Supplementary Information (SI) for RSC Medicinal Chemistry.

Supporting Information

Near-Infrared Photochemical Internalization: Design of a Distorted Zinc Phthalocyanine for Efficient Intracellular Delivery of Immunotoxins

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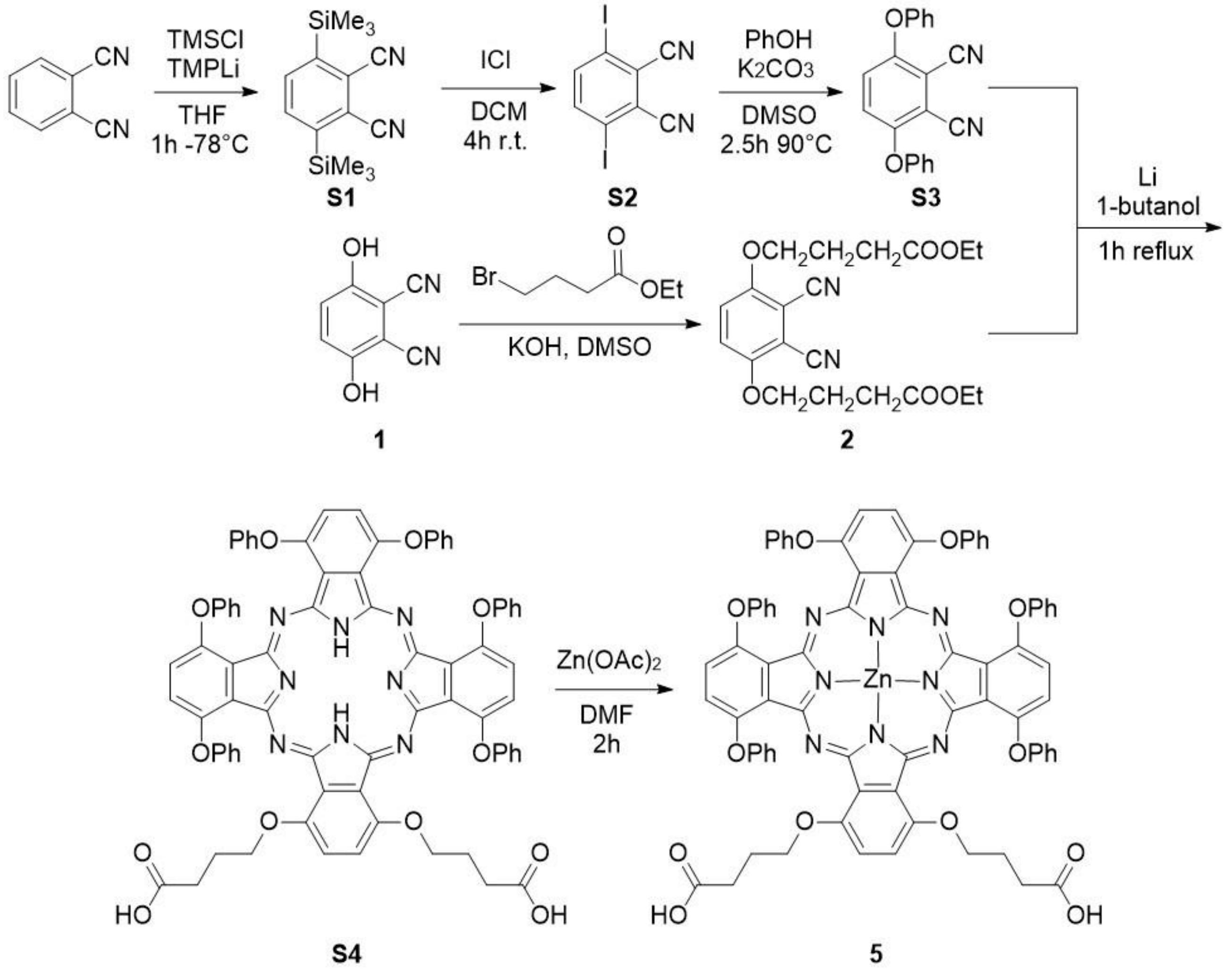
Synthesis of 5

Scheme S1. Synthesis of **5**.



S1. This compound was synthesized according to the previously reported paper (T. Furuyama *et al*., *J. Org. Chem*., **2019**, 84, 14306−14312). A solution of phthalonitirile (2.05 g, 16 mmol) and chlorotrimethylsilane (12.17 mL, 96 mmol) in tetrahydrofuran was added dropwise to a solution of freshly prepared 2,2,6,6-tetramethylpiperidine lithium (from 2,2,6,6-tetramethylpiperidine (6.8 mL, 40 mmol) and butyl lithium (1.6 M solution in hexane, 25 mL, 40 mmol) stirred for 30 min at 0 °C) at −78 °C, and then stirred at −78 °C for 1 h. After brought to room temperature, the light green solution was stirred another 1.5 h at room temperature. After addition of hydrochloric acid, the solution was extracted with ethyl acetate. The organic layer was washed with water and brine. After dried over with magnesium sulfate and concentrated, the precipitate was washed with hexane, then dried under reduced pressure to give **S1** (3.6 g, 83 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76 (s, 2H), 0.45 (s, 18H); ESI-MS [M+Na]$^+$ calcd. 295.1057, found 295.1042.

