Supporting Information

Monovalent and Clickable, Uncharged Water-Soluble Perylenediimide-Cored Dendrimers for Target-Specific, Fluorescent Biolabeling

Si Kyung Yang,[†] Xinghua Shi,[‡] Seongjin Park,[‡] Sultan Doganay,[‡] Taekjip Ha,[‡] and Steven C. Zimmerman*[,†]

Departments of Chemistry and Physics, Institute for Genomic Biology, and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

General Methods. All reagents were purchased from Acros Organics, Alfa Aesar, AK Scientific, or Sigma-Aldrich, and used without further purification unless otherwise noted. NMR spectra were recorded using a Varian Unity 400 or 500 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the corresponding residual nuclei in deuterated solvents. Mass spectral analyses were provided by the Mass Spectrometry Laboratory, School of Chemical Science, University of Illinois, using ESI on a Waters Micromass Q-Tof spectrometer, FD on a Waters 70-VSE spectrometer, or MALDI-TOF on an Applied Biosystems Voyager-DE STR spectrometer. GPC analyses were carried out using a Viscotek Model 300 TDA with DMF as the eluent and a flow rate of 1 mL/min on a series of three Viscogel columns. All GPCs were calibrated using poly(styrene) standards and carried out at 25 °C. M_w, M_n, and PDI represent the weight-average molecular weight, number-average molecular weight, and polydispersity index, respectively. UV-Vis absorbance spectra were recorded on a Shimadzu UV-2501PC spectrophotometer. Fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax-3 spectrophotometer and quantum yields were determined using cresyl violet (Φ = 0.54 in MeOH) as a reference. IR spectra were recorded using a Perkin-Elmer Spectrum BX FT-IR spectrophotometer. Preparative SEC was carried out on Bio-Beads S-X1 Beads gel permeation 200-400 mesh (Bio-Rad Laboratories, exclusion limits 600-14000). Dialysis was performed using dialysis tubing (Sigma-Aldrich MWCO 1200; Spectra-Por MWCO 3500 or 25000) against water at 25 °C. PDIs **8a**[1] and **9**,[2] PGD **20**,[3] and alkyne-functionalized biotin **25**[4] and maleimide **26**[5] were synthesized according to the previously published procedures.

Single-Molecule Imaging. PGD-PDI **4** or PDI **8** was immobilized on a PEG-coated surface through biotin-neutravidin binding by using a solution of the 100 pM compound as described before.[6] Prior to imaging, the sample was rinsed extensively with an imaging buffer at pH 8.0 referred to as T50, containing 10 mM Tris and 50 mM NaCl.

Single-molecule imaging was performed with prism-type total internal reflection microscopy as described before.[6] In brief, a 532 nm DPSS laser (Spectra Physics) was used to excite immobilized **4** or **8** on the surface through total internal reflection. Fluorescence was collected using a 60×, 1.2 NA, water immersion objective lens (Olympus America) and detected up to 60 s at 100 ms time resolution with an EMCCD camera (Andor). The optics for a Cy3/Cy5 FRET experiment[7] were used after removing the 630dcxr dichroic beamsplitter (Chroma). Using a custom program written in IDL,

individual molecules were identified from the raw data and their intensity time traces were thus extracted. The total intensity of all molecules identified within an imaging area was then calculated using a custom MATLAB program. The decay in average intensity of all the molecules as a function of time was fit to a single exponential, with the fitted lifetime defined as the corresponding single-molecule photobleaching time. Such a measurement was repeated six times in different imaging areas for each of the samples examined, which formed the basis for a statistical analysis.

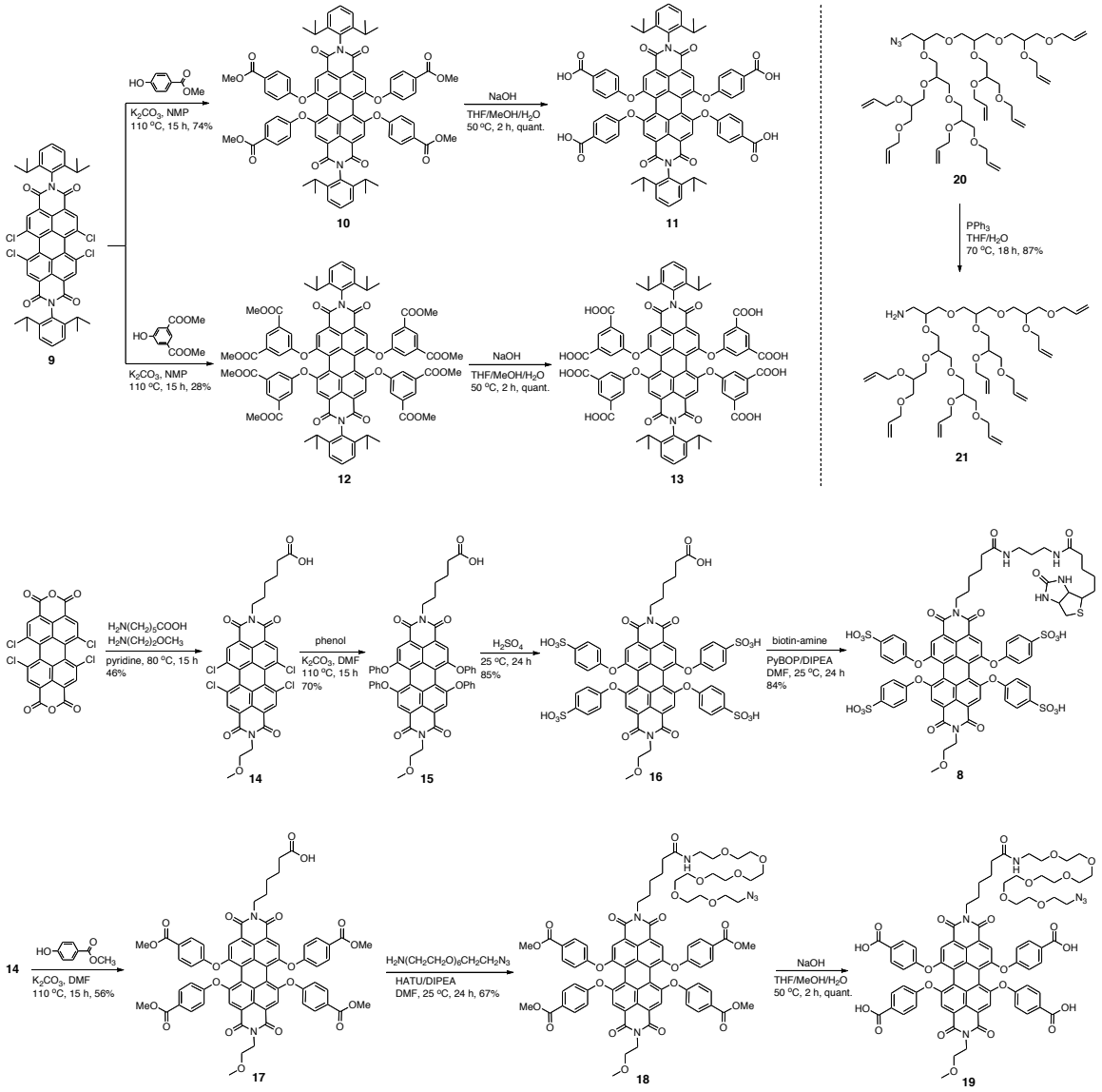
Live Bacteria Imaging. A bacteria strain named pLO16,[8] which produces biotinylated λ-receptors *in vivo*, was used in this study. Cells were spread on a LB/chloramphenicol agar plate and incubated at 37 °C overnight. An isolated colony was picked from this plate to inoculate a LB medium containing 25 μg/mL chloramphenicol and cultured at 37 °C with vigorous shaking overnight. This culture was diluted into a fresh LB/chloramphenicol medium in a ratio of 1:100 and cultured at 37 °C with vigorous shaking until OD_{600} reached 0.3. Then the expression of λ-receptor gene was induced at 37 °C by adding IPTG to a final concentration of 0.1 or 1 mM. After 45 min of incubation with rigorous shaking, cells were harvested at 4 °C by 3 min of centrifugation at 3,000 × rpm, resuspended in pre-chilled PBS buffer, and pelleted again by centrifugation. The cells collected were splitted into two aliquots each and a solution of 20 μg/mL streptavidin in PBS or the buffer alone was then added. After 30 min of incubation at 4 °C, cells were washed three times by resuspension in PBS buffer and centrifugation, and then labeled with 1 μM biotinylated **4** and **8** for 30 min at 4 °C. After this incubation, cells were washed three times as described above and then plated on a Lab-Tek II #1.5 coverglass (Nunc) that was pretreated with 0.1% w/v poly-L-lysine for 45 min.

Live bacteria imaging was carried out using an inverted epifluorescence microscope (Nikon) with a 100×, 1.4 NA, oil immersion objective lens and a TxRed filter cube for fluorescence. Images were taken with an exposure time of 100 or 1000 ms through the MetaMorph software (Molecular Devices) using a cooled EMCCD camera (Photometrics). A series of six images were also obtained for cells stained with each of the two biotinylated compounds mentioned above, with a step size of 200 nm along the z axis. Using the ImageJ software (National Institutes of Health), fluorescence images were analyzed and plotted in a green color on an intensity scale that is identical between sets of experiments for comparison, unless noted otherwise. From the images acquired at different z-positions, a movie was created with a frame rate of 3.5 fps using the same software.

Imaging of Live Mammalian Cells. HeLa cells plated on a Lab-Tek II #1.5 coverglass (Nunc) were transfected with the pACP-GPI plasmid (New England Biolabs) by following a standard protocol using Lipofectamine 2000 (Invitrogen). After five-hour incubation at 37 °C in a CO_2 incubator, cell culture media were changed. At 18 hours after transfection, a mixture of 5 μM **7**, 10 mM $MgCl_2$, and 1 μM SFP synthase (New England Biolabs) was prepared in complete medium and replaced the medium on the cells expressing the GPI-ACP fusion protein. After incubation for one hour at 37 °C in a CO_2 incubator, cells were washed three times with serum-containing medium.

Imaging of live HeLa cells was carried out using a LSM710NLO confocal fluorescence microscope (Zeiss) with a 63×, 1.4 NA, oil immersion objective lens and a 561 nm laser for excitation. Images were taken with fluorescence integrated over a spectral window from 580 to 700 nm.

Scheme S1. Synthesis of PDIs and PGDs



PDI 10. To a solution of PDI **9** (1.0 g, 1.2 mmol) and methyl 4-hydroxybenzoate (1.1 g, 7.1 mmol) in anhydrous *N*-methyl-2-pyrrolidone (NMP, 16 mL) was added K_2CO_3 (0.98 g, 7.1 mmol). After the mixture was stirred at 110 °C for 15 h, dilute aqueous hydrochloric acid (80 mL) was added at 0 °C and the precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel using dichloromethane/ethyl acetate (25:1) as eluent to yield PDI **10** (1.1 g, 74%). ^{1}H NMR ($CDCl_3$): δ 8.28 (s, 4H), 7.96 (d, J = 9.2 Hz, 8H), 7.45 (t, J = 7.9 Hz, 2H), 7.29 (d, J = 7.9

Hz, 4H), 6.96 (d, *J* = 9.2 Hz, 8H), 3.92 (s, 12H), 2.68 (sept, *J* = 6.8 Hz, 4H), 1.12 (d, *J* = 6.8 Hz, 24H). ^{13}C NMR ($CDCl_3$): δ 166.1, 162.7, 159.2, 154.9, 145.5, 133.0, 131.9, 130.2, 129.7, 126.4, 124.0, 123.5, 121.5, 121.1, 119.0, 52.2, 29.1, 24.0. MS-FD (*m*/*z*): $[M]^+$ calcd for $C_{80}H_{66}N_2O_{16}$, 1310.4; found, 1311.0.

PDI 12. To a solution of PDI **9** (0.52 g, 0.61 mmol) and dimethyl 5-hydroxyisophthalate (0.77 g, 3.7 mmol) in anhydrous NMP (10 mL) was added K_2CO_3 (0.51 g, 3.7 mmol). After the mixture was stirred at 110 °C for 15 h, dilute aqueous hydrochloric acid (50 mL) was added at 0 °C and the precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel using dichloromethane/ethyl acetate (15:1) as eluent to yield PDI **12** (0.26 g, 28%). ^{1}H NMR ($CDCl_3$): δ 8.36 (s, 4H), 8.26 (s, 4H), 7.74 (s, 8H), 7.44 (t, *J* = 7.8 Hz, 4H), 7.28 (d, *J* = 7.8 Hz, 8H), 3.87 (s, 24H), 2.68 (sept, *J* = 6.8 Hz, 4H), 1.13 (d, *J* = 6.8 Hz, 24H). ^{13}C NMR ($CDCl_3$): δ 164.9, 162.7, 155.6, 154.8, 145.5, 133.3, 132.5, 130.1, 129.6, 126.5, 124.4, 124.0, 123.7, 121.4, 121.4, 121.2, 52.5, 29.1, 24.0. MS-FD (*m*/*z*): $[M]^+$ calcd for $C_{91}H_{83}N_2O_{24}$, 1542.5; found, 1542.3.

