Electronic Supplementary Material (ESI) for Chemical Communications


*Supporting Information*

# Photostable, Hydrophilic and Functional Near Infrared Quaterrylenediimide-cored Dendrimers for Biomedical Imaging

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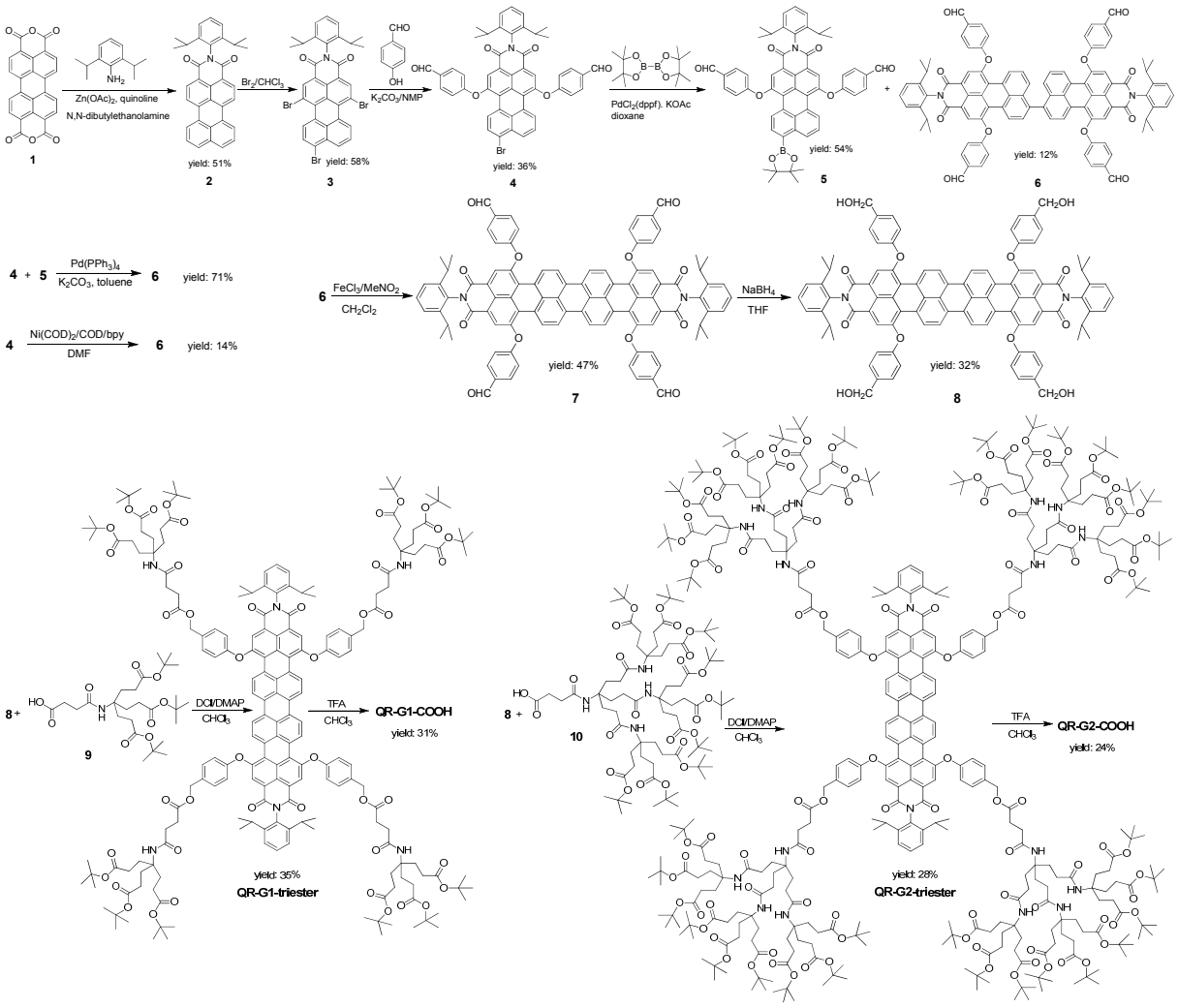
**General:** The solvents used are of commercial grade. Compound **2**, **3**, **9** and **10** were synthesized by following the procedures reported.[1-4] Column chromatography was performed on the silica gel (standard grade, 60A, Sorbtech). $^{1}$H and $^{13}$C NMR were recorded on the Brucker DRX 300 MHz and Brucker Avance III 400 MHz. MALDI-TOF mass spectra were recorded on a PerSeptive Voyager STR MS spectrometer. UV/Vis spectra were recorded on a Cary 100 Bio UV-Vis spectrophotometer, and fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer.