S2. This compound was synthesized according to the previously reported paper (T. Furuyama *et al*., *J.*

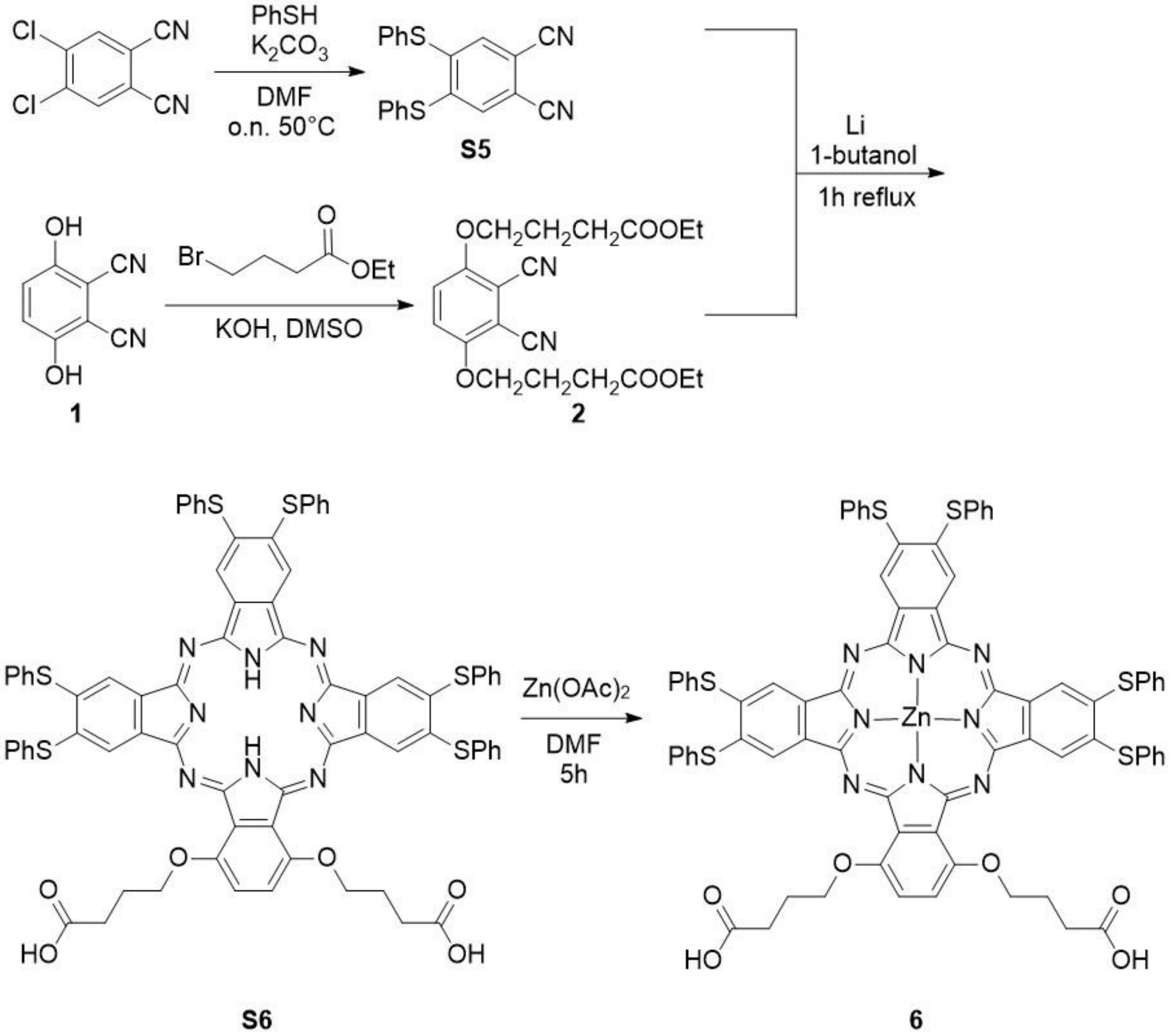
Org. Chem., **2019**, 84, 14306−14312). Iodine chloride (25 g, 154 mmol) was added to the solution of **S1** (3.6 g, 13.2 mmol) in dichloromethane (40 mL), and the solution was stirred at room temperature for 4 h. Aqueous sodium thiosulfate was added to the solution and the solution was extracted with dichloromethane. The organic layer was washed with water and then brine, and dried over magnesium sulfate. After filtration and concentration *in vacuo*, **S2** was obtained through recrystallization from acetone. (1.61 g, 33 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (s, 2H); ESI-MS $[M+Na]^+$ calcd. 402.8199, found 402.8165.

S3. This compound was synthesized according to the previously reported paper (T. Furuyama *et al.*, *J. Org. Chem.*, **2019**, 84, 14306−14312). **S2** (1.6 g, 4.2 mmol), potassium carbonate (6.7 g, 48 mmol), and phenol (4.2g, 45 mmol) were dissolved in dimethylsufoxide (100 mL) and stirred at 90 °C for 2.5 h. Water was added to the solution, then the solution was extracted with ethyl acetate. The organic layer was washed with aqueous sodium hydroxide, water and brine. The organic layer was dried over with sodium sulfate and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (chloroform) to give **S3** (156 mg, 12 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (t, J = 7.9, 4H), 7.25 (t, J = 7.3, 2H), 7.06 (d, J = 7.9, 4H), 7.01 (s, 2H); ESI-MS $[M+Na]^+$ calcd. 335.0791, found 335.0781.