PDI 14. To a solution of 6-aminohexanoic acid (0.14 g, 1.0 mmol) and 2-methoxyethylamine (77 mg, 1.0 mmol) in pyridine (5 mL) was added 1,6,7,12-tetrachloroperylene tetracarboxylic acid dianhydride (0.50 g, 0.94 mmol). After the mixture was stirred vigorously at 80 °C for 15 h, the solvent was removed under reduced pressure. The crude product was purified by column chromatograph on silica gel using dichloromethane/ethyl acetate (5:2) as eluent to obtain PDI **14** (0.30 g, 46%). ^{1}H NMR ($CDCl_3$): δ 8.69 (s, 2H), 8.68 (s, 2H), 4.48 (t, *J* = 7.5 Hz, 2H), 4.22 (t, *J* = 7.5 Hz, 2H), 3.77 (t, *J* = 5.5 Hz, 2H), 3.42 (s, 3H), 2.40 (t, *J* = 7.3 Hz, 2H), 1.76 (m, 4H), 1.52 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 178.1, 162.4, 162.2, 135.4, 135.4, 133.1, 133.0, 131.4, 128.6, 128.6, 123.3, 123.3, 123.2, 123.1, 69.4, 58.8, 40.6, 39.7, 33.6, 27.7, 26.4, 24.3. MS-FD (*m*/*z*): $[M]^+$ calcd for $C_{33}H_{22}Cl_4N_2O_7$, 700.0; found, 699.9.

PDI 15. To a solution of PDI **14** (0.25 g, 0.36 mmol) and phenol (0.27 g, 2.8 mmol) in anhydrous DMF (10 mL) was added K_2CO_3 (0.40 g, 2.9 mmol). After the mixture was stirred at 110 °C for 15 h, dilute aqueous hydrochloric acid (50 mL) was added at 0 °C and the precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (30:1) as eluent to yield PDI **15** (0.23 g, 70%). ^{1}H NMR ($CDCl_3$): δ 8.20 (s, 2H), 8.18 (s, 2H), 7.27 (br t, 8H), 7.15 (t, *J* = 7.0 Hz, 4H), 6.96 (d, *J* = 7.2 Hz, 8H), 4.37 (br t, 2H), 4.09 (br t, 2H), 3.66 (br t, 2H), 3.34 (s, 3H), 2.32 (t, *J* = 7.2 Hz, 2H), 1.68 (m, 4H), 1.43 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 163.4, 163.2, 155.9, 155.9, 155.3, 132.8, 130.0, 124.6, 122.6, 122.6, 120.5, 120.5, 120.1, 120.0, 119.7, 119.6, 69.5, 58.8, 40.3, 39.4, 33.3, 27.6, 26.4, 24.3. MALDI-TOF (*m*/*z*): $[M]^+$ calcd for $C_{57}H_{42}N_2O_{11}$, 930.3; found, 930.8.

PDI 16. PDI **15** (46 mg, 49 μmol) was dissolved in concentrated sulfuric acid (1 mL) and stirred at 25 °C for 24 h. Water (20 mL) was added and the solution was dialyzed against water at 25 °C for 2 d. The solvent was removed under reduced pressure to give PDI **16** (53 mg, 85%). ^{1}H NMR (CD_3OD): δ 8.12 (s, 4H), 7.82 (d, *J* = 8.4 Hz, 8H), 7.05 (d, *J* = 8.4 Hz, 8H), 4.27 (br t, 2H), 4.08 (br t, 2H), 3.77 (br t, 2H), 3.62 (s, 3H), 2.32 (t, *J*

= 7.6 Hz, 2H), 1.67 (m, 4H), 1.39 (m, 2H). ^{13}C NMR (CD_3OD): δ 164.1, 164.0, 158.9, 158.6, 156.2, 142.2, 133.6, 129.3, 124.5, 121.9, 121.8, 120.3, 60.0, 56.2, 43.4, 41.2, 34.6, 28.4, 27.4, 25.6. MALDI-TOF (*m*/*z*): $[M]^+$ calcd for $C_{57}H_{42}N_2O_{23}S_4$, 1250.1; found, 1251.9 $[M+H]^+$.

PDI 8. PDI **16** (9.0 mg, 7.2 μmol), *N*-(+)-biotinyl-3-aminopropylammonium trifluoroacetate (15 mg, 36 μmol) and PyBOP (23 mg, 44 μmol) were dissolved in anhydrous DMF (3 mL) under a nitrogen atmosphere. DIPEA (30 μL) was added and the mixture was stirred at 25 °C for 24 h. After the solvent was removed under reduced pressure, water was added and the precipitate was filtered off. The filtrate was dialyzed against water at 25 °C for 2 d. The solvent was removed under reduced pressure to give PDI **8** (9.2 mg, 84%). ^{1}H NMR (DMSO-d_6): δ 7.86 (s, 4H), 7.72 (br, 1H), 7.63 (m, 8H), 6.93 (m, 8H), 6.53 (br, 2H), 6.39 (br, 1H), 4.28 (br t, 2H), 4.10 (m, 2H), 3.93 (br t, 2H), 3.55 (br t, 2H), 3.28 (s, 3H), 3.08 (br t, 2H), 2.94 (br t, 2H), 2.75 (m, 1H), 2.66 (br, 1H), 2.30 (br, 1H), 2.12-1.90 (m, 4H), 1.72-1.14 (m, 14H). MALDI-TOF (*m*/*z*): $[M]^+$ calcd for $C_{70}H_{64}N_6O_{24}S_5$, 1532.3; found, 1533.7 $[M+H]^+$. UV-Vis (H_2O): λ_{max} (ε) = 561 nm (27 850 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.39).

PDI 17. To a solution of PDI **14** (0.29 g, 0.41 mmol) and methyl 4-hydroxybenzoate (0.38 g, 2.5 mmol) in anhydrous DMF (15 mL) was added K_2CO_3 (0.34 g, 2.5 mmol). After the mixture was stirred at 110 °C for 15 h, dilute aqueous hydrochloric acid (80 mL) was added at 0 °C and the precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (30:1) as eluent to yield PDI **17** (0.27 g, 56%). ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 2H), 8.20 (s, 2H), 7.94 (d, *J* = 8.6 Hz, 8H), 6.93 (d, *J* = 8.6 Hz, 8H), 4.38 (t, *J* = 6.3 Hz, 2H), 4.09 (t, *J* = 6.3 Hz, 2H), 3.92 (s, 12H), 3.67 (t, *J* = 5.8 Hz, 2H), 3.33 (s, 3H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.68 (m, 4H), 1.42 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 166.1, 162.9, 162.7, 159.2, 154.8, 132.6, 131.9, 126.4, 123.2, 121.3, 121.2, 120.8, 120.8, 120.5, 120.4, 119.1, 69.4, 58.7, 52.2, 40.4, 39.5, 33.2, 27.6, 26.4, 24.3. MS-FD (*m*/*z*): $[M]^+$ calcd for $C_{65}H_{50}N_2O_{19}$, 1162.3; found, 1162.7.

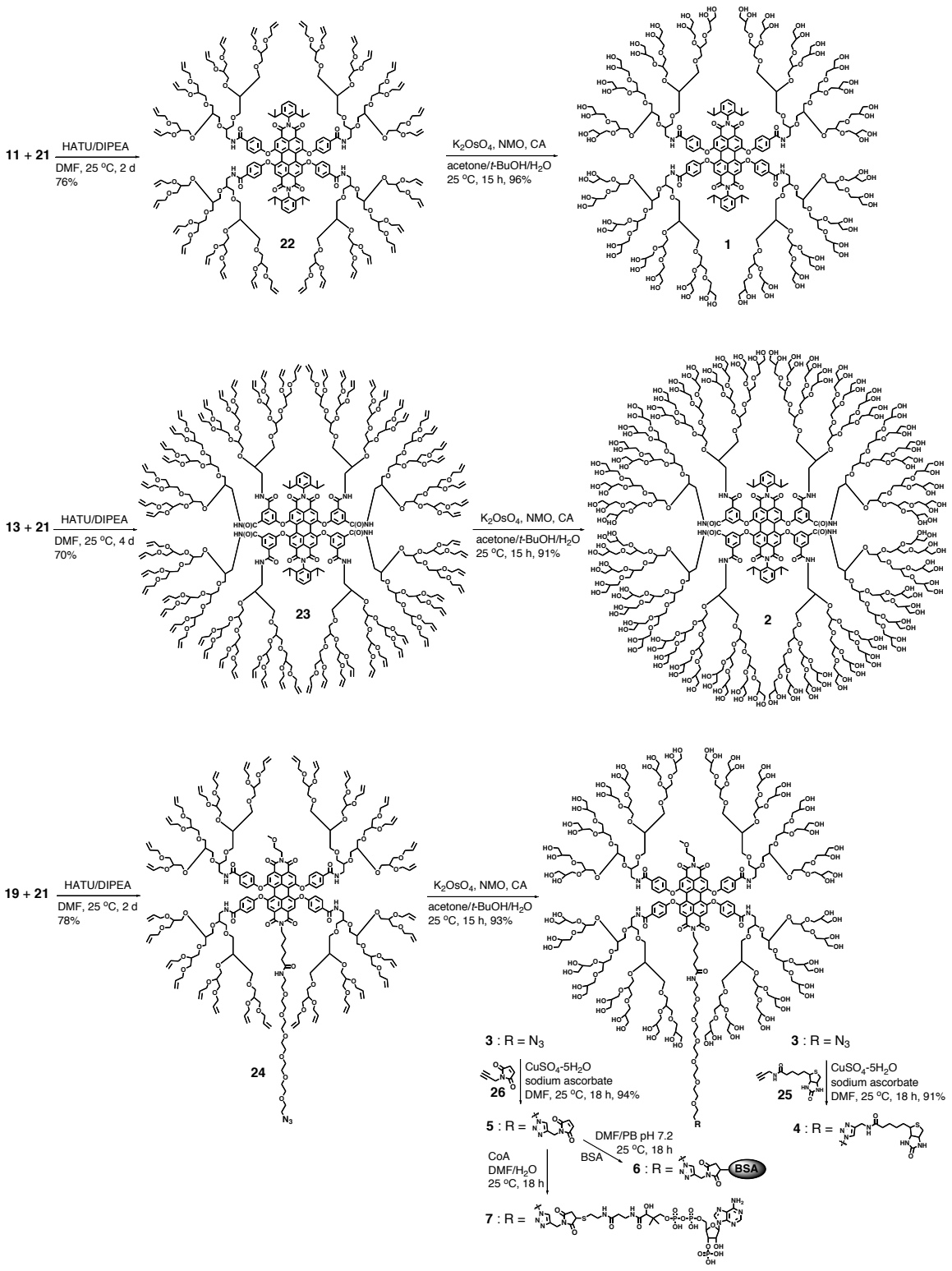
PDI 18. PDI **17** (58 mg, 50 μmol), *O*-(2-aminoethyl)-*O'*-(2-azidoethyl)pentaethylene glycol (35 mg, 0.10 mmol) and HATU (38 mg, 0.10 mmol) were dissolved in anhydrous DMF (3 mL) under a nitrogen atmosphere. DIPEA (35 μL) was added and the mixture was stirred at 25 °C for 24 h. After the solvent was removed under reduced pressure, water (50 mL) was added and the precipitate was collected by filtration. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (20:1) as eluent to yield PDI **18** (50 mg, 67%). ^{1}H NMR ($CDCl_3$): δ 8.21 (s, 2H), 8.20 (s, 2H), 7.95 (d, *J* = 6.8 Hz, 8H), 6.94 (m, 8H), 6.08 (br s, 1H), 4.38 (t, *J* = 6.5 Hz, 2H), 4.10 (t, *J* = 6.5 Hz, 2H), 3.94 (s, 12H), 3.64 (m, 24H), 3.52 (t, *J* = 5.0 Hz, 2H), 3.40 (m, 4H), 3.34 (s, 3H), 2.17 (t, *J* = 7.6 Hz, 2H), 1.68 (m, 4H), 1.41 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.8, 166.1, 166.1, 162.9, 162.7, 159.2, 154.8, 132.6, 131.9, 126.3, 123.3, 123.2, 121.3, 121.2, 120.9, 120.8, 120.5, 120.4, 119.1, 119.0, 70.7, 70.6, 70.6, 70.5, 70.5, 70.5, 70.2, 70.0, 69.9, 69.4, 58.7, 52.2, 50.6, 40.5, 39.5, 39.1,

36.3, 27.8, 26.7, 25.3. IR: 2103 cm^{-1} (N_3). MALDI-TOF (*m*/*z*): $[M]^+$ calcd for $C_{79}H_{78}N_6O_{24}$, 1494.5; found, 1518.1 $[M+Na]^+$.