***N*-(2,6-diisopropylphenyl)-1,6-bis(4-formylphenoxy)-9-bromoperylene-3,4-dicarboximide (4):**
**3** (3.59 g, 5 mmol), 4-hydroxybenzaldehyde (1.22 g, 10 mmol), and potassium carbonate (1.38 g, 10 mmol) were stirred in N-methyl-2-pyrrolidone (360 mL) at 80 °C under Argon atmosphere for 3.5 hours. After being cooled to room temperature, the mixture was poured into HCl/$H_2O$ (2 L, 1/5), filtered and dried. The crude product was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/50) as eluent resulting in **4** (1.44 g, 36 %) as a red solid. $^{1}$H NMR ($CDCl_3$): δ = 9.90 (s, 2H), 9.14 (d, 1 H, *J* = 7.8 Hz), 8.90 (d, 1 H, *J* = 8.4 Hz), 8.39-8.42 (m, 2 H), 8.28 (d, 1 H, *J* = 8.4 Hz), 7.88 (d, 4 H, *J* = 7.5 Hz), 7.80 (d, 1 H, *J* = 8.4 Hz), 7.62 (t, 1 H, *J* = 8.4 Hz), 7.46 (t, 1 H, *J* = 7.8 Hz), 7.32 (d, 2 H, *J* = 7.5 Hz), 7.17-7.21 (m, 4 H), 2.77 (sep, 2 H, *J* = 6.6 Hz), 1.17 (d, 12 H, *J* = 6.6 Hz). $^{13}$C NMR ($CDCl_3$): δ = 193.89, 166.30, 164.21, 164.10, 155.08, 154.98, 149.16, 136.00, 135.89, 135.70,

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135.50, 134.69, 133.90, 133.83, 133.62, 133.28, 133.09, 132.36, 131.76, 131.63, 131.53, 130.60, 130.10, 130.06, 130.00, 129.92, 128.33, 127.65, 126.03, 125.98, 121.45, 121.42, 32.77, 27.61.

***N*-(2,6-diisopropylphenyl)-1,6-bis(4-formylphenoxy)-9-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-perylene-3,4-dicarboximide (5):**

**4** (0.82 g, 1 mmol), bis(pinacolato)diborane (0.39 g, 1.5 mmol), and potassium acetate (0.31 g, 3 mmol) were stirred in dioxane (30 mL) under Argon atmosphere, and then $PdCl_2$(dppf) (45 mg, 0.06 mmol) was added. The mixture was heated to 70 °C for 24 hours. After being cooled to room temperature, the solvent was removed by rotary evaporation. The crude product was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/50) as eluent resulting in **5** (0.46 g, 54 %) as a red solid. $^1$H NMR ($CDCl_3$): δ = 9.89 (s, 1H), 9.88 (s, 1H), 9.07 (d, 1 H, *J* = 7.8 Hz), 9.02 (d, 1 H, *J* = 7.8 Hz), 8.89 (d, 1 H, *J* = 8.4 Hz), 8.43 (s, 2H), 8.11 (d, 1 H, *J* = 7.8 Hz), 7.85 (dd, 4 H, *J* = 2.1, 8.7 Hz), 7.58 (t, 1 H, *J* = 8.1 Hz), 7.46 (t, 1 H, *J* = 7.5 Hz), 7.32 (d, 2 H, *J* = 7.5 Hz), 7.17 (dd, 4 H, *J* = 1.8, 8.7 Hz), 2.78 (sep, 2 H, *J* = 6.6 Hz), 1.41 (s, 12H), 1.18 (d, 12 H, *J* = 6.6 Hz). $^{13}$C NMR ($CDCl_3$): δ = 190.51, 162.90, 160.97, 160.95, 151.33, 150.98, 145.74, 145.66, 137.03, 136.10, 132.35, 132.31, 132.19, 132.03, 131.96, 130.48, 129.72, 129.55, 129.13, 128.96, 128.89, 128.65, 127.85, 127.14, 127.01, 126.91, 126.37, 125.11, 124.11, 122.42, 121.86, 117.62, 117.54, 84.31, 83.51, 29.22, 25.07, 24.99, 24.08. And compound **6** was obtained as purple red solid (0.09 g, 12 %).

**9,9-Bis[[*N*-(2,6-diisopropylphenyl)-1,6-di(4-formylphenoxy)]perylene-3,4-dicarboximide] (6):**

**Method 1 (Suzuki Coupling Reaction)**

**4** (252 mg, 0.32 mmol) and **5** (267 mg, 0.32 mmol) were dissolved in freshly distilled toluene (35 mL). A solution of potassium carbonate (134 mg, 0.97 mmol) in $H_2O$ (2 mL) and ethanol (0.2 mL) was purged with Argon for 10 minutes and added to the toluene solution under Argon atmosphere. After $Pd(PPh_3)_4$ was added, the resulting mixture was stirred at 80 °C for 24 hours. The reaction mixture was then cooled to room temperature and concentrated by rotary evaporation. The crude product was purified by column chromatography (silica gel) using ethyl acetate/dichloromethane (1/50 to 1/20) as eluent resulting in **6** (0.328 g, 71 %) as a purple red solid. $^1$H NMR ($CDCl_3$): δ = 9.95 (s, 2H), 9.94 (s, 2H), 9.23 (d, 2 H, *J* = 8.1 Hz), 9.16 (d, 2 H, *J* = 7.2 Hz), 8.43 (s, 2 H), 8.42 (s, 2H), 7.89-7.93 (m, 8 H), 7.58 (d, 2 H, *J* = 8.1 Hz), 7.52 (d, 2 H, *J* = 8.7 Hz), 7.48 (t, 2 H, *J* = 7.8 Hz), 7.41 (t, 2 H, *J* = 7.8 Hz), 7.33 (d, 4 H, *J* = 7.8 Hz), 7.20-7.25 (m, 8 H), 2.71-2.77 (m, 4 H), 1.17 (dd, 24 H, *J* = 6.6 Hz). $^{13}$C NMR ($CDCl_3$): δ = 190.57, 162.88, 160.98, 160.96, 151.61, 151.58, 145.71, 145.68, 140.56, 132.84, 132.53, 132.49, 132.48, 132.46, 132.04, 130.42, 129.84, 129.59, 129.43, 129.34, 129.27, 128.94, 128.67, 128.53, 127.36, 127.18, 126.76, 126.66, 125.05, 124.22, 122.44, 122.42, 118.06, 117.99, 29.30, 24.16, 24.15.

