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Photophysical Characterization and Self-Assembly Properties of Mono- and bis-Pyrene Derivatives for Cell Imaging Applications

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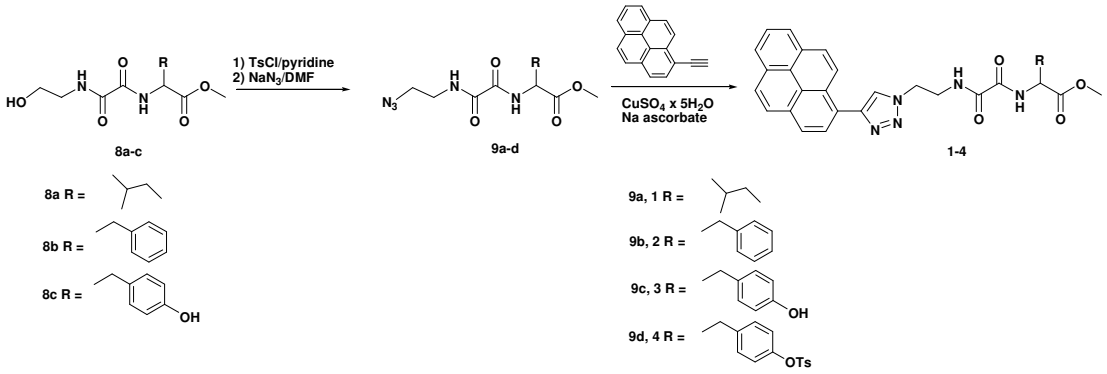


General procedure for the synthesis of ligands 8a-c:

Ethyl oxalyl chloride (9 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise into a mixture of amino acid methyl ester hydrochloride (9 mmol) and triethylamine (19.2 mmol) in dry methylene chloride (50 mL) over 1 h at 0 °C, and stirred for 18 h at room temperature. The mixture was washed with water (2 × 50 mL), saturated aqueous ammonium chloride (3 × 50 mL), and water again (2 × 50 mL). The organic layer was dried over $MgSO_4$ and evaporated to obtain an oxamic acid ethyl esters as colorless oil which was used in next step without further purification.

2-aminoethanol (6.5 mmol) was added to oxamic acid ethyl ester (5.42 mmol) dissolved in dry CH_2Cl_2 (30 mL) and catalytically amount of DMAP.

Synthesis of Leu-2-OH (8a): The reaction mixture was stirred overnight at room temperature. The solvent was removed under vacuum until dryness and the residue was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH= 20:1). A compound was obtained as colourless oil (0.91 g, 62.3 % yield). Spectroscopic analysis: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (d, J = 8.4 Hz, 1H), 8.59 (t, J = 5.8 Hz, 1H), 4.73 (t, J = 5.6 Hz, 1H), 4.42 – 4.29 (m, 1H), 3.61 (s, 3H), 3.44 (dd, J = 11.8, 6.0 Hz, 2H), 3.20 (dd, J = 9.1, 8.6 Hz, 2H), 1.81 (dd, J = 10.6, 9.2 Hz, 1H), 1.58 – 1.45 (m, 2H), 0.84 (2 x d, J = 6.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.95 (s), 160.19 (s), 159.50 (s), 59.14 (s), 52.03 (s), 50.48 (s), 41.72 (s), 38.83 (s), 24.28 (s), 22.85 (s), 20.98 (s).

Synthesis of Phe-2-OH (8b): The reaction mixture was stirred for 2 days at room temperature. The solvent was removed under vacuum until dryness and the residue was recrystallized from EtOH. A compound was obtained as white solid (3.16 g, 87.9 % yield;

m.p. 146-148°C). Spectroscopic analysis: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.95 (d, J = 8.4 Hz, 1H), 8.53 (t, J = 5.9 Hz, 1H), 7.29 – 7.17 (m, 5H), 4.72 (t, J = 5.6 Hz, 1H), 4.58 (td, J = 8.9, 5.3 Hz, 1H), 3.64 (s, 3H), 3.42 (q, J = 6.0 Hz, 2H), 3.23 – 3.08 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.05 (s), 159.93 (s), 159.32 (s), 137.27 (s), 129.00 (s), 128.26 (s), 126.55 (s), 59.11 (s), 53.58 (s), 52.14 (s), 41.70 (s), 35.71 (s).

