. Each fraction was further analyzed by LC/MS performed on an Agilent 1100 liquid chromatography system with an Agilent 6130 single quadrupole mass spectrometer (Applied Biosystems). Fractions containing pure product (>97% purity) were collected, lyophilized, and stored at -80 °C.

Figure S1. Chemical structures of porphyrin-pepotid conjugates.

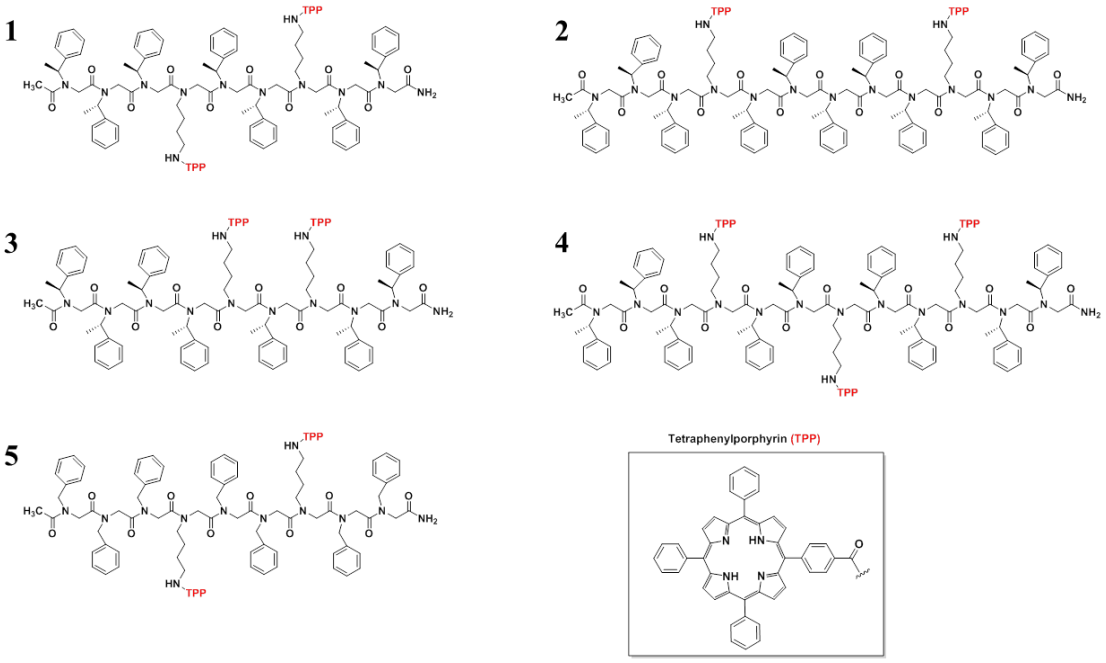
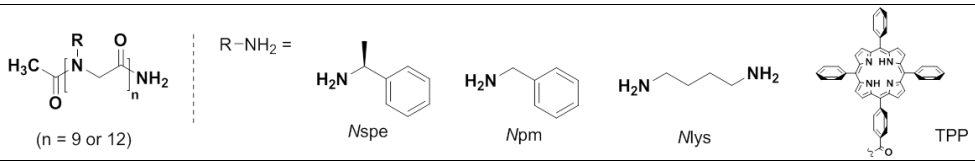


Table S1. Sequences and structures of porphyrin-peptoid conjugates (**1 – 5**) and their control peptoids (**6 – 9**).