**Method 2 (Yamamoto Coupling Reaction)**

A mixture of bis(1,5-cyclooctadiene)nickel (0) ($Ni(COD)_2$) (138 mg, 0.5 mmol), 2,2'-bipyridine (Bpy) (78 mg, 0.5 mmol) and 1,5-cyclooctadiene (COD) (45 mg, 0.42 mmol) in anhydrous DMF (20 mL) was heated at 65 °C under Argon atmosphere for 0.5 h. Next, **4** (252 mg, 0.32 mmol) was added and the resulting mixture was allowed to stir for another 4 hours. After being cooled to room temperature, the mixture was poured into HCl/$H_2O$ (300 mL, 1/5), filtered and dried. The crude product was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/50 to 1/20) as eluent resulting in **6** (31 mg, 14 %) as a purple red solid.

***N,N'*-(2,6-diisopropylphenyl)-1,6,11,16-tetra(4-formylphenoxy)quaterrylene (7)**

A solution of $FeCl_3$ (530 mg, 3.3 mmol) in nitromethane (3 mL) was added dropwise to a stirred solution of **6** (246 mg, 0.17 mmol) in dichloromethane (14 mL) under Argon atmosphere. The resulting mixture was stirred at room temperature for 17 hours. The solvent was then removed by rotary evaporation and the resulting solid was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/20 to 1/10) as eluent resulting in **7** (115 mg, 47 %) as a green solid. $^1$H NMR ($CDCl_3$): δ = 9.93 (s, 4H), 9.32 (d, 4 H, *J* = 8.7 Hz), 8.52 (d, 4 H, *J* = 9 Hz), 8.43 (s, 4 H), 7.90 (d, 8 H, *J*

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= 6.9 Hz), 7.48 (t, 2 H, *J* = 7.8 Hz), 7.33 (d, 4 H, *J* = 7.8 Hz), 7.23 (d, 8 H, *J* = 6.9 Hz), 2.72 (sep, 4 H, *J* = 6.6 Hz), 1.16 (d, 24 H, *J* = 6.6 Hz). $^{13}$C NMR ($CDCl_3$): δ = 190.55, 162.86, 161.03, 151.81, 145.71, 132.58, 132.54, 132.00, 131.44, 130.50, 129.88, 129.86, 129.66, 128.37, 127.64, 127.43, 126.48, 125.30, 124.27, 123.42, 122.27, 117.96, 29.35, 24.17. MS (MALDI-TOF): m/z = 1439.52, calcd. for $C_{96}H_{66}N_2O_{12}$: 1439.46.

***N,N'*-(2,6-diisopropylphenyl)-1,6,11,16-tetra(4-hydroxymethylphenoxy)quaterrylene (8)**
$NaBH_4$ (40 mg, 1.1 mmol) was added to a solution of **7** (62 mg, 0.043 mmol) in anhydrous THF (50 mL). The resulting mixture was stirred at room temperature for 20 hours. The solvent was removed and the resulting solid was purified over silica gel column chromatography using methanol/dichloromethane (1/20 to 1/12) as eluent resulting in **8** (20 mg, 32 %) as a green solid. $^1$H NMR ($CDCl_3$): δ = 9.23 (d, 4H, *J* = 8.8 Hz), 8.28 (s, 4H), 8.16(d, 4 H, *J* = 8.8 Hz), 7.46 (t, 2 H, *J* = 8 Hz), 7.29-7.34 (m, 12 H), 7.03 (d, 8 H, *J* = 8.4 Hz), 4.62 (s, 8 H), 2.72 (sep, 4 H, *J* = 6.4 Hz), 1.13 (d, 24 H, *J* = 6.4 Hz). MS (MALDI-TOF): m/z $(M+H)^+$ = 1448.05, calcd. for $C_{96}H_{75}N_2O_{12}$: 1448.54.