Synthesis of Tyr-2-OH (8c): The reaction mixture was stirred for 1 day at room temperature. The solvent was removed under vacuum until dryness and the residue was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH= 10:1). A compound was obtained as white solid (1.51 g, 82.3 % yield; m.p. 157-160°C). Spectroscopic analysis: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.82 (d, J = 8.3 Hz, 1H), 8.55 (t, J = 5.8 Hz, 1H), 6.98 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.5 Hz, 2H), 4.73 (t, J = 5.6 Hz, 1H), 4.56 – 4.42 (m, 1H), 3.63 (s, 3H), 3.42 (dd, J = 11.8, 6.0 Hz, 3H), 3.26 – 3.13 (m, 2H), 3.00 (d, J = 7.9 Hz, 2H). ^{13}C NMR 75 MHz, DMSO-d_6) δ 171.17 (s), 159.87 (s), 159.37 (s), 156.00 (s), 129.96 (s), 127.11 (s), 115.11 (s), 59.13 (s), 53.90 (s), 52.09 (s), 41.72 (s), 35.05 (s).

Synthesis of Leu-2-azide (9a): The solution of **8a** (2.6415 g, 10 mmol) in dry pyridine (25 mL), *p*-TsCl (2.2878 g, 12 mmol) was added and the reaction mixture was stirred for 1 day at room temperature. When the reaction was complete (thin-layer chromatography), the solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O, HOAc (5%) and then again with H_2O. The organic phase was dried (Na_2SO_4) and evaporated. The crude tosylate was used in the next step without further purification. The tosylate was reacted with sodium azide (1.300 g, 20 mmol) in DMF (30 mL). The reaction mixture was heated for 1 h at 100°C and stirred overnight at room temperature. The salt was removed by filtration and the filtrate was evaporated under reduced pressure. The remaining solid was purified by column chromatography using CH_2Cl_2/MeOH (100:1) and ethyl acetate/petroleum ether (3:1) as eluens. A compound was obtained as white solid (1.385 g, 48.5 % yield; m.p. 74-76°C). Spectroscopic analysis: ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.03 (d, J = 8.4 Hz, 1H), 8.96 (t, J = 5.9 Hz, 1H), 4.36 (ddd, J = 10.9, 8.4, 3.8 Hz, 1H), 3.63 (s, 3H), 3.48 – 3.42 (m, 2H), 3.38 – 3.33 (m, 2H), 1.87 – 1.79 (m, 1H), 1.59 – 1.50 (m, 2H), 0.91 – 0.81 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.95 (s), 159.96 (s), 159.91 (s), 52.06 (s), 50.52 (s), 49.26 (s), 38.82 (s), 38.47 (s), 24.30 (s), 22.86 (s), 20.99 (s).

Synthesis of Phe-2-azide (9b): The solution of **8b** (1.50 g, 5.10 mmol) in dry pyridine (20 mL), *p*-TsCl (2.33 g, 12.24 mmol) was added and the reaction mixture was stirred for 1 day at room temperature. When the reaction was complete (thin-layer chromatography), the solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O, HOAc (5%) and then again with H_2O. The organic phase was dried (Na_2SO_4) and evaporated. The crude tosylate was used in the next step without further purification. The tosylate was reacted with sodium azide (0.67 g, 10.31 mmol) in DMF (40 mL). The reaction mixture was heated for 1h at 100°C and stirred for 4 days at room temperature. The salt was removed by filtration and the filtrate was evaporated under reduced pressure. The remaining solid was purified by column chromatography using petroleum ether/ethyl acetate (1:1). A compound was obtained as white solid (0.72 g, 44 % yield; m.p. 84-85°C). Spectroscopic analysis: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.99 (d, J = 8.4 Hz, 1H), 8.89 (t, J = 5.7 Hz, 1H), 7.30 – 7.15 (m, 5H), 4.58 (td, J = 9.0, 5.3 Hz, 1H), 3.64 (s, 3H), 3.43 – 3.37 (m, 2H), 3.20 – 3.07 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.12 (s), 159.79 (s), 159.75 (s), 137.29 (s), 129.09 (s), 128.37 (s), 126.67 (s), 53.70 (s), 52.26 (s), 49.32 (s), 38.53 (s), 35.78 (s).