5. **S3** (50.5 mg, 0.16 mmol) and **2** (20.9 mg, 0.053 mmol) were added to a solution of lithium (6 mg, 0.84 mmol) in 2 mL of *n*-butanol and heated under reflux for 1 h. After evaporation to reduce the volume to get crude **S4** (^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.64−6.76 (m, 36H), 6.68−6.64 (m, 2H), 3.48 (t, J = 6.4, 4H), 2.27 (t, J = 7.4, 4H), 1.74−1.68 (m, 4H)), zinc acetate (52.0 mg, 0.28 mmol) in *N*,*N*-dimethylformamide (2 mL) was added and the mixture was heated at 100 °C for 2 h. After the solvent was removed *in vacuo*, the mixture was purified by silica gel column chromatography (chloroform/methanol = 20:1, containing 1 % acetic acid) to get **5** (8.8 mg, 13 %) as a dark green powder. The product can be stored in a cool, dark place under nitrogen atmosphere for 3 months, although room light and air can induce decomposition of the product. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.15−6.57 (m, 38H), 3.52−3.16 (br, 4H), 2.35−1.43 (br, 8H); MALDI-TOF-MS (α-cyano-4-hydroxycinamic acid) $[M+H]^+$ calcd. 1333.3074, found 1333.3052.

Synthesis of 6

Scheme S2. Synthesis of **6**.

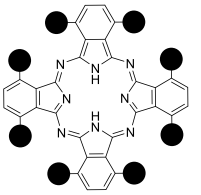
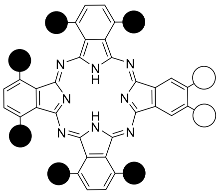
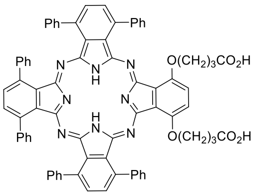
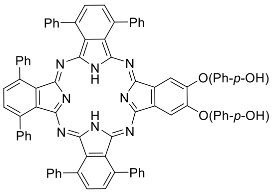


S5. This compound was synthesized according to the previously reported paper (R. Steffen *et al.*, *Chem. Eur. J.*, **2017**, 23, 13660−13668). Thiophenol (330.6 mg, 3 mmol), 4,5-dichlorophthalonitrile (195.0 mg, 1.0 mmol), and potassium carbonate (829.2 mg, 6 mmol) were dissolved in *N*,*N*-dimethylformamide (12 mL) and stirred at 50 °C overnight. Water was added to the solution, and the precipitate was collected and washed with water to give **S5** (337 mg, 99 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59−7.49 (m, 10H), 6.98 (s, 2H); ESI-MS $[M+Na]^+$ calcd. 367.0334, found 367.0350.

6. S5 (237.0 mg, 0.69 mmol) and **2** (98.0 mg, 0.25 mmol) were added to a solution of lithium (60.1 mg, 8.66 mmol) in 4.5 mL of *n*-butanol and heated under reflux for 1 h. After excess lithium was removed by celite filtration, followed by evaporation to reduce the volume, the mixture was purified by silica gel column chromatography (chloroform: methanol = 1:0→10:1, containing 1 % acetic acid) to get crude **S6**. Zinc acetate

(56.6 mg, 0.31 mmol) in *N*,*N*-dimethylformamide (5 mL) was added to the black crude product and heated at 100 °C for 5 h. After the solvent was removed *in vacuo*, the residue was purified by silica gel column chromatography (chloroform/methanol = 20:1→10:1, containing 1 % acetic acid) to give **6**. (39.8 mg, 12%). The product can be stored in a cool, dark place under nitrogen atmosphere for 3 months, although room light and air can induce decomposition of the product. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.64−7.73 (br, 2H), 7.33−7.61 (m, 32H), 7.01−7.14 (m, 4H), 3.61−3.44 (m, 4H), 2.42−2.31 (br, 4H), 2.00−1.85 (br, 4H); MALDI-TOF-MS (α-cyano-4-hydroxycinamic acid) $[M+H]^+$ calcd. 1429.1704, found 1429.1671.

Table S1. Effect of substitution at the α-position on HOMO-LUMO gap reduction.[a]

	Full α-position substitution	Partially containing β-position substitution
		
		
LUMO / eV	-2.994	-3.094
HOMO / eV	-4.721	-5.088
Gap / eV	1.727	1.994

[a] B3LYP/3-21G

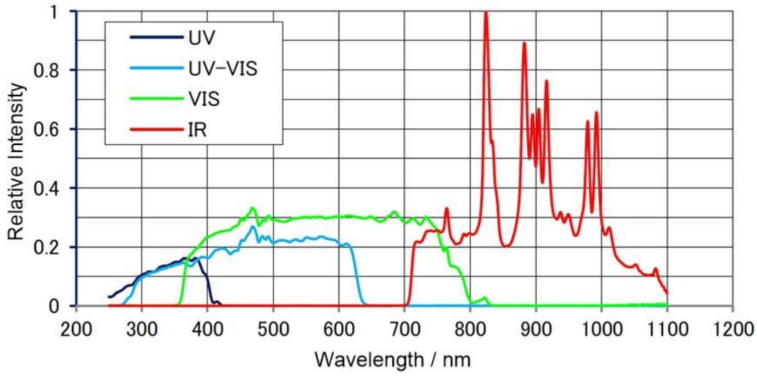
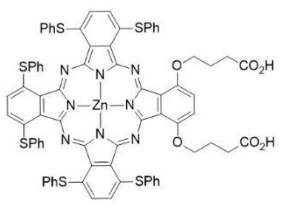
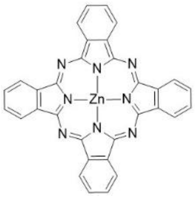
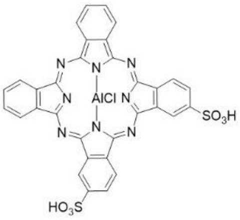
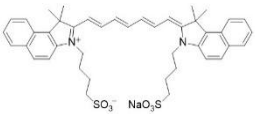
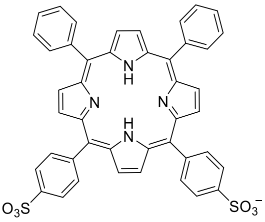
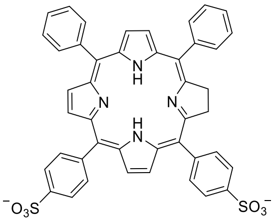


Figure S1. NIR light source. The IR range (red) in the figure was used for irradiation. This figure was prepared by translating the language of the figure drawn by the light source developer (Asahi Bunko HP, https://www.asahi-spectra.co.jp/kiki/kougen/max-303/max-303.htm).