General Procedure for Hydrolysis. The desired amount of PDI **10**, **12**, or **18** was dissolved in MeOH/THF (1:1). A solution of NaOH (10 equiv. per methyl ester) in water was added and the mixture was stirred at 50 °C for 2 h. The solution was concentrated under reduced pressure and acidified with dilute aqueous hydrochloric acid to form a precipitate. The precipitated PDI **11**, **13**, or **19** was isolated by filtration (for PDIs **11** and **13**) or centrifugation (for PDI **19**) and used without further purification.

PGD 21. PGD **20** (1.2 g, 1.3 mmol) and PPh_3 (0.86 g, 3.3 mmol) were dissolved in THF (20 mL) and stirred at 60 °C for 15 h. Water (1 mL) was added and the mixture was stirred at 60 °C for an additional 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel using dichloromethane/methanol (15:1) as eluent to yield PGD **21** (0.98 g, 87%). ^{1}H NMR ($CDCl_3$): δ 5.91 (m, 8H), 5.28 (m, 8H), 5.17 (m, 8H), 4.14 (m, 8H), 4.00 (m, 8H), 3.80-3.42 (m, 35H). ^{13}C NMR ($CDCl_3$): δ 135.4, 135.3, 135.0, 134.9, 117.1, 117.0, 116.9, 78.7, 77.5, 77.2, 72.5, 72.4, 71.8, 71.7, 71.5, 71.4, 71.3, 70.6, 70.5, 70.4, 70.3, 70.1. MS-ESI (*m*/*z*): $[M]^+$ calcd for $C_{45}H_{77}NO_{14}$, 855.5; found, 856.5 $[M+H]^+$.

Scheme S2. Synthesis of PGD-PDIs



General Procedure for Amidation. The desired amount of PDI **11, 13,** or **19**, PGD **21** (2 equiv. per acid) and HATU (4 equiv. per acid) were dissolved in anhydrous DMF under a nitrogen atmosphere. DIPEA (8 equiv. per acid) was added and the mixture was stirred at 25 °C for 2-4 days. After the solvent was removed under reduced pressure, water was added and the mixture was extracted with dichloromethane. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/methanol (30:1) as eluent to obtain PGD-PDI **22**, **23**, or **24** that was further purified by SEC using toluene as eluent.

PGD-PDI 22. PGD-PDI **22** (0.28 g, 76%) was obtained from PDI **11** (0.10 g, 80 μmol). ^{1}H NMR ($CDCl_3$): δ 8.24 (s, 4H), 7.79 (br, 8H), 7.44 (t, J = 8.2 Hz, 2H), 7.27 (br, 4H), 7.15-6.85 (br, 12H), 5.84 (m, 32H), 5.36-4.98 (m, 64 H), 4.09 (m, 32 H), 3.95 (m, 32 H), 3.84-3.23 (br, 140 H), 2.65 (br, 4H), 1.09 (br, 24H). MALDI (*m/z*): $[M]^+$ calcd for $C_{256}H_{358}N_6O_{68}$, 4604.5; found, 4630.4 $[M+Na]^+$, 4646.4 $[M+K]^+$. GPC: M_w = 6.1 kDa, M_n = 5.9 kDa, and PDI = 1.05.

PGD-PDI 23. PGD-PDI **23** (60 mg, 70%) was obtained from PDI **13** (15 mg, 10 μmol). ^{1}H NMR ($CDCl_3$): δ 8.38 (br, 4H), 8.17 (br, 4H), 7.88-7.21 (br, 22H), 5.84 (br, 64H), 5.37-5.01 (br, 128 H), 4.10 (br, 64 H), 3.97 (br, 64 H), 3.82-3.18 (br, 280 H), 2.67 (br, 4H), 1.08 (br, 24H). MALDI (*m/z*): $[M]^+$ calcd for $C_{440}H_{658}N_{10}O_{128}$, 8130.5; found, 8149.4 $[M+Na]^+$, 7986.4 [M+Na-diisopropylphenyl]$^+$, 7336.4 (seven substitution). GPC: M_w = 8.2 kDa, M_n = 7.9 kDa, and PDI = 1.04.

PGD-PDI 24. PGD-PDI **24** (96 mg, 78%) was obtained from PDI **19** (37 mg, 26 μmol). ^{1}H NMR ($CDCl_3$): δ 8.18 (br, 4H), 7.80 (br, 8H), 7.09 (br, 4H), 6.93 (br, 8H), 6.23 (br, 1H), 5.86 (br, 32H), 5.36-5.03 (br, 64H), 4.34 (br, 2H), 4.12 (br, 34H), 3.98 (br, 32H), 3.85-3.37 (br, 170H), 3.34 (s, 3H), 2.18 (br, 2H), 1.68 (br, 4H), 1.42 (br, 2H). IR: 2103 cm^{-1} (N_3). MALDI (*m/z*): $[M]^+$ calcd for $C_{255}H_{370}N_{10}O_{76}$, 4788.5; found, 4816.6 $[M+Na]^+$, 4781.9 $[M+Na-N_2]^+$. GPC: M_w = 7.5 kDa, M_n = 7.1 kDa, and PDI = 1.05.

General Procedure for Dihydroxylation. The desired amount of PGD-PDI **22**, **23** or **24**, K_2OsO_4•$2H_2O$ (catalytic amount), *N*-methylmorpholine-*N*-oxide (4 equiv. per alkene), and citric acid (1 equiv. per alkene) were dissolved in acetone/water/*t*-butanol (5:5:1) and stirred at 25 °C for 15 h. Excess Smopex-105 (an osmium scavenger) was added to the stirred solution. Following stirring at 25 °C for an additional 24 h, the mixture was filtered to remove the osmium scavenger and the filtrate was evaporated under reduced pressure to afford the crude PGD-PDI **1**, **2**, or **3** that was further purified by dialysis against water at 25 °C for 2 d.

PGD-PDI 1. PGD-PDI **1** (30 mg, 96%) was obtained from PGD-PDI **22** (25 mg, 5.4 μmol). ^{1}H NMR (CD_3OD): δ 8.22 (br, 4H), 7.84 (br, 8H), 7.44 (br, 2H), 7.32 (br, 4H), 7.18 (br, 8H), 3.80-3.38 (br, 300 H), 2.69 (br, 4H), 1.10 (br, 24H). MALDI (*m/z*): $[M]^+$ calcd for $C_{256}H_{422}N_6O_{132}$, 5692.7; found, 5715.7 $[M+Na]^+$, 5731.4 $[M+K]^+$. GPC: M_w = 9.4

kDa, M_n = 8.5 kDa, and PDI = 1.11. UV-Vis (H_2O): λ_{max} (ε) = 567 nm (22 750 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.62).

PGD-PDI 2. PGD-PDI **2** (29 mg, 91%) was obtained from PGD-PDI **23** (25 mg, 3.1 μmol). ^{1}H NMR (CD_3OD): δ 8.31 (br, 4H), 8.03 (br, 4H), 7.84-7.26 (br, 22H), 3.92-3.35 (br, 600 H), 2.72 (br, 4H), 1.12 (br, 24H). MALDI (*m/z*): $[M]^+$ calcd for $C_{440}H_{786}N_{10}O_{256}$, 10306.9; found, 10344.0 $[M+K]^+$, 10184.3 [M+K-diisopropylphenyl]$^+$, 9254.9 (seven substitution). UV-Vis (H_2O): λ_{max} (ε) = 569 nm (35 600 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.83).

PGD-PDI 3. PGD-PDI **3** (57 mg, 93%) was obtained from PGD-PDI **24** (50 mg, 10 μmol). ^{1}H NMR (CD_3OD): δ 8.40 (br, 4H), 8.16 (br, 4H), 7.98 (br, 1H), 7.83 (br, 8H), 7.08 (br, 8H), 4.34 (br, 2H), 4.09 (br, 2H), 3.90-3.39 (br, 330H), 3.36 (s, 3H), 2.20 (br, 2H), 1.67 (br, 4H), 1.42 (br, 2H). IR: 2105 cm^{-1} (N_3). MALDI (*m/z*): $[M]^+$ calcd for $C_{255}H_{434}N_{10}O_{140}$, 5876.7; found, 5899.7 $[M+Na]^+$, 5916.0 $[M+K]^+$, 5868.5 $[M+Na-N_2]^+$, 5884.7 $[M+K-N_2]^+$. GPC: M_w = 11.2 kDa, M_n = 10.6 kDa, and PDI = 1.06. UV-Vis (H_2O): λ_{max} (ε) = 559 nm (29 300 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.57).

PGD-PDI 4. To a solution of compounds **3** (10 mg, 1.7 μmol) and **25** (5.0 mg, 18 μmol) in DMF (2 mL) were added $CuSO_4$•$5H_2O$ (0.11 mg, 0.44 μmol) and sodium ascorbate (0.34 mg, 1.7 μmol). The mixture was stirred at 25 °C for 18 h and then filtered. Subsequently, the filtrate was evaporated under reduced pressure. Water was added and the precipitate was filtered off. The filtrate was dialyzed against water at 25 °C for 2 d to afford PGD-PDI **4** (9.5 mg, 91%). ^{1}H NMR (CD_3OD): δ 8.24-7.72 (br, 19H), 7.09 (br, 8H), 4.70-4.04 (br, 8H), 3.90-3.39 (br, 330H), 3.34 (s, 3H), 3.14 (br, 1H), 2.88 (br, 1H), 2.67 (br, 1H), 2.20 (br, 4H), 1.68 (br, 8H), 1.40 (br, 4H). IR: no N_3 peak at ~2100 cm^{-1}. MALDI (*m/z*): $[M]^+$ calcd for $C_{268}H_{453}N_{13}O_{142}S$, 6157.8; found, 6196.2 $[M+K]^+$. GPC: M_w = 12.2 kDa, M_n = 11.3 kDa, and PDI = 1.08. UV-Vis (H_2O): λ_{max} (ε) = 560 nm (28 950 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.57).

PGD-PDI 5. To a solution of compounds **3** (4.0 mg, 0.68 μmol) and **26** (2.0 mg, 15 μmol) in DMF (0.5 mL) were added $CuSO_4$•$5H_2O$ (68 μg, 0.27 μmol) and sodium ascorbate (0.11 mg, 0.54 μmol). The mixture was stirred at 25 °C for 18 h and then filtered. Subsequently, the filtrate was evaporated under reduced pressure. Water was added and the precipitate was filtered off. The filtrate was dialyzed against water at 25 °C for 2 d to afford PGD-PDI **5** (3.8 mg, 94%). ^{1}H NMR (CD_3OD): δ 8.40 (br, 4H), 8.16 (br, 4H), 8.04-7.72 (br, 10H), 7.09 (br, 8H), 6.82 (br, 2H), 4.71-4.05 (br, 6H), 3.90-3.39 (br, 330H), 3.36 (s, 3H), 2.21 (br, 2H), 1.67 (br, 4H), 1.43 (br, 2H). IR: no N_3 peak at ~2100 cm^{-1}. MALDI (*m/z*): $[M]^+$ calcd for $C_{262}H_{439}N_{11}O_{142}$, 6011.7; found, 6032.2 $[M+Na]^+$. GPC: M_w = 12.5 kDa, M_n = 11.5 kDa, and PDI = 1.08. UV-Vis (H_2O): λ_{max} (ε) = 559 nm (28 200 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.58).

PGD-PDI 6. To a solution of PGD-PDI **5** (0.18 mg, 30 nmol) in DMF (0.1 mL) was added BSA (0.2 mg, 3 nmol) in phosphate buffer (0.1 M, pH = 7.2, 0.2 mL). The mixture was stirred at 25 °C for 18 h and then dialyzed against water at 25 °C for 2 d to remove excess PGD-PDI **6**. MALDI (*m*/*z*): $[M]^+$ calcd, 72442; found, 72400 $[M]^+$, 66431 [native BSA]$^+$.

PGD-PDI 7. To a solution of PGD-PDI **5** (1.0 mg, 0.17 μmol) in DMF (0.2 mL) was added CoA (2.5 mg, 2.8 μmol) in H_2O (20 μL). The mixture was stirred at 25 °C for 18 h and then dialyzed against water at 25 °C for 2 d to afford PGD-PDI **7** (1.1 mg, 93%). MALDI (*m*/*z*): $[M]^+$ calcd for $C_{283}H_{475}N_{18}O_{158}P_3S$, 6778.9; found, 6771.3 $[M]^+$. UV-Vis (H_2O): λ_{max} (ε) = 561 nm (27 800 $M^{-1}cm^{-1}$). Fluorescence (H_2O): λ_{max} (Φ) = 612 nm (0.57).