compounds	chain lentgh	sequence
1	9	Ac-*N*spe-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-NH_2
2	12	Ac-*N*spe-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-*N*spe-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-NH_2
3	9	Ac-*N*spe-*N*spe-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-NH_2
4	12	Ac-*N*spe-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-*N*lys(TPP)-*N*spe-*N*spe-NH_2
5	9	Ac-*N*pm-*N*pm-*N*pm-*N*lys(TPP)-*N*pm-*N*pm-*N*lys(TPP)-*N*pm-*N*pm-NH_2
6	9	Ac-*N*spe-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-NH_2
7	9	Ac-*N*spe-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-*N*spe-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-NH_2
8	12	Ac-*N*spe-*N*spe-*N*spe-*N*spe-*N*lys-*N*spe-*N*lys-*N*spe-*N*spe-NH_2
9	12	Ac-*N*spe-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-*N*lys-*N*spe-*N*spe-NH_2

Figure S2. HPLC chromatograms of **1** - **5** with UV detection at 220 nm**.**

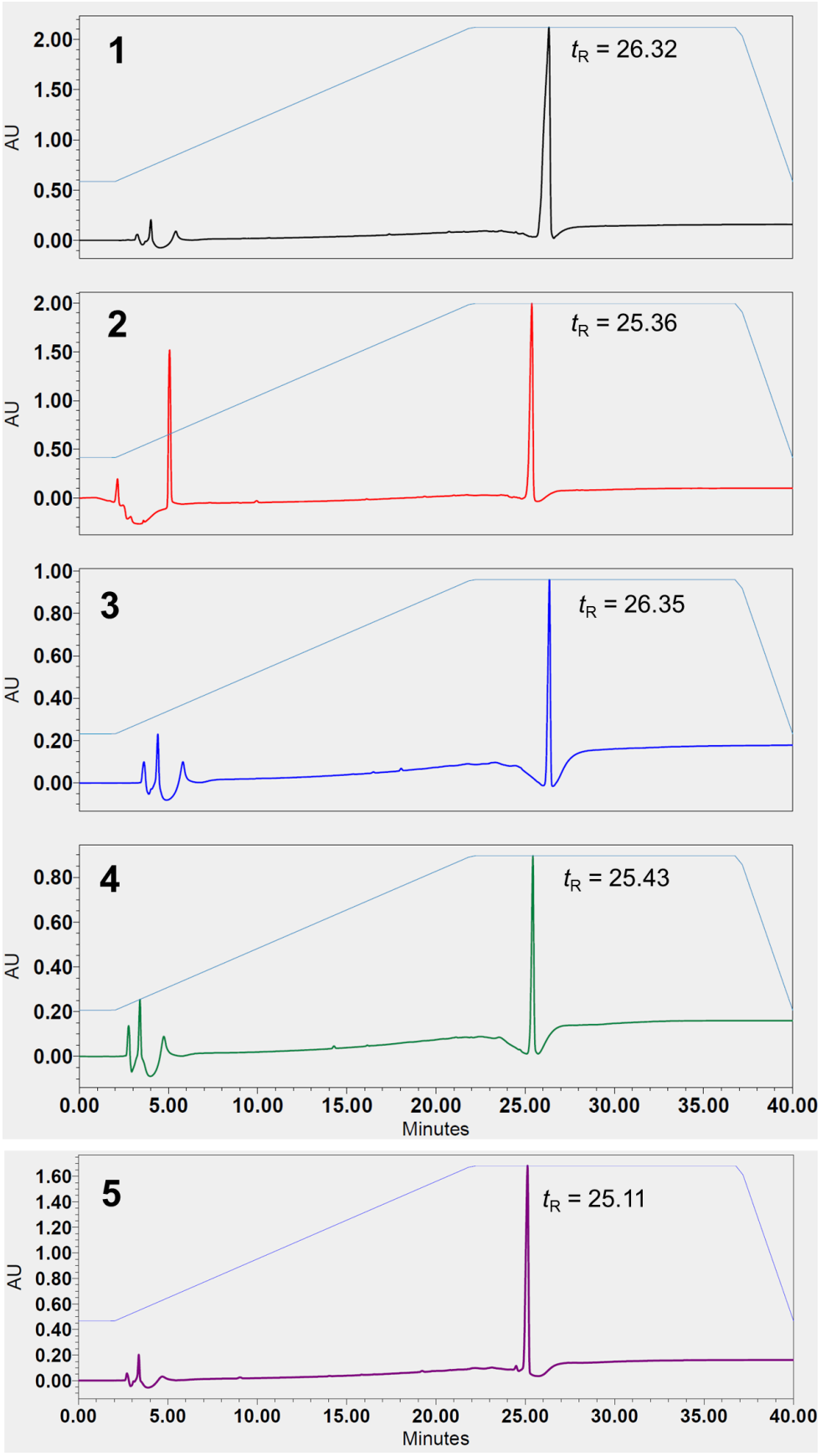


Figure S3. HPLC chromatograms of **6** - **9** with UV detection at 220 nm.