Synthesis of Tyr-OTs-2-azide (9c-d): The solution of **8c** (0.60 g, 1.93 mmol) in dry pyridine (10 mL), *p*-TsCl (0.44 g, 2.32 mmol) was added and the reaction mixture was stirred for 2 days at room temperature. When the reaction was complete (thin-layer chromatography), the solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O, HOAc (5%) and then again with H_2O. The organic phase was dried (Na_2SO_4) and evaporated. The crude tosylate was used in the next step without further purification. The tosylate was reacted with sodium azide (0.25 g, 3.86 mmol) in DMF (20 mL). The reaction mixture was heated for 1h at 100°C and stirred for 2 days at room temperature. The salt was removed by filtration and the filtrate was evaporated under reduced pressure. The remaining solid was purified by column chromatography using CH_2Cl_2/MeOH (100:1).

9c: A compound was obtained as white solid (0.19 g, 25.9 % yield; m.p. 139-141°C). Spectroscopic analysis: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.96 – 8.78 (m, 2H),6.98 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 8.5 Hz, 2H), 4.59 – 4.38 (m, 1H), 3.63 (s, 3H), 3.42 (dd, J = 8.8, 3.5 Hz, 2H), 3.09 – 2.94 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.1, 159.7, 156.0, 130.0, 127.1, 115.1, 53.9, 52.1, 49.2, 38.4, 35.0

9d: A compound was obtained as white solid (0.206 g, 21.8 % yield; m.p. 137-139°C). Spectroscopic analysis: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (d, J = 8.4 Hz, 1H), 8.90 (s, 1H), 7.67 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 4.62 – 4.48 (m, 1H), 3.61 (s, 3H), 3.45 – 3.37 (m, 4H), 3.19 – 3.05 (m, 2H), 2.42 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.68 (s), 145.69 (s), 136.74 (s), 130.52 (s), 130.15 (s), 128.16 (s), 121.78 (s), 53.68 – 53.10 (m), 52.47 – 52.03 (m), 49.20 (s), 34.89 (s), 28.50 – 27.81 (m), 21.78 – 20.82 (m).

Synthesis of Leu-2-pyrene (1): In the flask were added **9a** (1.70 mmol) and 1-ethynylpyrene (2.125 mmol) in 18 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 4.25 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.255 mmol $CuSO_4 \bullet 5H_2O$ in 0.5 mL of water. The solution was stirred vigorously at 70°C for 3 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The organic phase was dried (Na_2SO_4) and evaporated. The precipitate was washed with hot EtOH, filtered and washed with diethyl-ether. The remaining solid was purified by column chromatography using CH_2Cl_2/MeOH (50:1) and ethyl acetate/petroleum ether (2:1). A compound was obtained as yellow solid (0.661 g, 75.1 % yield; m.p. 145-147°C). Spectroscopic analysis: FTIR ($\tilde{\nu}$, cm^{-1}): 3305, 2953, 1742, 1661, 1509, 1433, 1206; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (t, J = 6.0 Hz, 1H), 9.02 (d, J = 8.4 Hz, 1H), 8.84 (d, J = 9.3 Hz, 1H), 8.72 (s, 1H), 8.41 – 8.29 (m, 4H), 8.27 (s, 1H), 8.23 (d, J = 3.5 Hz, 2H), 8.11 (t, J = 7.6 Hz, 1H), 4.71 (t, J = 5.8 Hz, 2H), 4.41 – 4.30 (m, 1H), 3.86 – 3.71 (m, 2H), 3.59 (s, 3H), 1.86 – 1.71 (m, 1H), 1.59 – 1.42 (m, 2H), 0.77 (t, J = 5.2 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.36 (s), 160.39 (s), 160.34 (s), 146.38 (s), 131.41 (s), 131.00 (s), 130.85 (s), 128.43 (s), 128.13 (s), 127.95 (s), 127.82 (s), 127.44 (s), 126.94 (s), 125.98 (s), 125.60 (s), 125.56 (s), 125.36 (s), 125.24 (s), 124.76 (s), 124.40 (s), 52.48 (s), 50.97 (s), 49.07 (s), 39.15 (s), 24.70 (s), 23.22 (s), 21.41 (s); ESI-MS (m/z): calculated for $C_{29}H_{29}N_5O_4Na^+$ (M+Na^+): 534.5, found: 534.4; Elemental analysis for $C_{29}H_{29}N_5O_4$ (M_r = 511.52) theoretical (%): C 68.09; H 5.71; N 13.69; O 12.51, found (%): C 68.12; H 5.77; N 13.73; O 12.55.