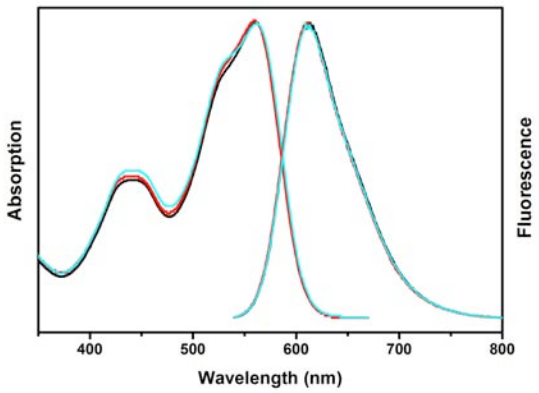


Figure S1. Normalized absorption and emission spectra of PGD-PDIs **4**, **5**, and **7** in water. $\lambda_{abs, max}$ (nm), $\lambda_{em, max}$ (nm), Φ (in water) = 560, 612, 0.57 (**4**, black); 559, 612, 0.58 (**5**, red); 561, 612, 0.57 (**7**, cyan), respectively.

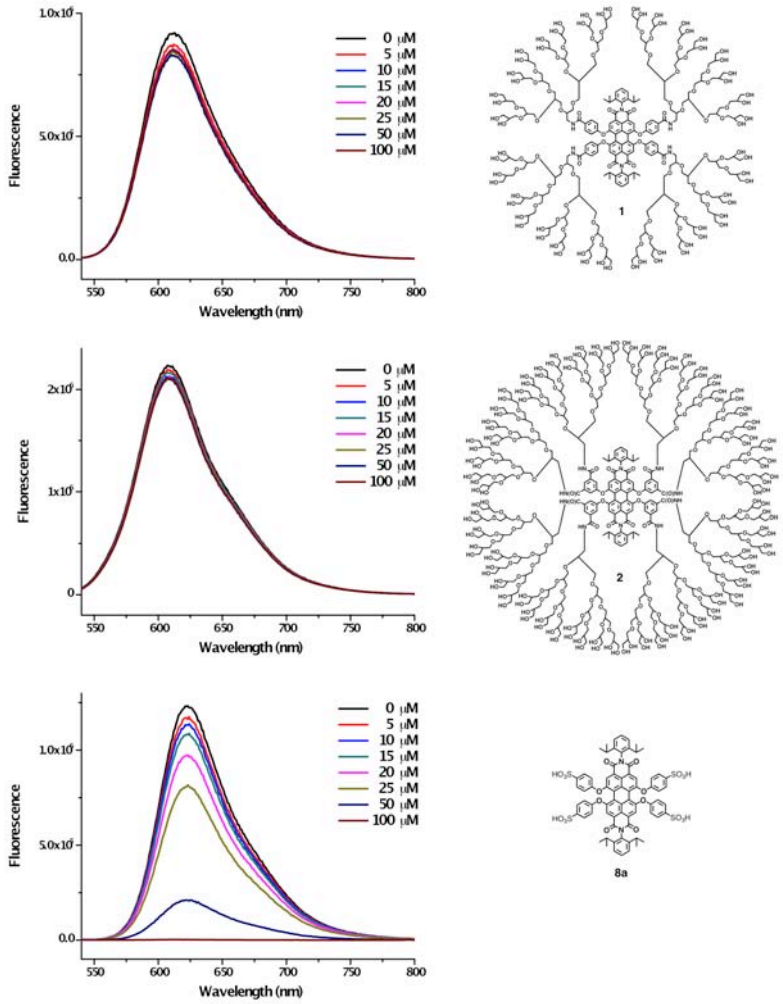


Figure S2. Emission spectra of PGD-PDIs **1** and **2**, and PDI **8a** in water (1 μM) in the presence of Cu^{2+} ions (0–100 μM).

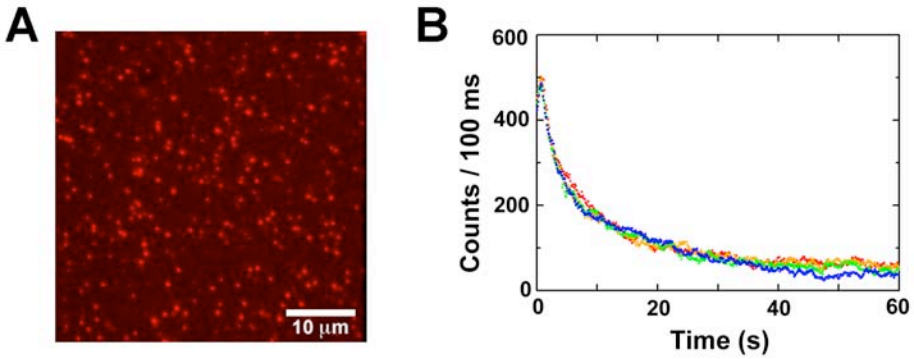


Figure S3. Single-molecule analysis of PGD-PDI **8**. (A) Fluorescence image of immobilized molecules with pseudo colors. (B) Average fluorescence intensity as a function of time in T50 buffer.

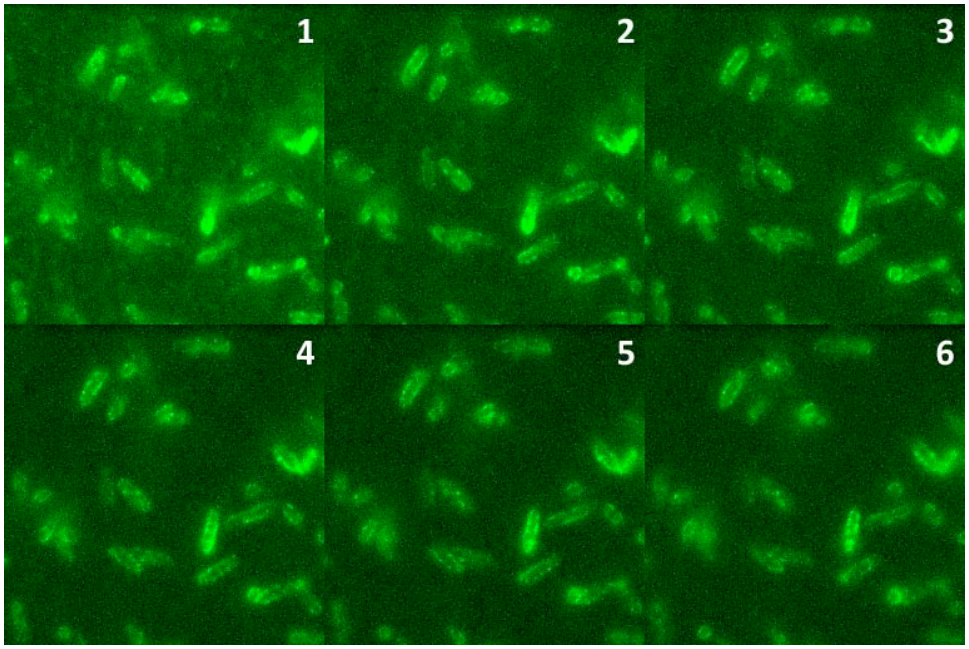


Figure S4. A series of fluorescence images of *E. coli* in the same x-y region, with a step size of 200 nm along the z axis (1-6). Cells were labeled with PGD-PDI **4** after preincubation with streptavidin. For all the z-planes imaged, fluorescence is localized to the cell surface rather than inside the cells.

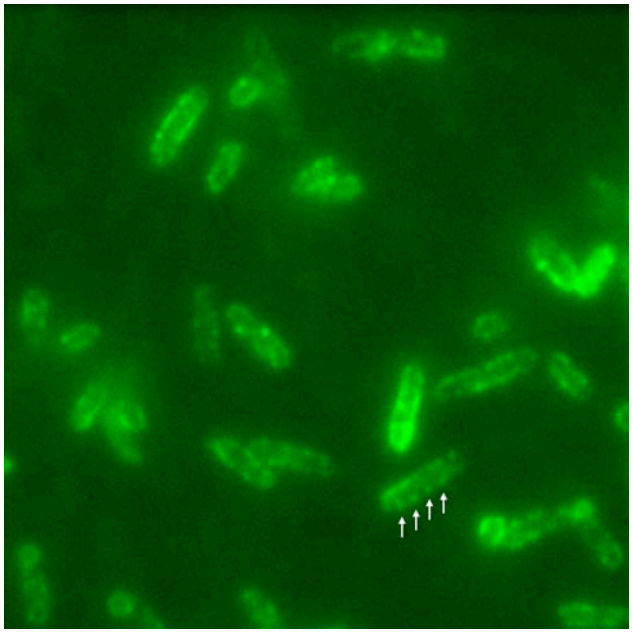


Figure S5. Image averaged over all z-locations in Figure S4. The discontinuous distribution in fluorescence and presence of evenly-spaced spots on the surface are

highlighted for one cell (white arrows), indicative of the helical pattern of λ-receptors and the highly specific labeling with PGD-PDI **4**.

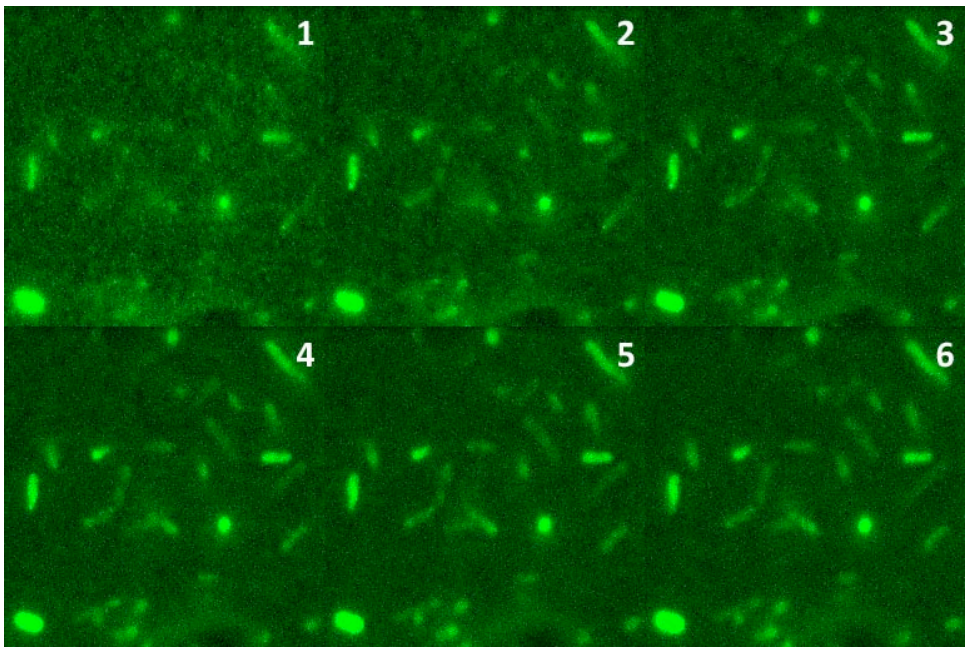


Figure S6. A series of fluorescence images of *E. coli* labeled with nondendronized PDI **8** with streptavidin preincubation, with a step size of 200 nm along the z axis (1-6). Fluorescence is observed throughout the cell for all the z-planes imaged, suggesting nonspecific binding of nondendronized PDI **8**.

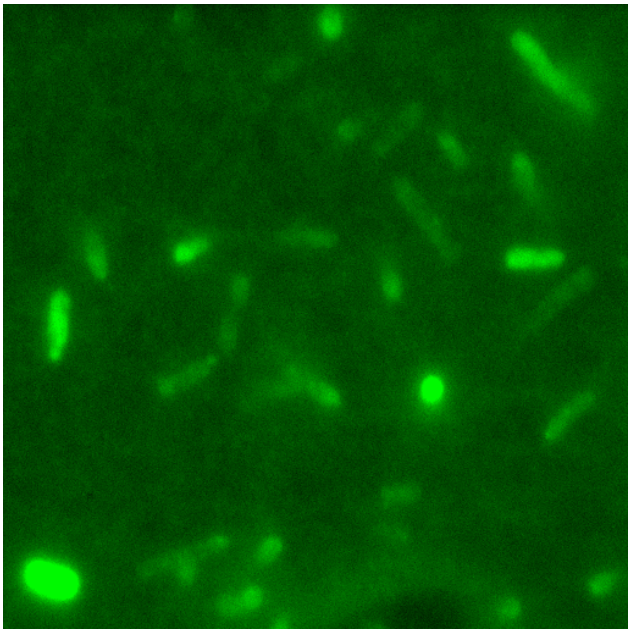


Figure S7. Image averaged over all z-locations in Figure S6.