Table S2. Absorption wavelength and singlet oxygen quantum yields of conventional photosensitizers for photodynamic therapy.

	 Zn6PTPc	 ZnPc	 AlPcS2α	 Indocyanine Green
λ_{ab} / nm	775	672	677	795
Φ_Δ	0.84	0.67[a]	0.17[b]	0.12[c]

[a] Ogunsipe, A.; Maree, D.; Nyokong, T. Solvent effects on the photochemical and fluorescence properties of zinc phthalocyanine derivatives. *J. Mol. Struct*. **2003**, 650, 131–140. [b] Petrášek, Z.; Phillips, D. A time-resolved study of concentration quenching of disulfonated aluminium phthalocyanine fluorescence. *Photochem. Photobiol. Sci*. **2003**, 2, 236–244. [c] Cardillo, J. A.; Jorge, R.; Costa, R. A.; Nunes, S. M. T.; Lavinsky, D.; Kuppermann, B. D.; Tedesco, A. C.; Farah M. E. Experimental selective choriocapillaris photothrombosis using a modified indocyanine green formulation. *Br. J. Ophthalmol.* **2008**, *92*, 276–280.

	 TPPS$_{2a}$	 TPCS$_{2a}$
λ_{ab} / nm	500–650 [d,e] (four weak Q-bands)	500–650 [d,e] (four weak Q-bands)
Φ_Δ	unknown[e]	unknown

[d] Jerjes, W.; Theodossiou, T. A.; Hirschberg, H.; Høgset, A.; Weyergang, A.; Selbo, P. K.; Hamdoon, Z.; Hopper, C.; Berg, K. Photochemical Internalization for Intracellular Drug Delivery. From Basic Mechanisms to Clinical Research. *J. Clin. Med*. **2020**, 9, 528. [e] Lilletvedt, M.; Tønnesen, H. H.; Høgset, A.; Nardo, L.; Kristensen, S. Physicochemical characterization of the photosensitizers TPCS$_{2a}$ and TPPS$_{2a}$ 1. Spectroscopic evaluation of drug - Solvent interactions. *Pharmazie* **2010**, 65, 588–595.

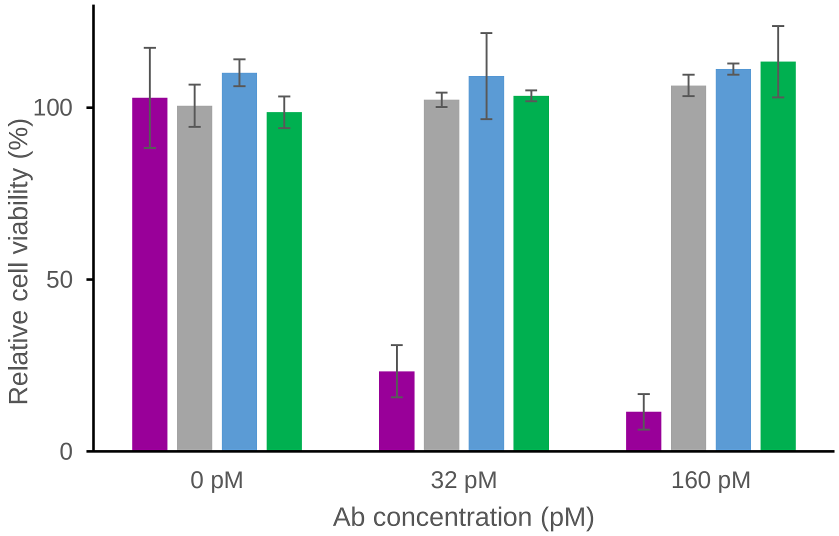


Figure S2. Cell viability of A431 cells after addition of saporin-modified Panitumumab and phosensitizers followed by NIR irradiation. Purple, Zn6PTPc; gray, ZnPc; light blue, AlPcS2α; green, Indocyanine Green. The data are displayed in the format of standard error of the mean (n = 3).