**Dendrimer QR-G1-triester:**
To a mixture of **8** (6 mg, 4.15 μmol), **9** (13 mg, 25 μmol), and DMAP (3.1 mg, 25 μmol) in chloroform (7 mL), DIC (3.9 μL, 25 μmol) was added. The resulting mixture was stirred at room temperature under Argon atmosphere for 48 hours. The solvent was removed and the resulting solid was purified over silica gel column chromatography using ethyl acetate/dichloromethane (1/4) as eluent resulting in **QR-G1-triester** (5 mg, 35 %) as a green solid. $^1$H NMR ($CDCl_3$): δ = 9.47 (d, 4H, *J* = 8.8 Hz), 8.56 (d, 4 H, *J* = 8.8 Hz), 8.34 (s, 4 H), 7.46 (d, 2 H, *J* = 8.4 Hz), 7.41 (d, 8 H, *J* = 8.8 Hz), 7.30 (d, 4 H, *J* = 8 Hz), 7.16 (d, 8 H, *J* = 8.8 Hz), 5.94 (s, 4 H), 5.11 (s, 8 H), 2.72 (sep, 4 H, *J* = 6.4 Hz), 2.67 (t, 8 H, *J* = 6.8 Hz), 2.42 (t, 8 H, *J* = 6.8 Hz), 2.19-2.23 (m, 24 H), 1.93-1.97 (m, 24 H), 1.41 (s, 108 H), 1.14 (d, 24 H, *J* = 6.8 Hz). $^{13}$C NMR ($CDCl_3$): δ = 172.98, 172.90, 170.71, 163.10, 156.02, 153.43, 145.78, 132.02, 131.81, 131.41, 130.81, 130.10, 129.63, 128.13, 127.48, 124.86, 124.09, 124.07, 123.17, 121.76, 118.82, 80.73, 65.98, 57.62, 31.76, 30.14, 29.88, 29.84, 29.64, 29.50, 28.21, 24.17. MS (MALDI-TOF): $(M+Na)^+$ m/z = 3460.72, calcd. for $C_{200}H_{246}N_6NaO_{44}$: 3461.00.

**Dendrimer QR-G1-COOH:**
**QR-G1-triester** (5 mg, 1.46 μmol) was dissolved in chloroform/TFA (2 mL/0.5 mL). The resulting mixture was stirred at room temperature in dark for 12 hours. The solvent was removed and the resulting solid was dissolved in methanol, and then precipitated in ether. The green solid was collected by centrifuge and dried in vacuum oven overnight resulting in **QR-G1-COOH** (1.25 mg, 31 %) as a green solid. $^1$H NMR (*d*6-DMSO): δ = 9.07 (br.s, 8H), 8.05 (br.s, 4 H), 7.40-7.44 (m, 10 H), 7.09-7.31 (m, 16 H), 5.05 (s, 8 H), 2.67-2.69 (m, 8 H), 2.32-2.34 (m, 12 H), 2.06-2.08 (m, 24 H), 1.79 (br.s, 24 H), 0.99 (br.s, 24 H). Due to the sample aggregation, the $^{13}$C NMR spectrum could not be obtained. MS (MALDI-TOF): $(M+Na)^+$ m/z = 2786.40, calcd. for $C_{152}H_{150}N_6NaO_{44}$: 2786.96.

**Dendrimer QR-G2-triester:**
To a mixture of **8** (14 mg, 9.45 μmol), **10** (99 mg, 64 μmol), and DMAP (8 mg, 65 μmol) in chloroform (6 mL), DIC (10 μL, 65 μmol) was added. The resulting mixture was stirred at room temperature under Argon atmosphere for 48 hours. The solvent was removed and the resulting solid was purified over a short silica gel column using ethyl dichloromethane as eluent resulting in **QR-G2-triester** (20 mg, 28 %) as a green solid. $^1$H NMR ($CDCl_3$): δ = 9.51 (d, 4H, *J* = 8.8 Hz), 8.58 (d, 4 H, *J* = 9.2 Hz), 8.31 (s, 4 H), 7.47 (d, 8 H, *J* = 8.4 Hz), 7.46 (s, 4H), 7.41-7.43 (m, 2 H), 7.28 (d, 4 H, *J* = 7.6 Hz), 7.18 (d, 8 H, *J* = 8.4 Hz), 6.07 (s, 12 H), 5.13 (s, 8 H), 2.68-2.71 (m, 12 H), 2.42 (t, 8 H, *J* = 6.4 Hz), 2.17-2.20 (m, 96 H), 1.92-2.00 (m, 96 H), 1.40 (s, 324 H), 1.13 (d, 24 H, *J* = 6.8 Hz). $^{13}$C NMR ($CDCl_3$): δ = 173.51, 173.11, 172.80, 171.21, 163.05, 155.85, 153.74, 145.76, 132.13, 131.75, 131.42, 131.01, 130.84, 130.20, 129.63, 128.24, 127.51, 127.10, 124.27, 124.05, 123.80, 123.06, 121.75, 119.07, 80.66, 66.25, 57.93, 57.52, 31.98,

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31.93, 31.31, 29.98, 29.90, 29.22, 29.17, 28.22, 24.16. MS (MALDI-TOF): m/z = 7534.35, calcd. for $C_{416}H_{618}N_{18}O_{104}$: 7534.38.