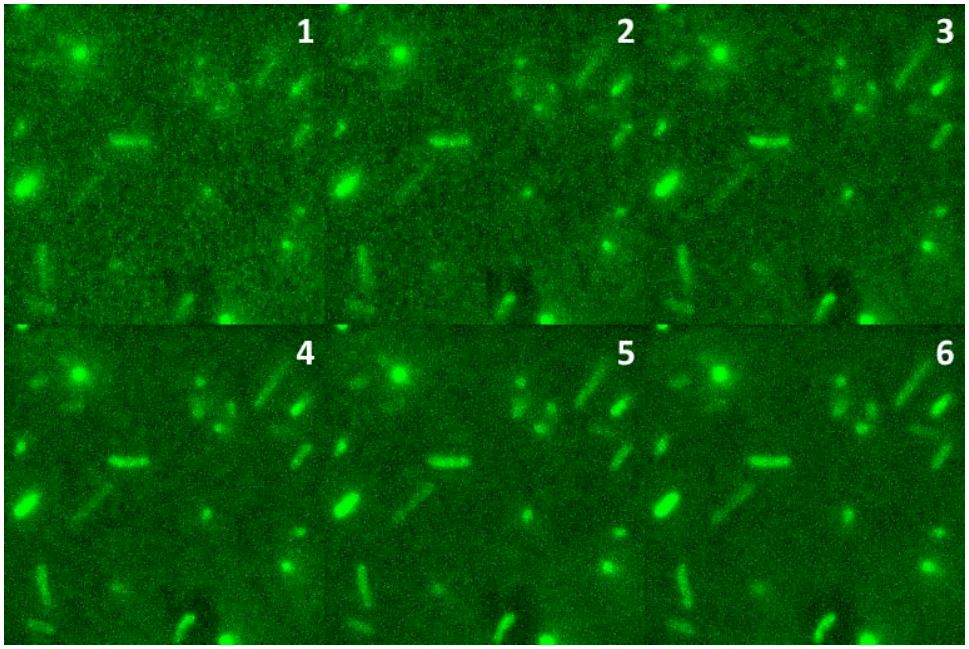


Figure S8. A series of fluorescence images of *E. coli* labeled with nondendronized PDI **8** without preincubation with streptavidin, with a step size of 200 nm along the z axis (1-6). Fluorescence is observed throughout the cell for all the z-planes imaged, consistent with the nonspecific binding of nondendronized PDI **8** shown in Figure S6.

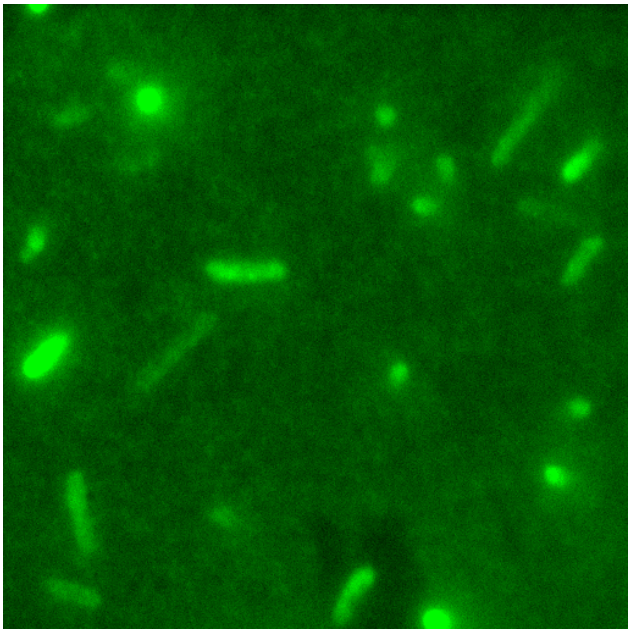


Figure S9. Image averaged over all z-locations in Figure S8.

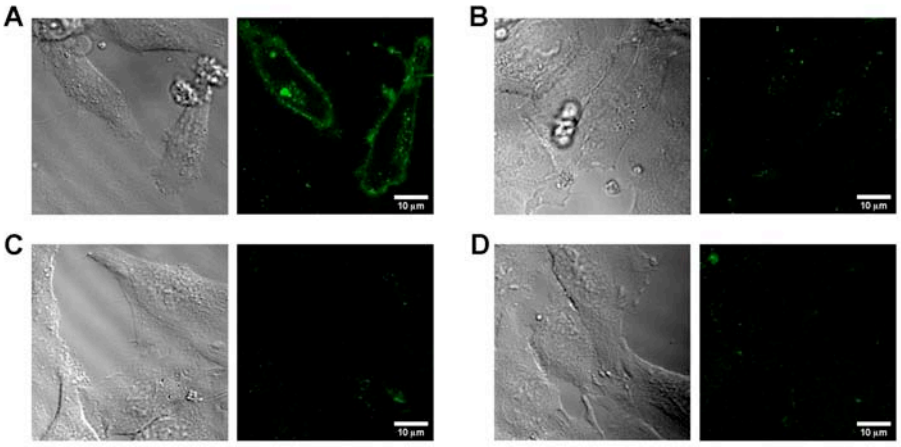


Figure S10. Fluorescent labeling of GPI-ACP fusion proteins on the surface of living mammalian cells. (A) Brightfield and fluorescence images of HeLa cells labeled with PGD-PDI **7** after incubation in the presence of SFP synthase. As controls, cells were also labeled without SFP synthase (B), the pACP-GPI plasmid during transient transfection (C), or the transient-transfection treatment (D).

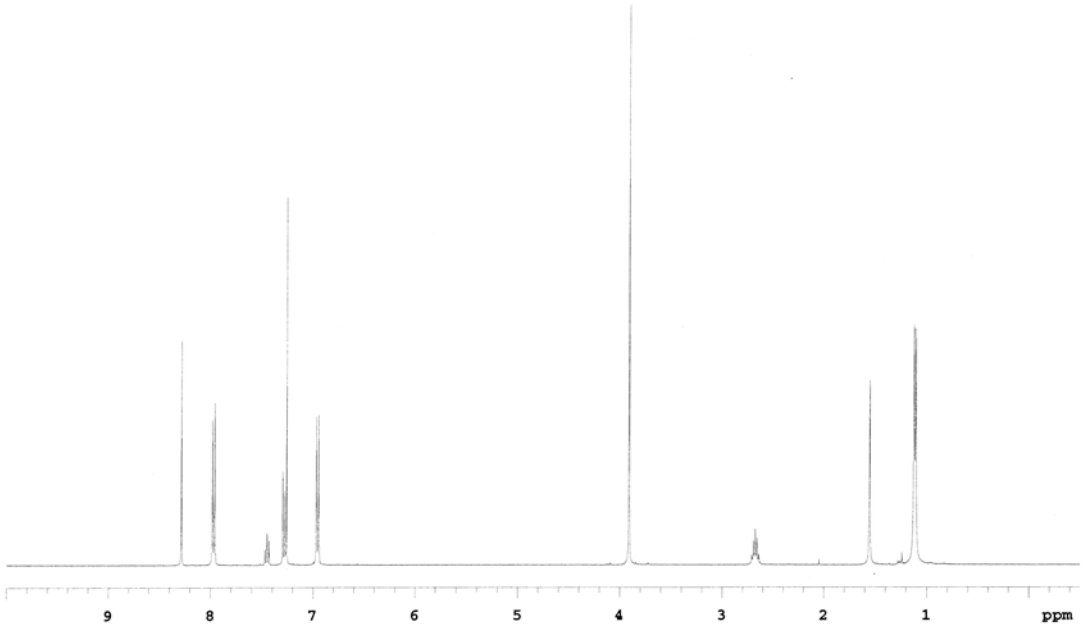


Figure S11. ^{1}H NMR spectrum of PDI **10** in $CDCl_3$.

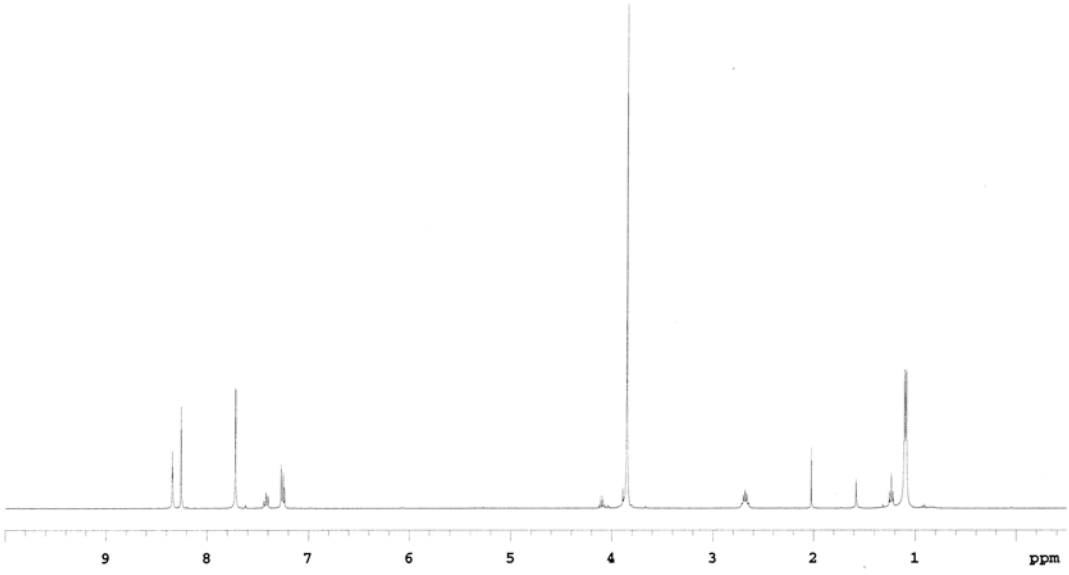


Figure S12. ^{1}H NMR spectrum of PDI **12** in $CDCl_3$.

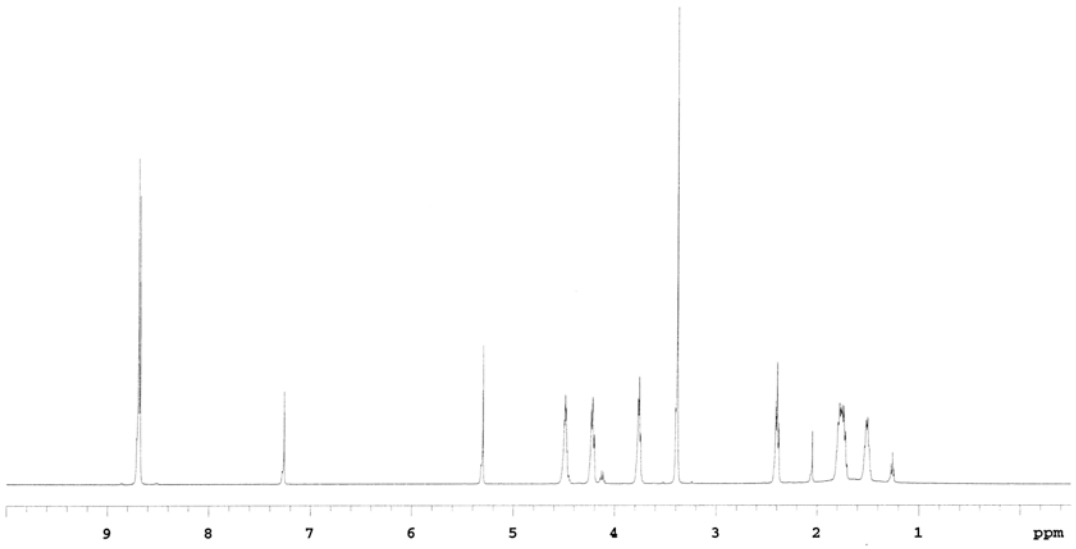


Figure S13. ^{1}H NMR spectrum of PDI **14** in $CDCl_3$.

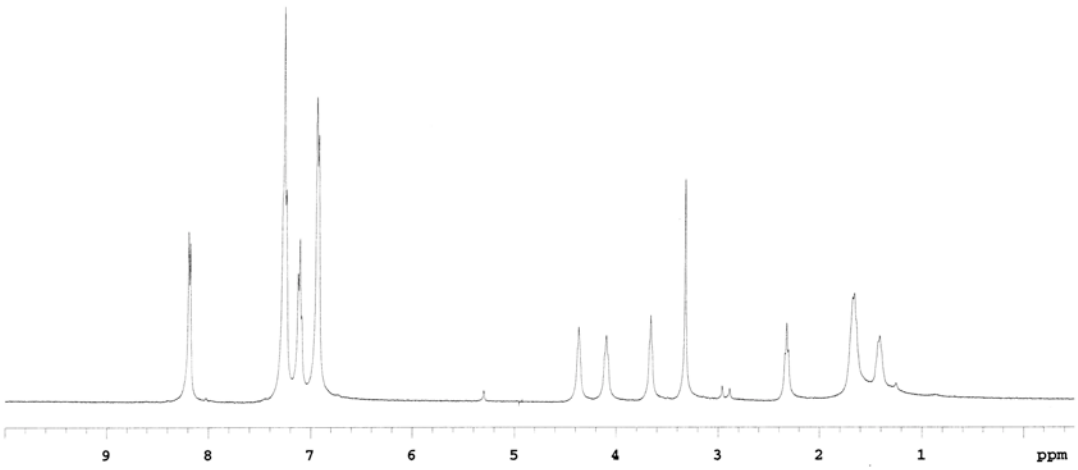


Figure S14. ^{1}H NMR spectrum of PDI **15** in $CDCl_3$.