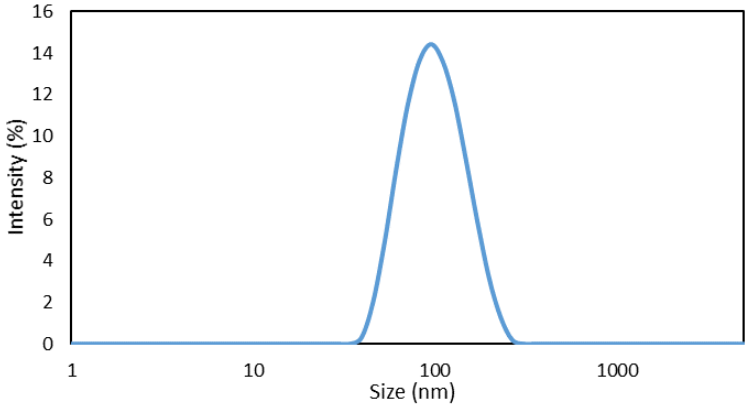
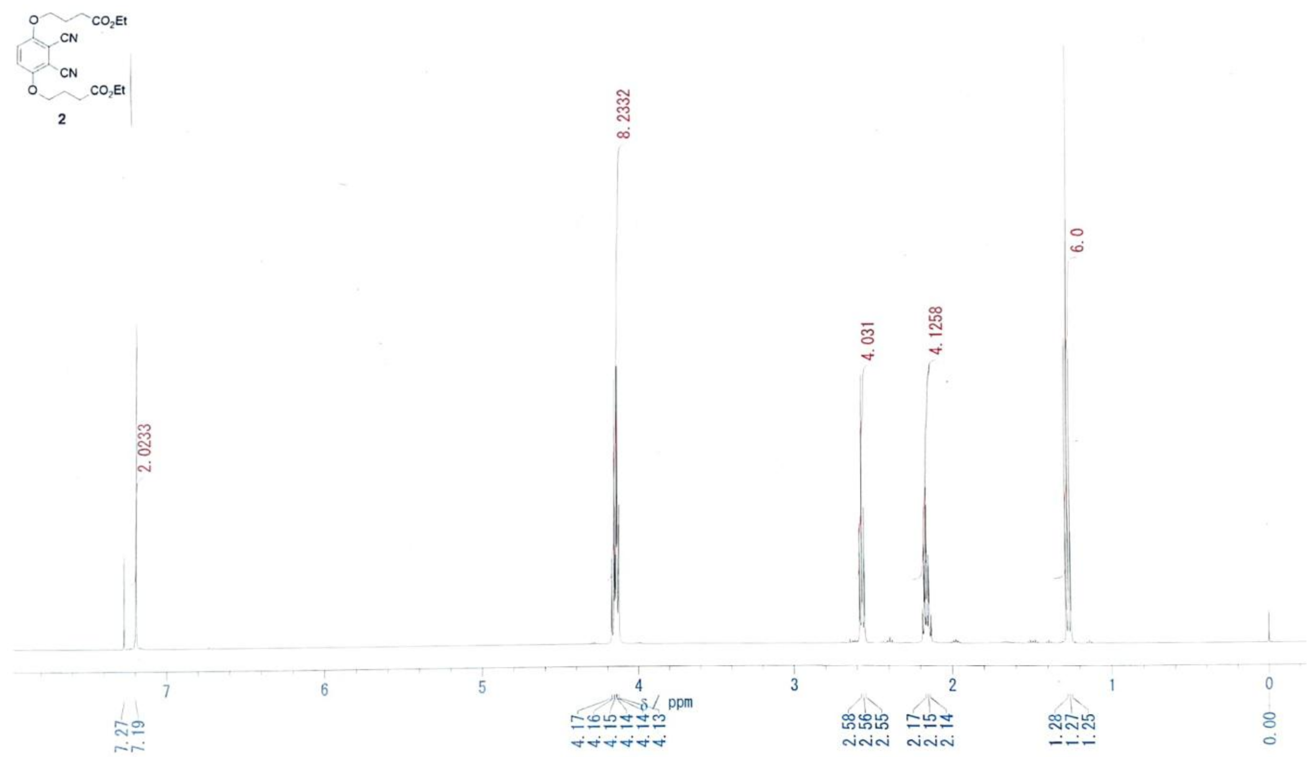


Figure S3. Aggregation size of Zn6PTPc (1 μM) in PBS.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CN
CO2Et
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8.2332
4.031
4.1258
6.0
7
6
5
4
3
2
1
0
δ / ppm
7.27
7.19
4.17
4.16
4.15
4.14
4.14
4.13
2.58
2.56
2.55
2.17
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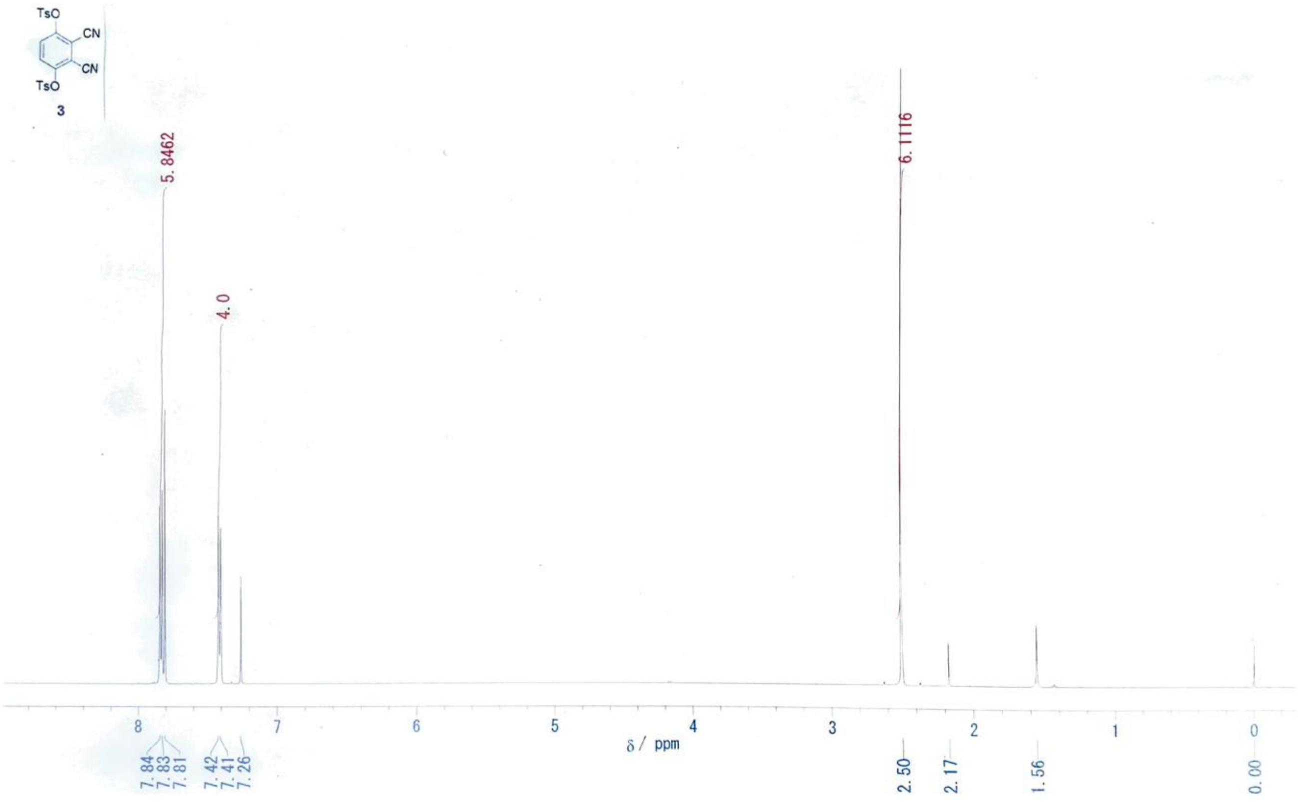
1.28
1.27
1.25
0.00