**Dendrimer QR-G1-COOH:**
**QR-G1-triester** (20 mg, 2.65 μmol) was dissolved in chloroform/TFA (8 mL/2 mL). The resulting mixture was stirred at room temperature in dark for 12 hours. The solvent was removed and the resulting solid was dissolved in methanol, and then precipitated in ether. The green solid was collected by centrifuge and dried in vacuum oven overnight resulting in **QR-G2-COOH** (3.5 mg, 24 %) as a green solid. $^{1}$H NMR (*d*6-DMSO): δ = 9.49 (br.s, 4H), 8.94 (br.s, 4H), 8.08 (br.s, 4 H), 7.25-7.50 (m, 34 H), 5.09 (s, 8 H), 2.64-2.67 (m, 8 H), 2.54 (br.s, 4H), 2.37 (br.s, 8 H), 1.98-2.09 (m, 96 H), 1.81 (br.s, 96 H), 1.02 (br.s, 24 H). Due to the sample aggregation, the $^{13}$C NMR spectrum could not be obtained. MS (MALDI-TOF): m/z = 5515.20, calcd. for $C_{272}H_{330}N_{18}O_{104}$: 5515.59.

**Solvent-dependent Absorption and Emission Spectra:**

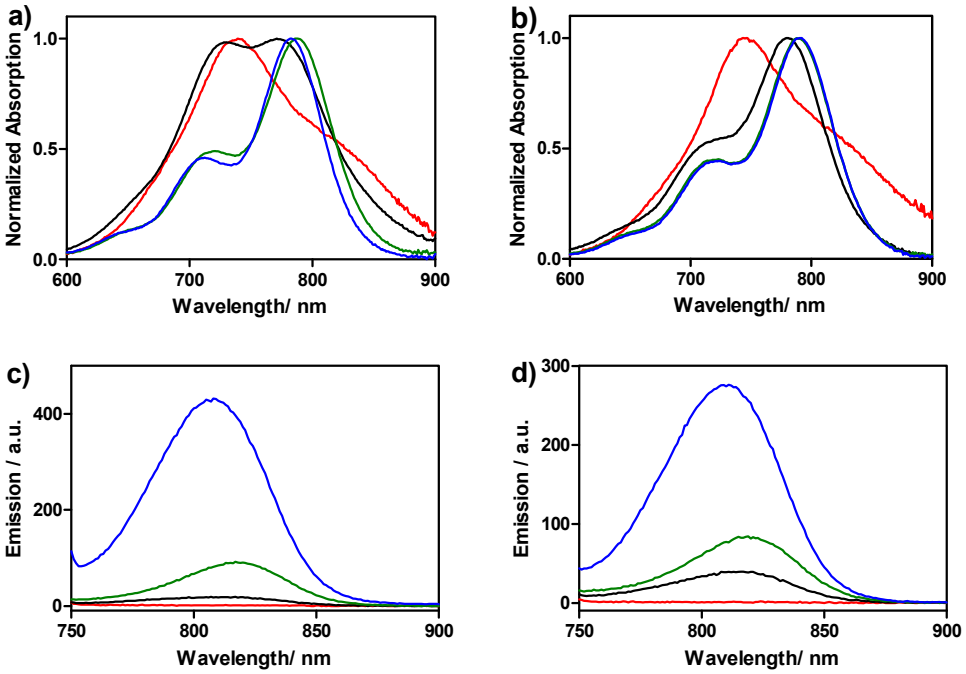


**Figure S1**. Normalized UV/Vis spectra of **QR-G1-COOH** (a) and **QR-G2-COOH** (b) in water (red), methanol (black), DMSO (green) and Pluronic P123 (blue) ($1\times10^{-6}$ M). Emission spectra of **QR-G1-COOH** (c) and **QR-G2-COOH** (d) in water (red), methanol (black), DMSO (green) and Pluronic P123 (blue) ($1\times10^{-6}$ M, $\lambda_{ex}$= 720 nm). The Pluronic concentration is ~0.4% wt/wt for **QR-G1-COOH** and ~10% wt/wt for **QR-G2-COOH**.

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**Photostability**

To test the photostability of the quaterrylene dendrimers, we compared the photobleaching behavior of **QR-G1-COOH** and **QR-G2-COOH** with that of indocyanine green (ICG), a commercially available and FDA approved NIR dye (Sigma-Aldrich, CAS: 3599-32-4). Solutions of the respective dyes with initial absorbance of 1.2 at maximum absorption wavelength in DMSO were kept in capped clear glass vials and exposed to ambient light. The absorption spectra of the dyes solutions were recorded in 1 cm quartz cuvettes (3 ml) over time. Figures S2 shows the absorption spectra of **QR-G1-COOH**, **QR-G2-COOH** and ICG over time.