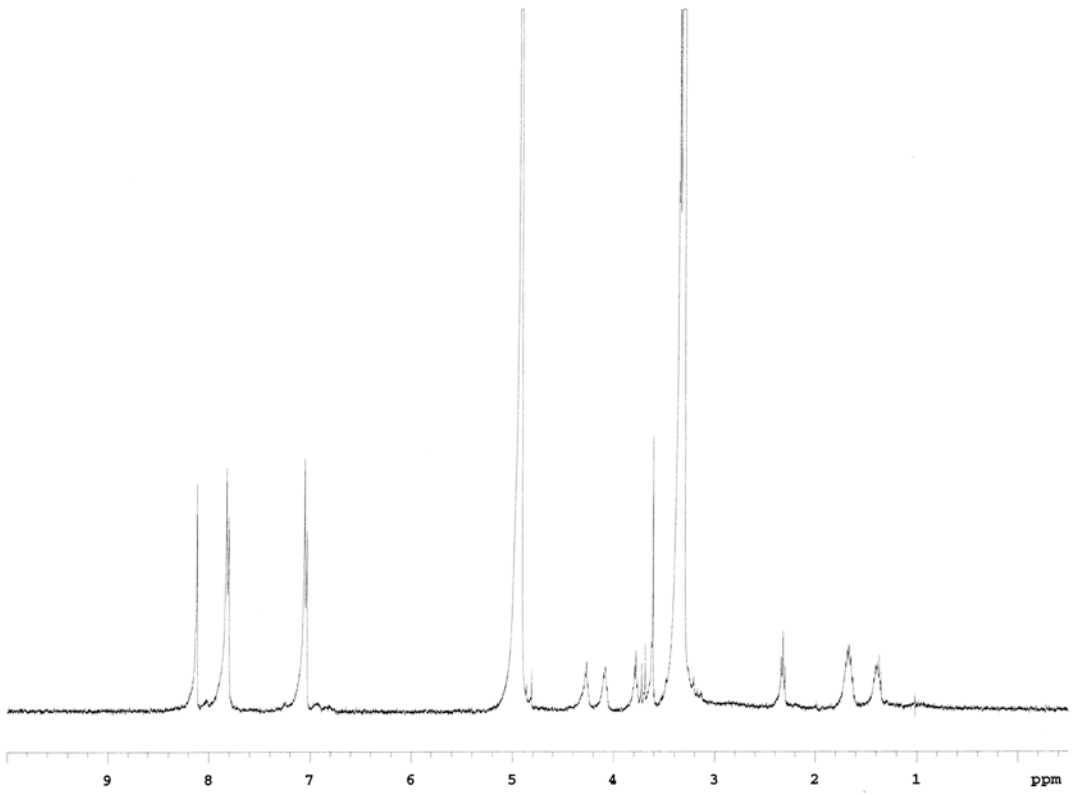


Figure S15. ^{1}H NMR spectrum of PDI **16** in CD_3OD.

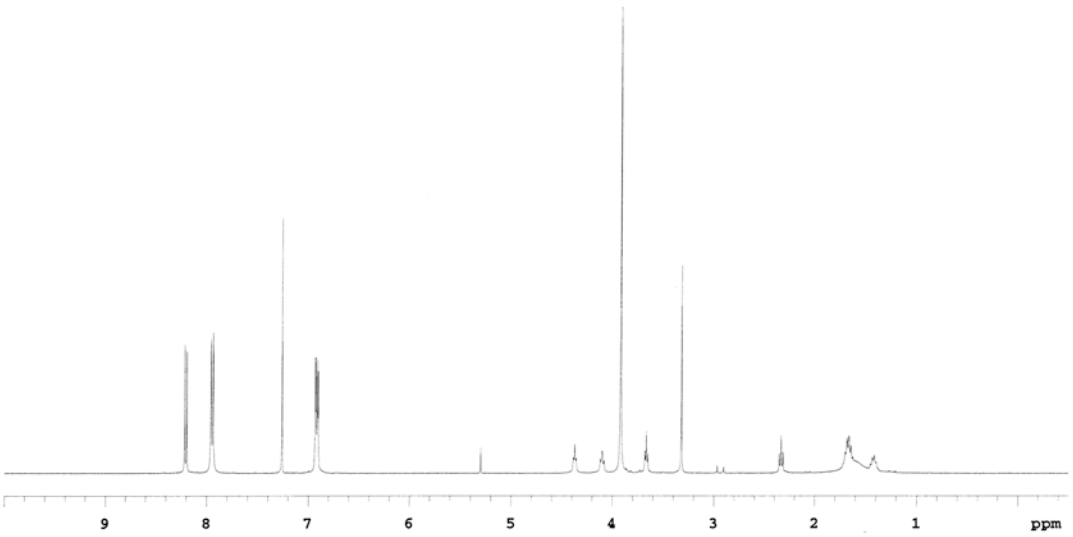


Figure S16. ^{1}H NMR spectrum of PDI **17** in $CDCl_3$.

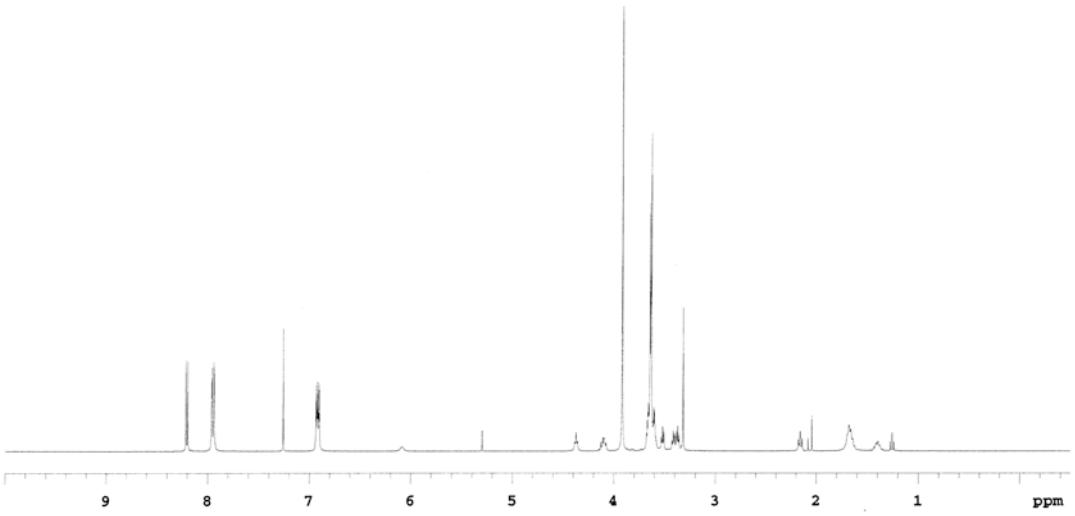


Figure S17. ^{1}H NMR spectrum of PDI **18** in $CDCl_3$.

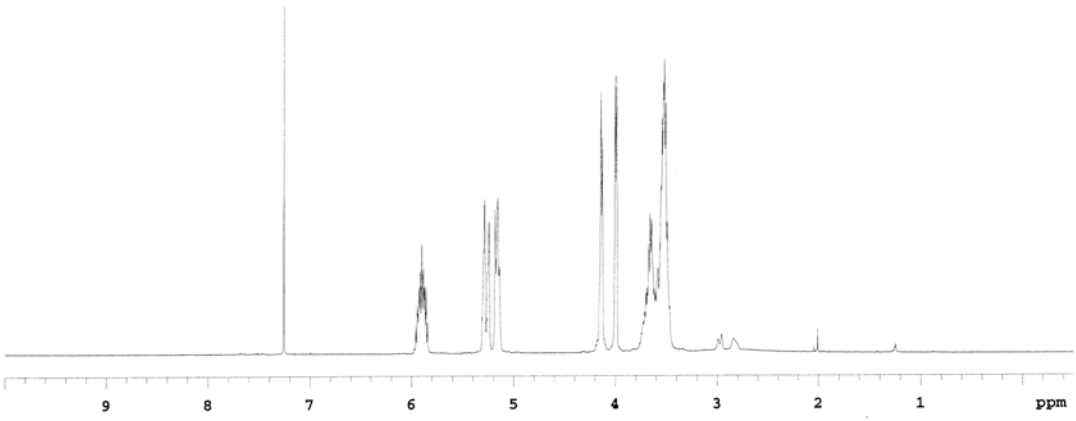


Figure S18. ^{1}H NMR spectrum of PGD **21** in $CDCl_3$.

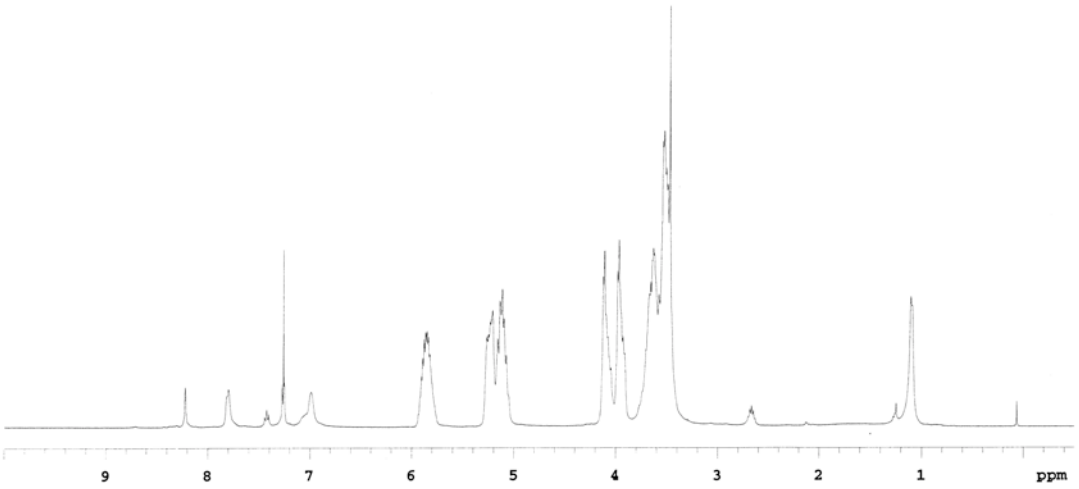


Figure S19. ^{1}H NMR spectrum of PGD-PDI **22** in $CDCl_3$.

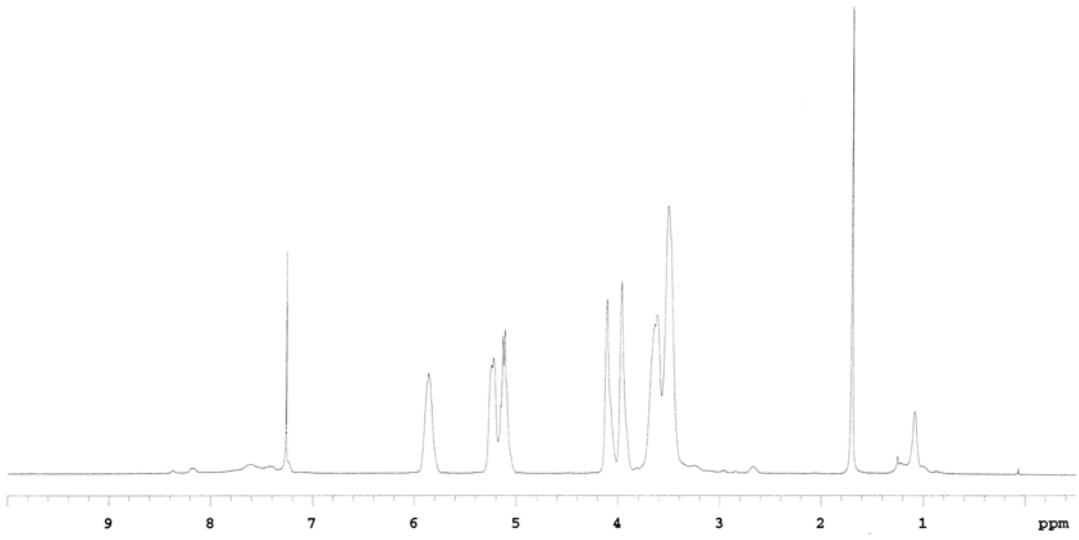


Figure S20. ^{1}H NMR spectrum of PGD-PDI **23** in $CDCl_3$.