TsO
CN
CN
TsO
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4.0
6.1116
8
7
6
5
4
3
2
1
0
δ / ppm
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7.83
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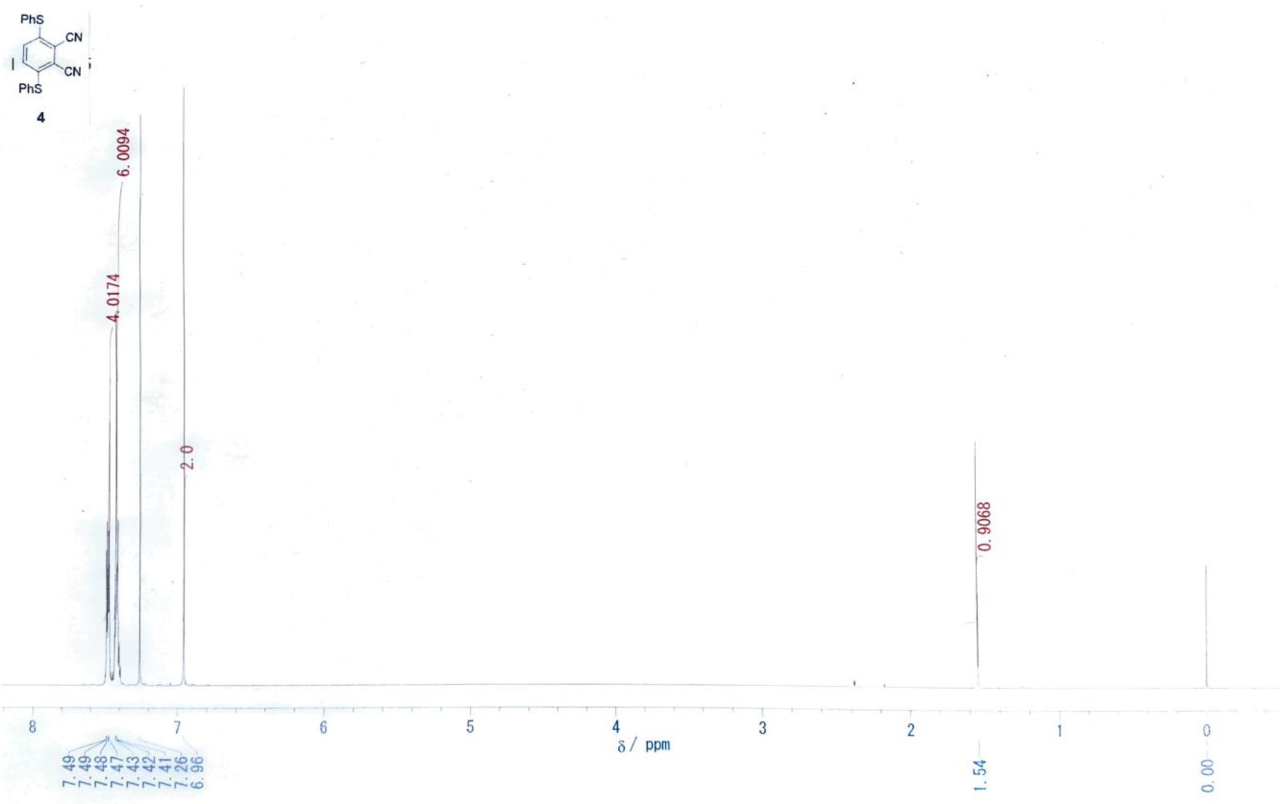
7.42
7.41
7.26
2.50
2.17
1.56
0.00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CN
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PhS
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1
0
δ / ppm
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7.49
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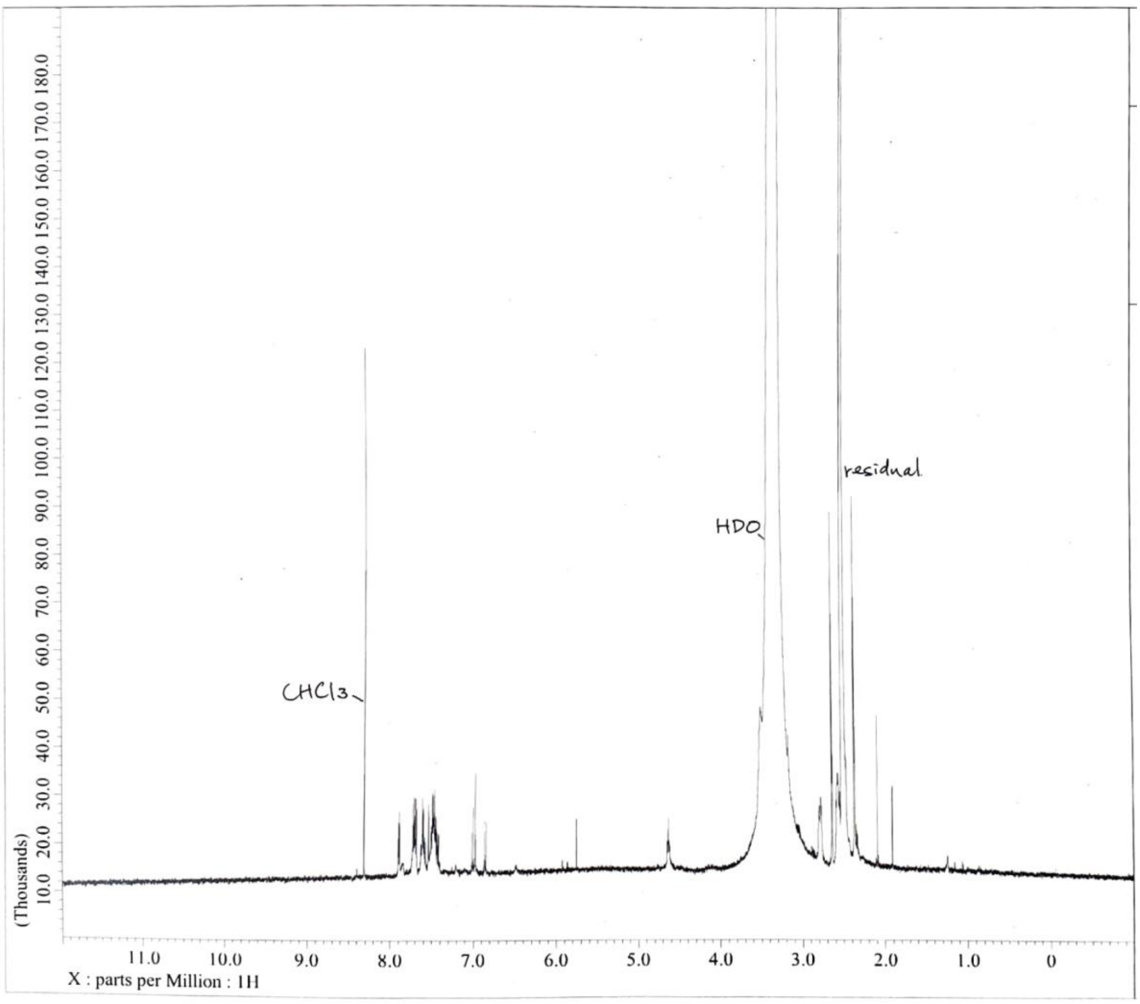
7.26
6.96
1.54
0.00