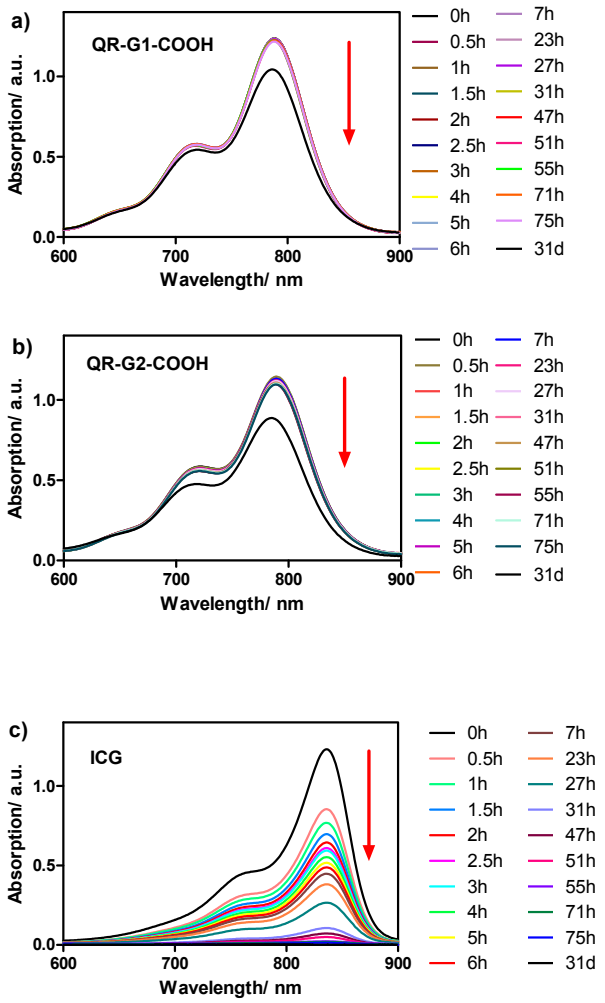


**Figure S2**. The absorption spectra of **QR-G1-COOH** (a), **QR-G2-COOH (b)** and ICG (c) in DMSO over time.

**Quantum yield measurement:**

Fluorescence quantum yield was measured following the method reported,[5] and ICG in methanol was used as a reference standard.

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### Cytotoxicity

The cytotoxicity of **QR-G1-COOH** and **QR-G2-COOH** against the wt-DBT cells was assessed by a Hemocytometer-based trypan blue dye exclusion method.[6] Wt-DBT cells were seeded in two 24-well plates at density of ~$7\times10^4$ cells/mL/0.5 mL (for 24 hours test) and $4\times10^4$ cells/mL/1 mL (for 48 hours test). After 24 hours incubation, the culture medium was removed and the cell culture medium containing test substances was immediately applied to the each well. The negative control group was treated with medium only, 1.6 % DMSO was used as solvent control group, and the positive control group was treated with 5% DMSO. To measure the viable cells, the cells from all the test groups were harvested after 24 or 48 hours by trypsinization using a 0.05% trypsin–EDTA solution and the viable cells were then determined by trypan blue dye exclusion using Hemacytometer.

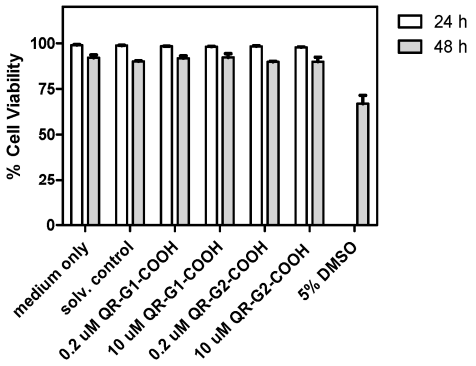


**Figure S3.** Cell viability tests of QR-G1-COOH and QR-G2-COOH on the wt-DBT line. Values are presented as mean ± standard deviation.