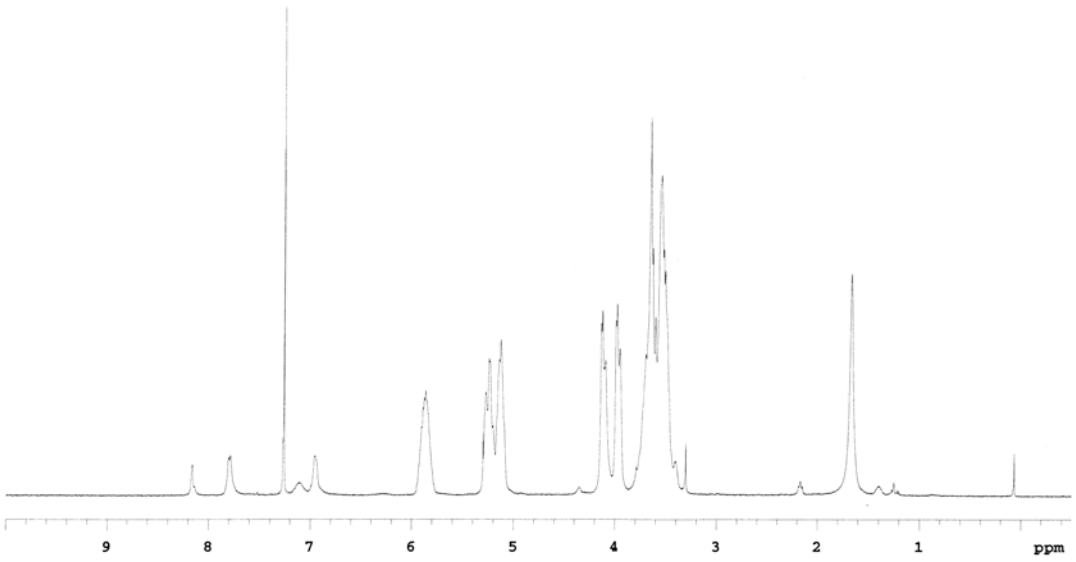


Figure S21. ^{1}H NMR spectrum of PGD-PDI **24** in $CDCl_3$.

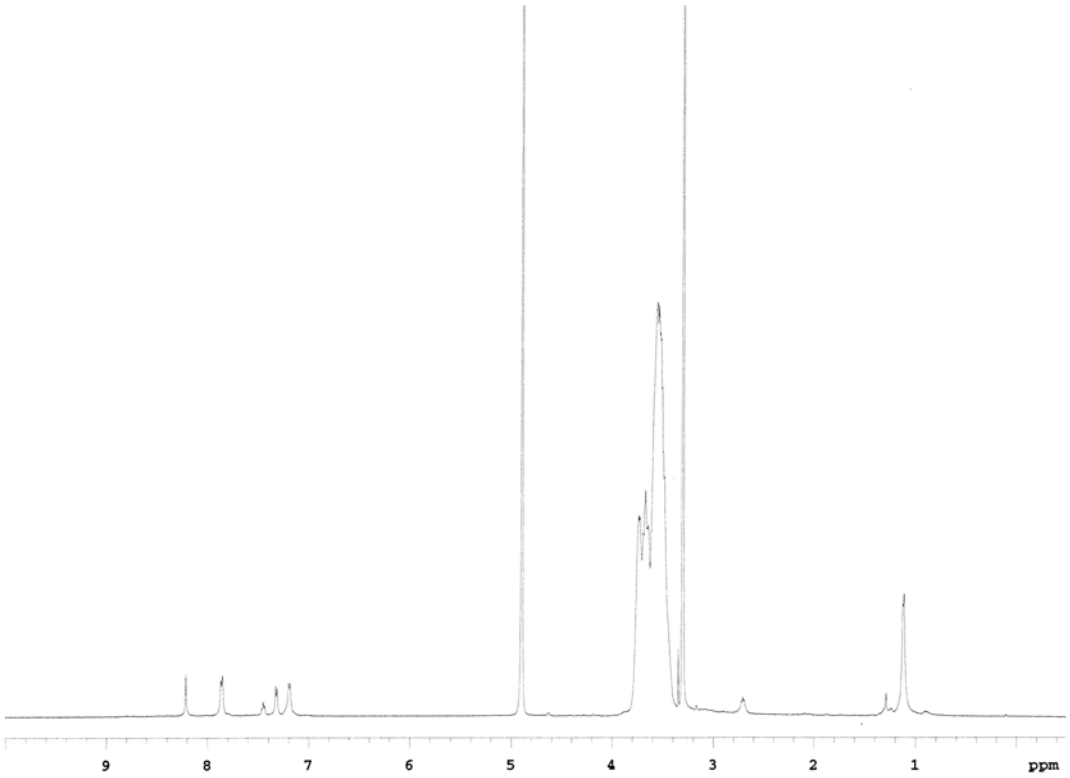


Figure S22. ^{1}H NMR spectrum of PGD-PDI **1** in CD_3OD.

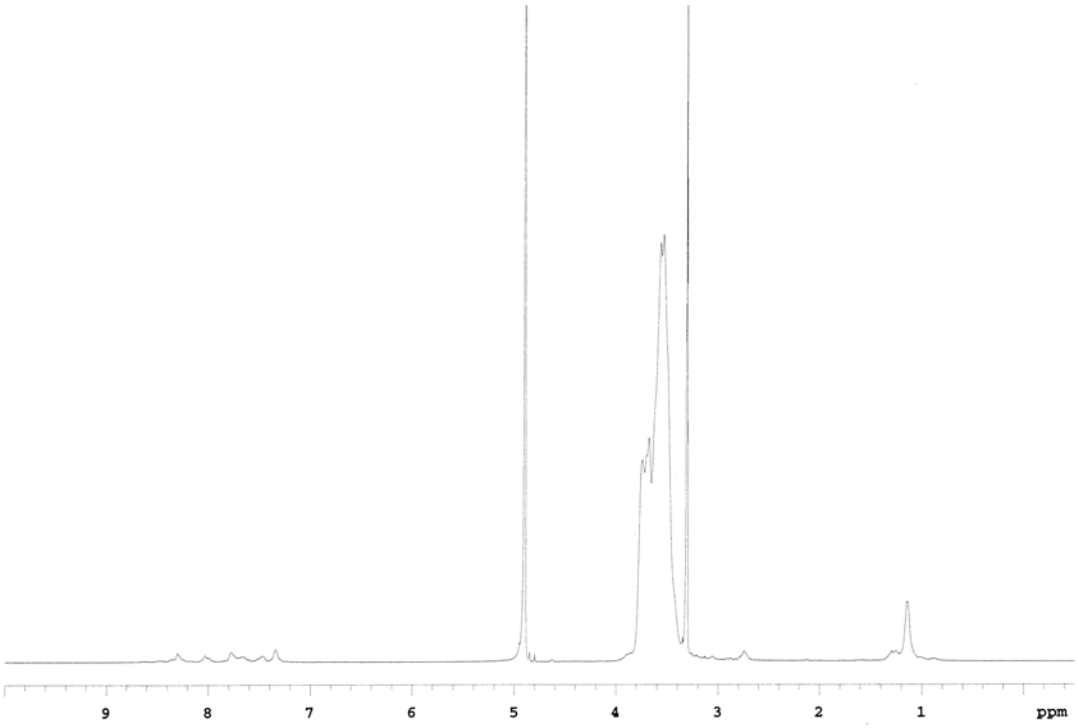


Figure S23. ^{1}H NMR spectrum of PGD-PDI **2** in CD_3OD.

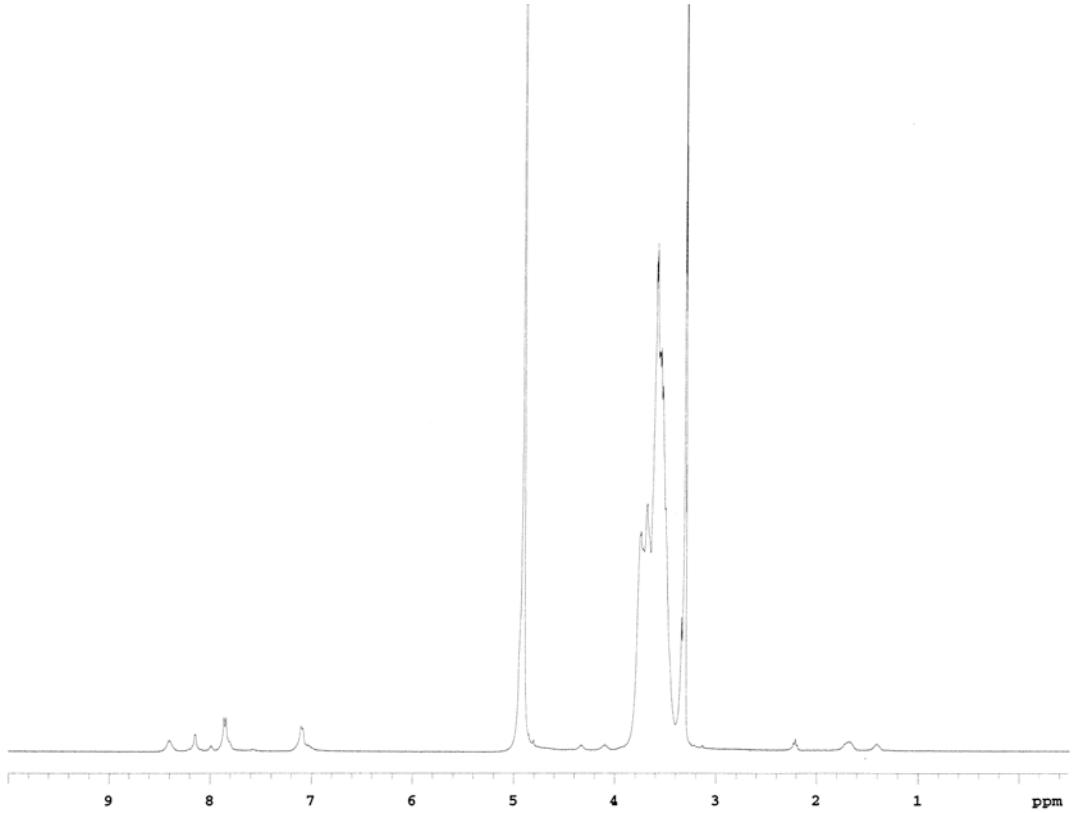


Figure S24. ^{1}H NMR spectrum of PGD-PDI **3** in CD_3OD.

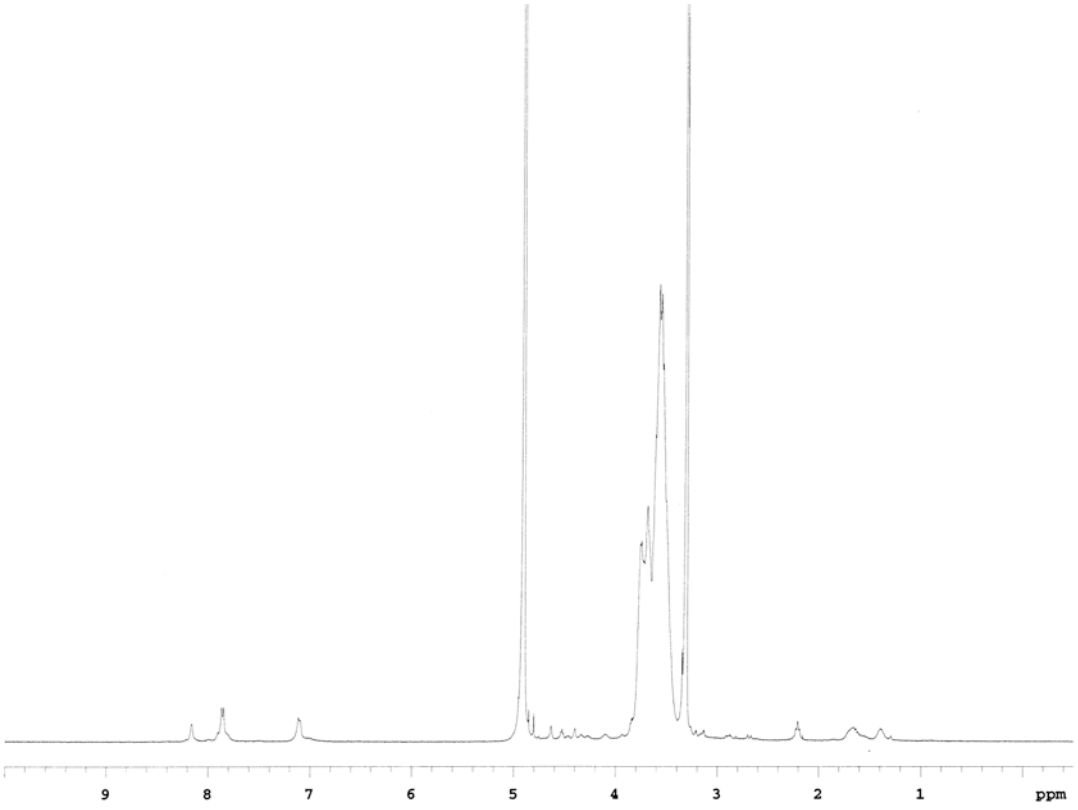


Figure S25. ^{1}H NMR spectrum of PGD-PDI **4** in CD_3OD.