H_26PTPc


(Thousands)
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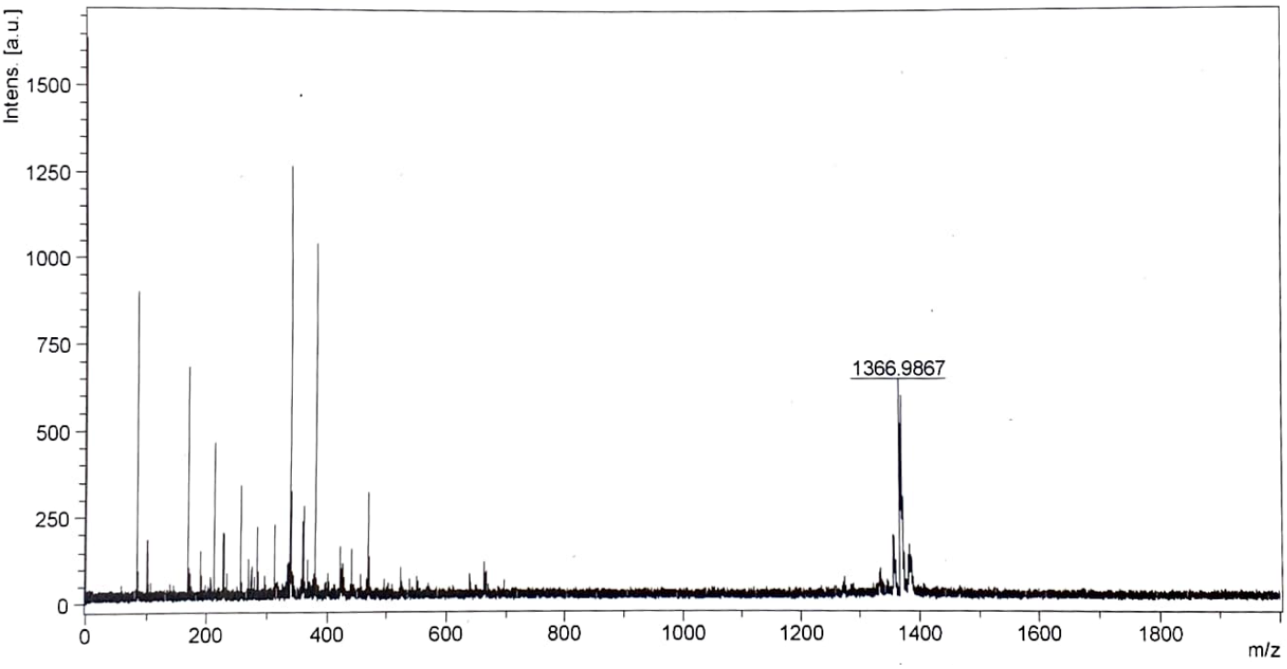
120.0
130.0
140.0
150.0
160.0
170.0
180.0
CHCl3
HDO
residual
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H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800
1000