### Reference

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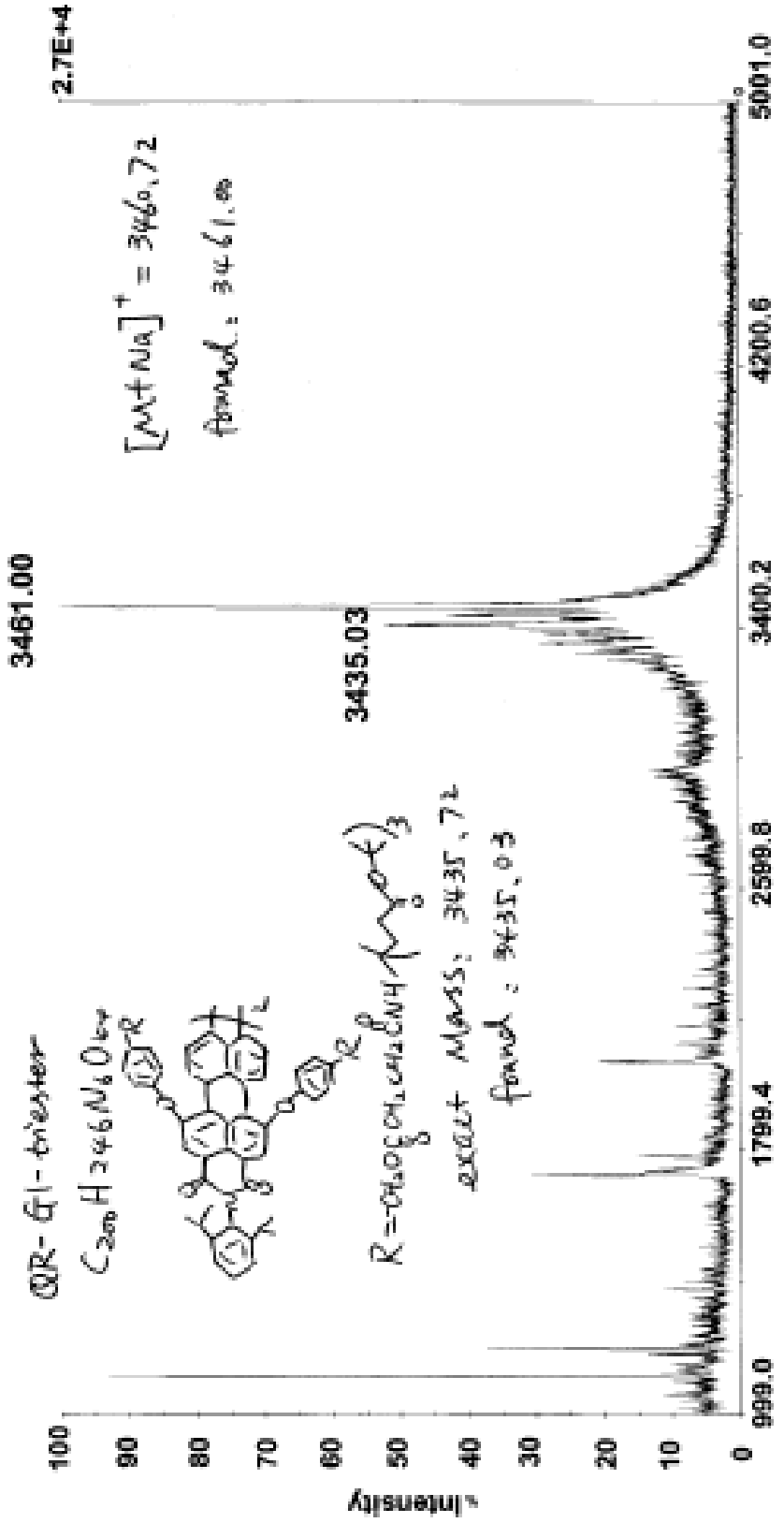

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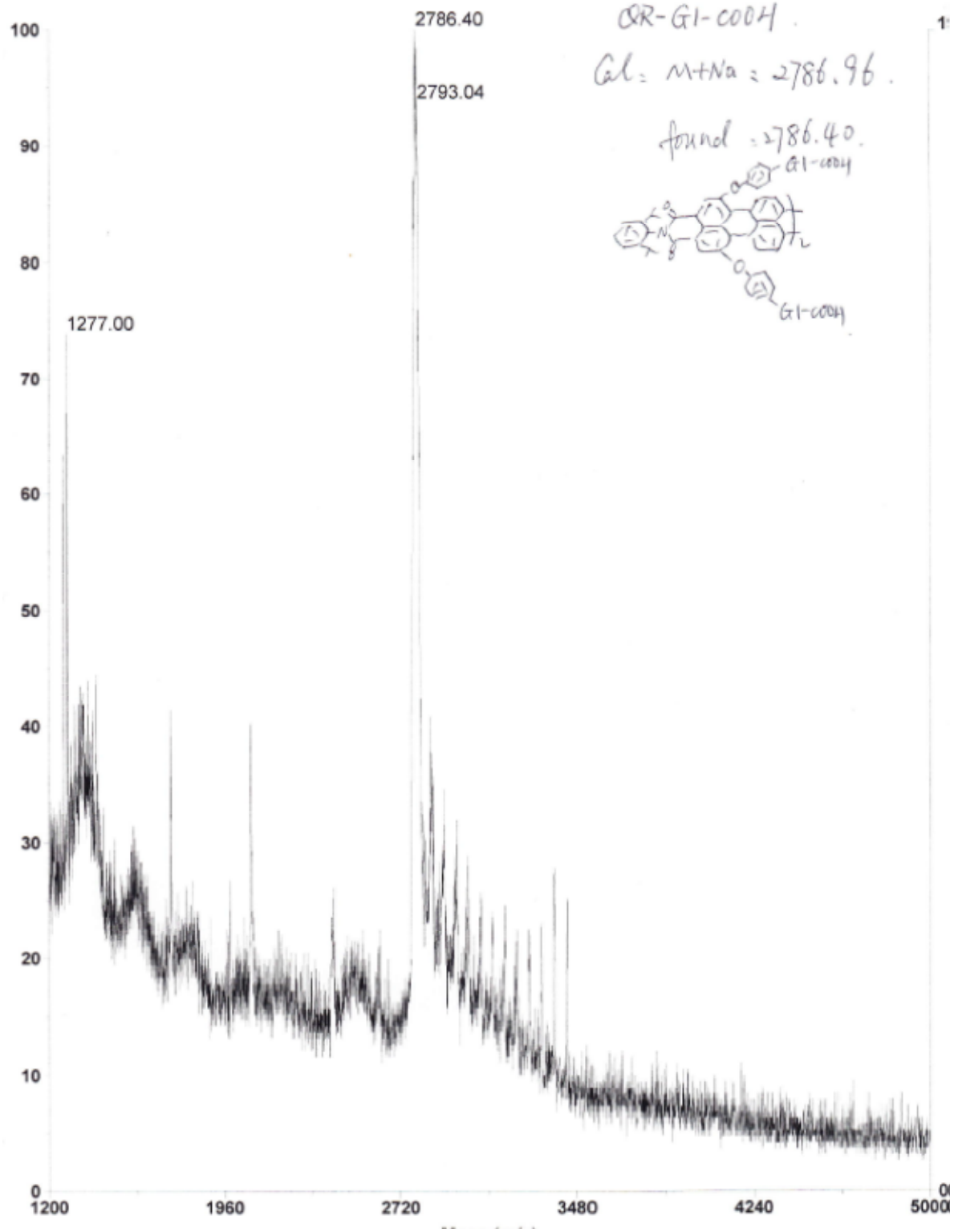