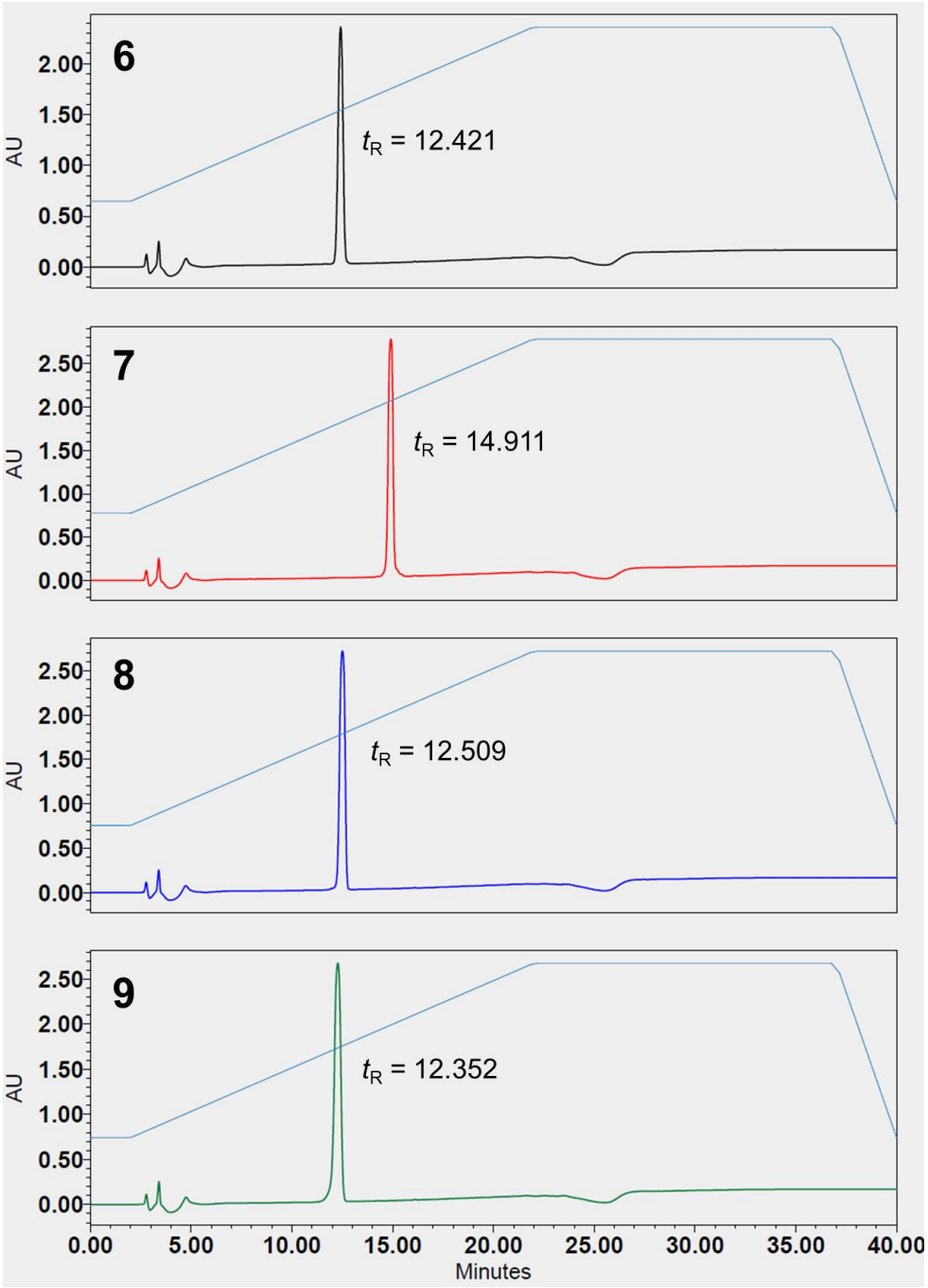


Table S2. ESI-MS data of **1** - **9**.

compounds	mass calculated	mass observed[a]
1[b]	2723.27	1363.2 ($2H^+$), 1385.3 ($2Na^+$)
2[b]	3206.52	1605.0 ($2H^+$), 1070.3 ($3H^+$)
3[b]	2723.27	1363.2 ($2H^+$), 909.2 ($3H^+$)
4[b]	3813.76	1908.5 ($2H^+$), 1272.9 ($3H^+$)
5[b]	2625.16	1314.2 ($2H^+$), 883.8 (Na^++$2H^+$)
6	1442.82	1443.9 (H^+), 1465.9 (Na^+)
7	1926.07	1928.0 (H^+), 1951.4 (Na^+)
8	1442.82	1444.3 (H^+), 1466.2 (Na^+)
9	1893.08	1894.9 (H^+), 1918.0 (Na^+)

[a]Observed in ESI-MS. [b]Parent peaks were not observed due to the mass range of the instrument (up to *m*/*z* 2000).

The observed masses are doubly charged ($2H^+$) and triply charged ($3H^+$) peaks, not fragments.

Figure S4. Concentration dependent normalized UV-Vis absorption spectra of **1 – 5** in acetonitrile. Line color was chosen to match the actual solution color. For high concentrations, Soret band reached instrument′s maximum absorption.