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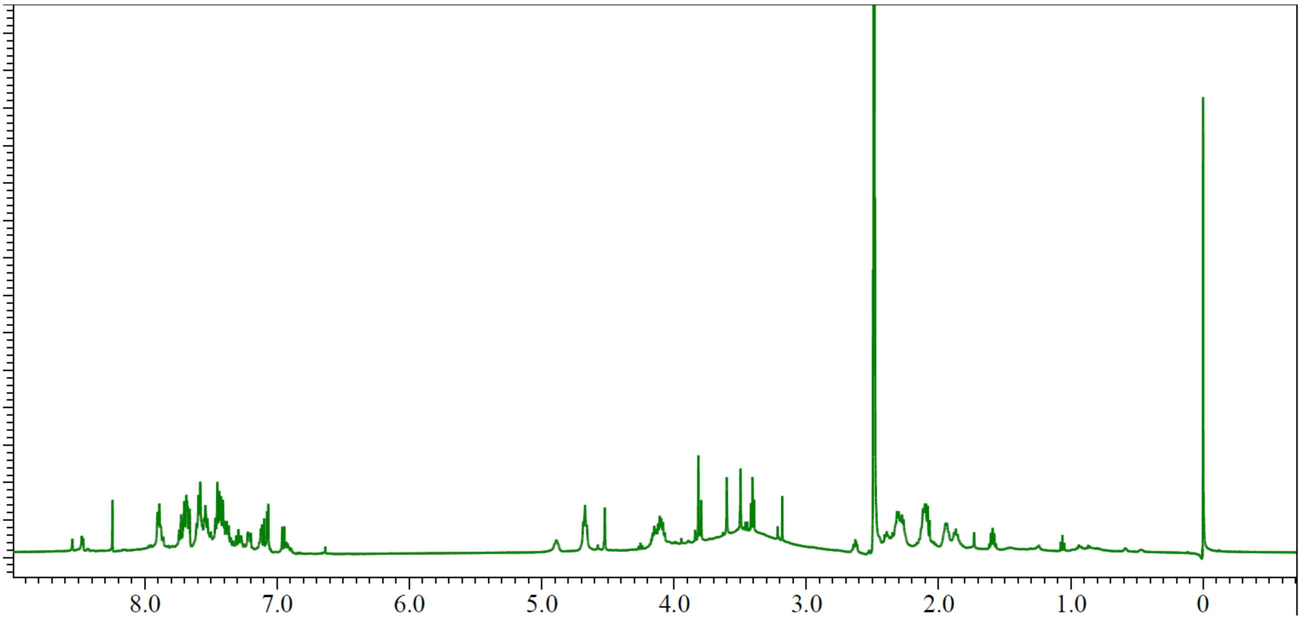
1600
1800
m/z

Zn6PTPc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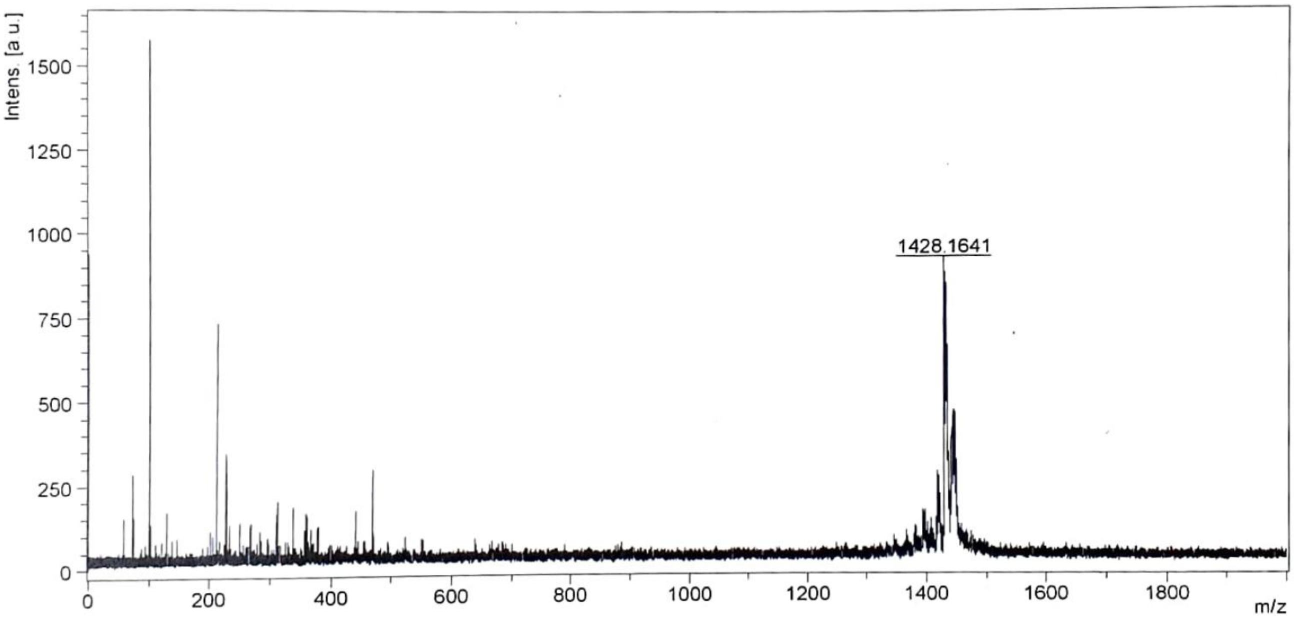
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1600
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m/z