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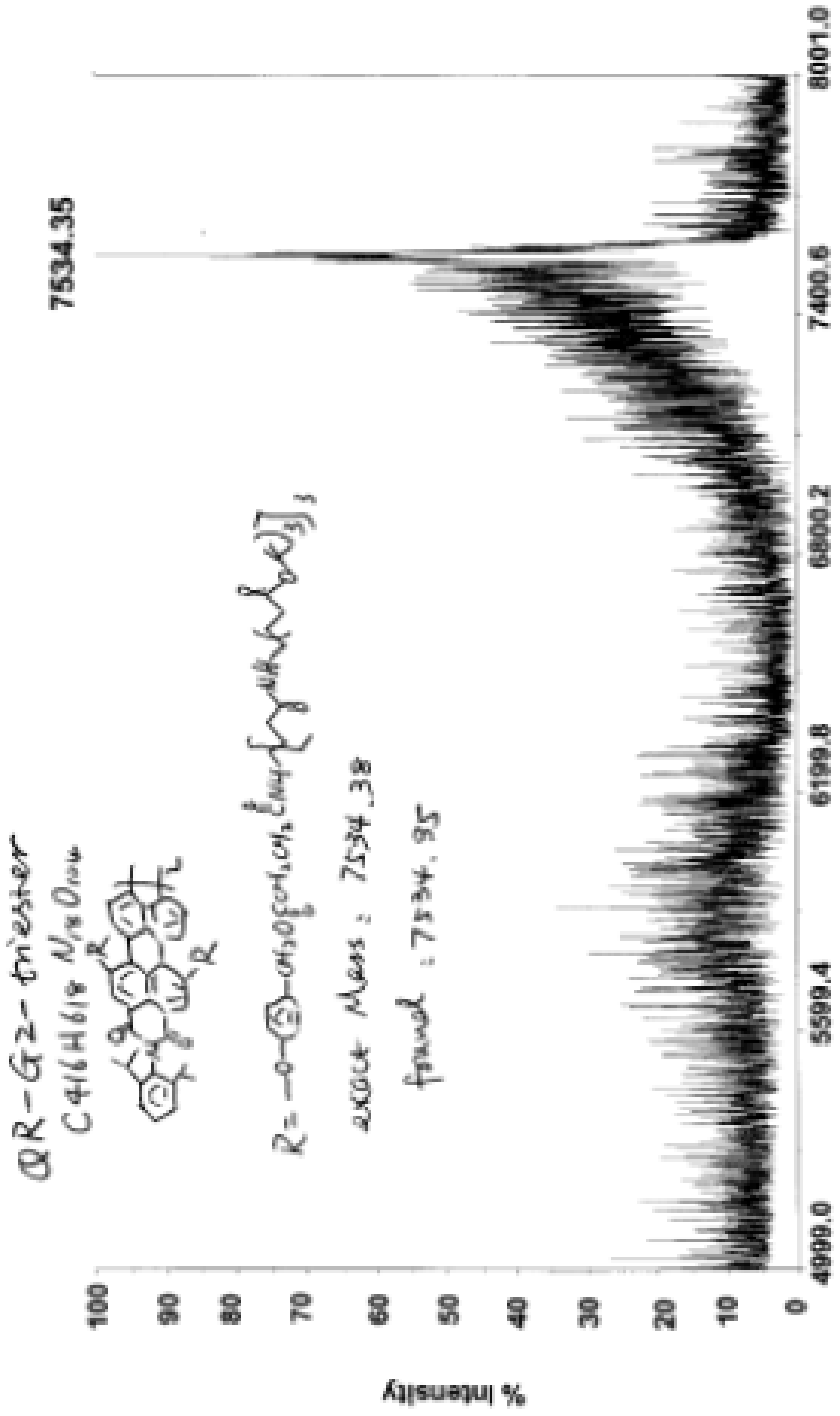

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