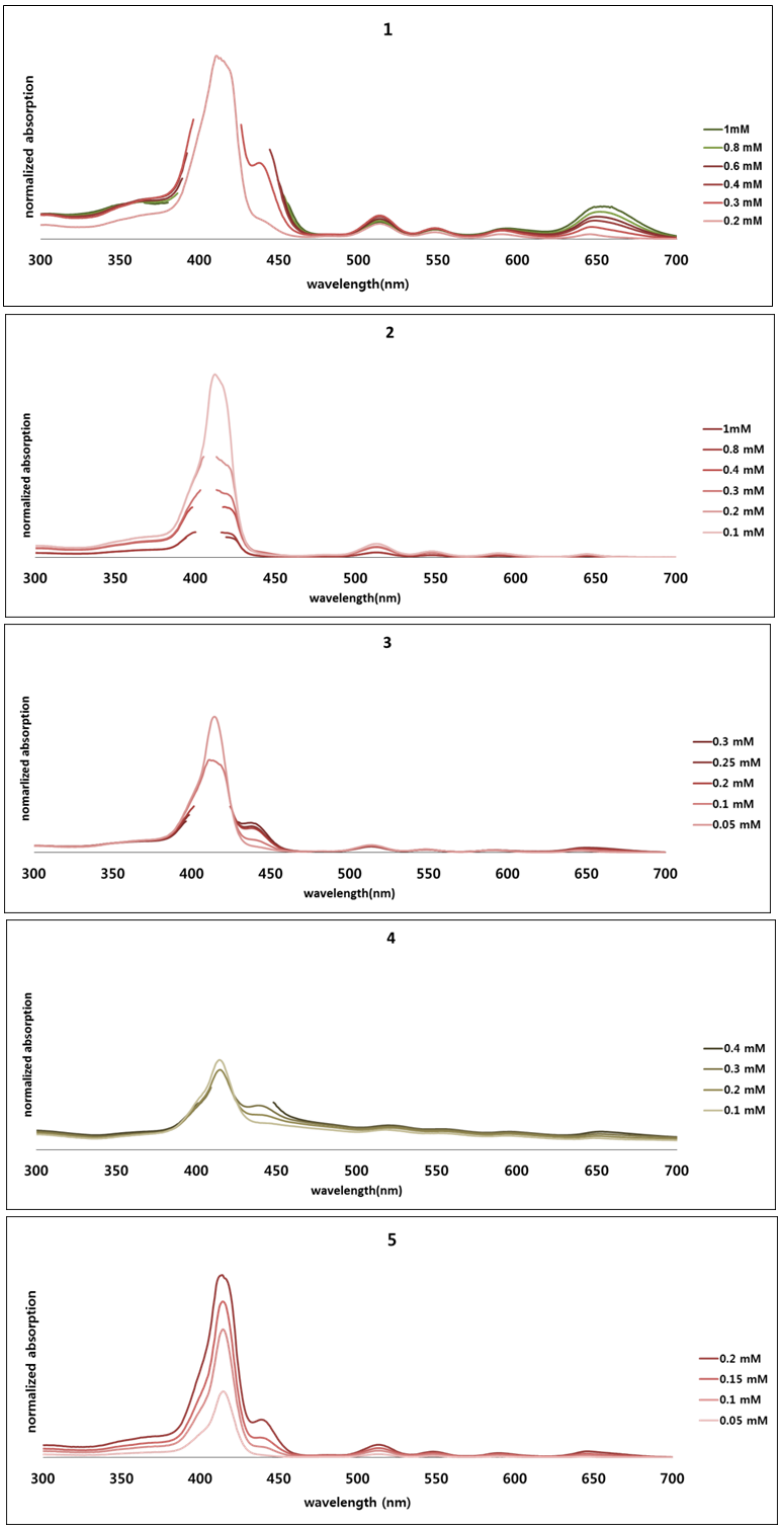
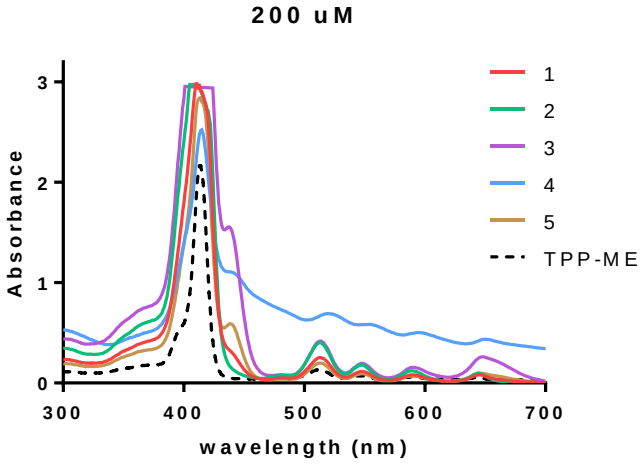


Figure S5. UV-Vis absorption spectra of **1 – 5** and TPP-ME at fixed concentration (0.2 mM). For PPC **3**, Soret band reached instrument′s maximum absorption.



Reference:

(1) Adler, A. D. *J. Org. Chem.* **1967**, *32*, 476-477.

(2) Fungo, F.; Otero, L. A.; Sereno, L.; Silber, J. J.; Durantini, E. N. *J. Mater. Chem.* **2000**, *10*, 645-650.

(3) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827-836.

(4) Boisbrun, M.; Vanderesse, R.; Engrand, P.; Olié, A.; Hupont, S.; Regnouf-de-Vains, J.; Frochot, C. *Tetrahedron* **2008**, *64*, 3494-3504.