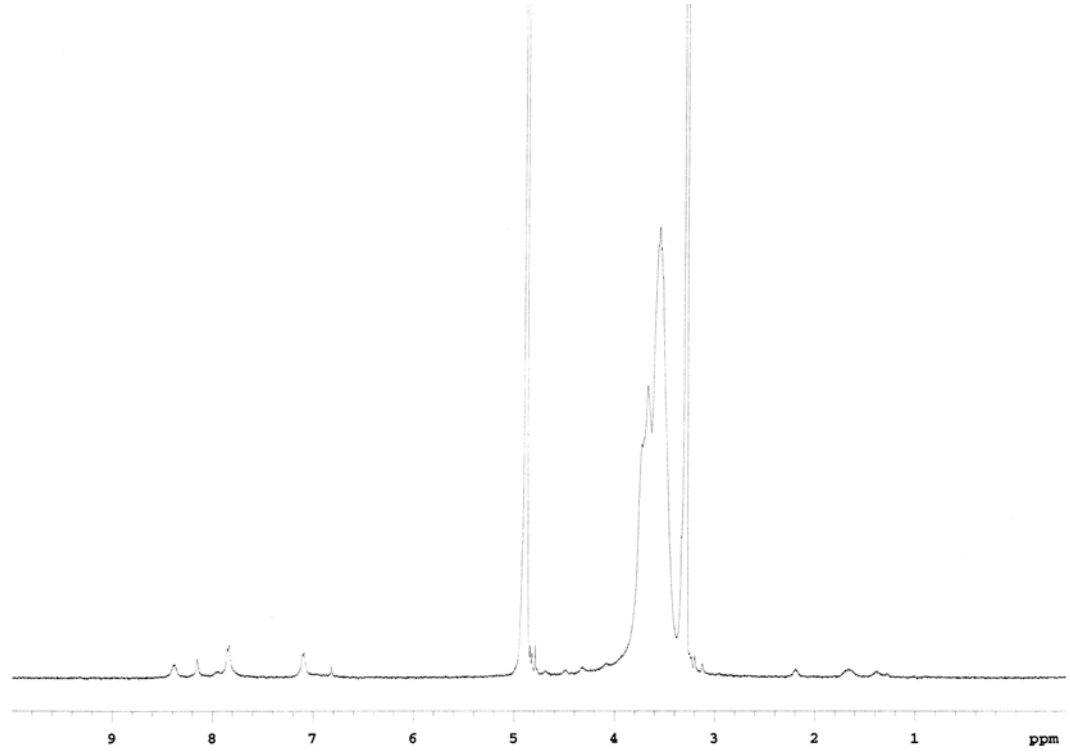


Figure S26. ^{1}H NMR spectrum of PGD-PDI **5** in CD_3OD.

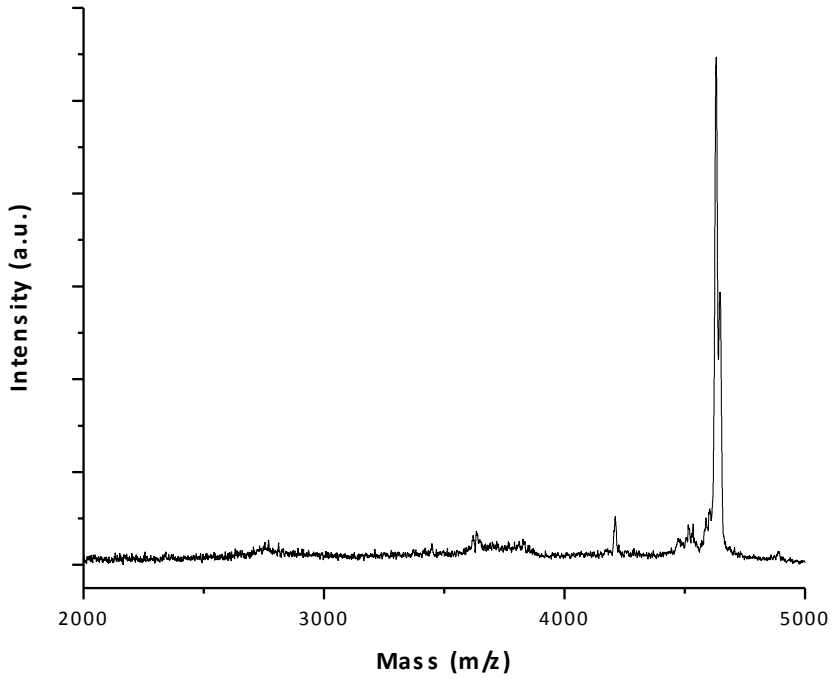


Figure S27. MALDI-TOF mass spectrum of PGD-PDI **22**.

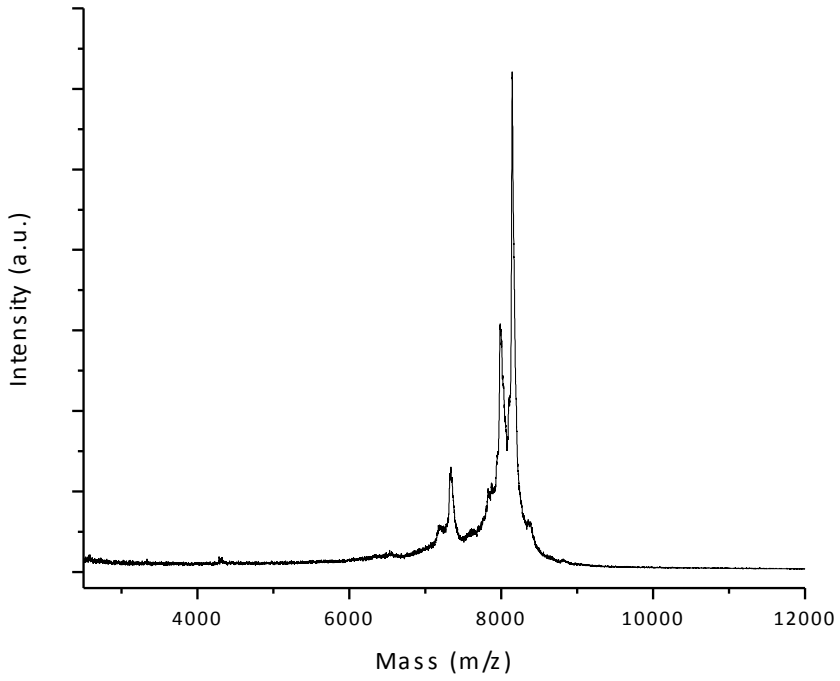


Figure S28. MALDI-TOF mass spectrum of PGD-PDI **23**.

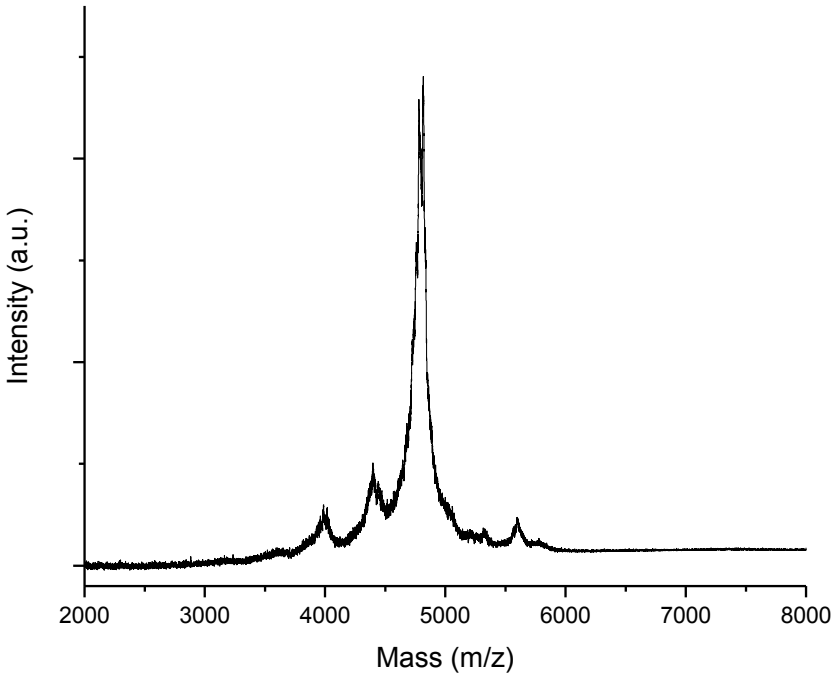


Figure S29. MALDI-TOF mass spectrum of PGD-PDI **24**.

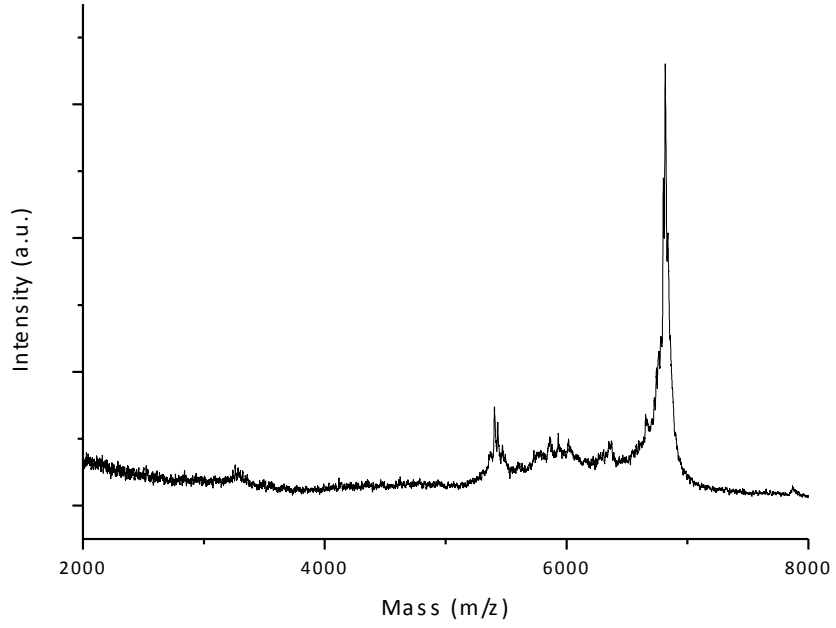


Figure S30. MALDI-TOF mass spectrum of PGD-PDI **7**.

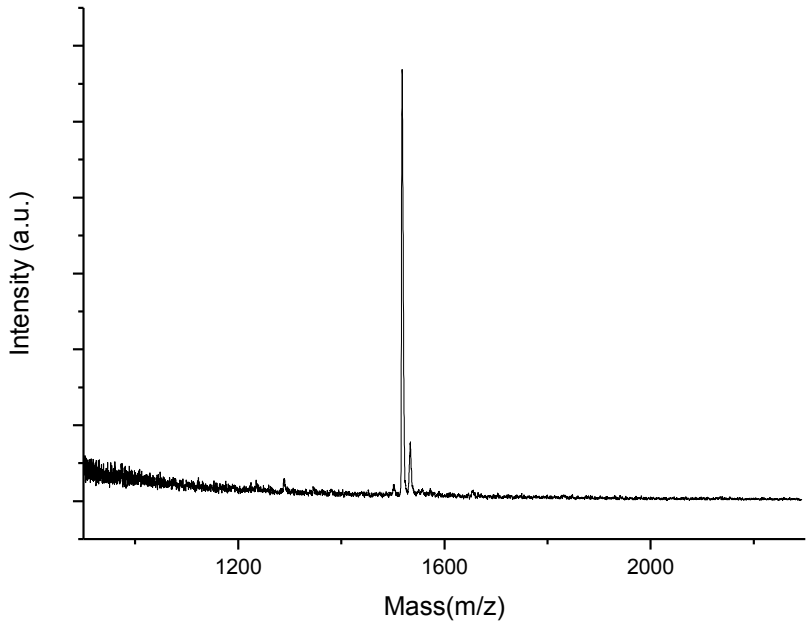


Figure S31. MALDI-TOF mass spectrum of PDI **8**.

References.

(1) Kohl, C.; Weil, T.; Qu, J.; Müllen, K. *Chem.-Eur. J.* **2004**, *10*, 5297-5310.
(2) Klok, H.-A.; Hernández, J. R.; Becker, S.; Müllen, K. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 1572–1583.
(3) Elmer, S. L.; Man, S.; Zimmerman, S. C. *Eur. J. Org. Chem.* **2008**, 3845-3851.
(4) Bonnet, D.; Ilien, B.; Galzi, J.-L.; Riché, S.; Antheaune, C.; Hibert, M. *Bioconjugate Chem.* **2006**, *17*, 1618-1623.
(5) Karlén, B.; Lindeke, B.; Lindgren, S.; Svensson, K.-G.; Dahlbom, R.; Jenden, D. J.; Giering, J. E. *J. Med. Chem.* **1970**, *13*, 651.
(6) Shi, X.; Lim, J.; Ha, T. *Anal. Chem.* **2010**, *82*, 6132-6138.
(7) Roy, R.; Hohng, S.; Ha, T. *Nat. Methods* **2008**, *5*, 507-516.
(8) Oddershede. L.; Dreyer, J. K.; Grego, S.; Brown, S.; Berg-Sørensen, K. *Biophys. J.* **2002**, *83*, 3152-3161.