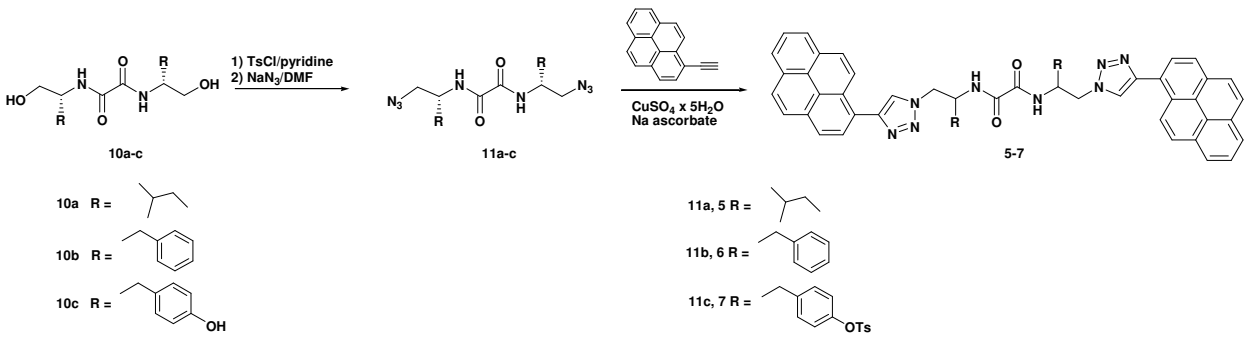
Synthesis of Phe-2-pyrene (2): In the flask were added **9b** (1.80 mmol) and 1-ethynylpyrene (2.25 mmol) in 21 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 4.50 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.27 mmol $CuSO_4 \bullet 5H_2O$ in 0.6 mL of water. The solution was stirred vigorously

at 70°C for 1.5 days and at room temperature for 1.5 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The newly formed percipitate was filtered and washed with diethyl-ether. The residue was recrystallized from a mixture of DMF/water. A compound was obtained as yellow solid (0.63 g, 64.3 % yield; m.p. 197-199°C). Spectroscopic analysis: FTIR ($\tilde{\nu}$, cm^{-1}): 3300, 1734, 1659, 1514, 1277; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.05 (t, J = 6.0 Hz, 1H), 8.98 (d, J = 8.3 Hz, 1H), 8.84 (d, J = 9.3 Hz, 1H), 8.69 (s, 1H), 8.42 – 8.19 (m, 8H), 8.11 (t, J = 7.6 Hz, 1H), 7.20 – 7.13 (m, 4H), 7.08 (dd, J = 7.4, 4.8 Hz, 1H), 4.68 (t, J = 6.0 Hz, 2H), 4.57 (td, J = 8.8, 5.3 Hz, 1H), 3.75 (dd, J = 11.9, 6.0 Hz, 2H), 3.60 (s, 3H), 3.16 – 3.06 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.96 (s), 159.75 (s), 159.57 (s), 145.91 (s), 137.16 (s), 130.94 (s), 130.53 (s), 130.38 (s), 128.96 (s), 128.18 (s), 127.97 (s), 127.65 (s), 127.48 (s), 127.35 (s), 126.98 (s), 126.46 (s), 126.46 – 126.42 (m), 125.51 (s), 125.49 (s), 125.14 (s), 125.10 (s), 124.90 (s), 124.71 (s), 124.29 (s), 123.93 (s), 53.63 (s), 52.10 (s), 48.53 (s), 35.73 (s); ESI-MS (m/z): calculated for $C_{32}H_{27}N_5O_4Na^+$ (M+Na^+): 568.6, found: 568.4; Elemental analysis for $C_{32}H_{27}N_5O_4$ (M_r = 545.59) theoretical (%): C 70.45; H 4.99; N 12.84; O 11.73, found (%): C 70.49; H 5.06; N 12.87; O 11.77.

Synthesis of Tyr-2-pyrene (3): In the flask were added **9c** (0.65 mmol) and 1-ethynylpyrene (0.81 mmol) in 18 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 1.63 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.10 mmol $CuSO_4 \bullet 5H_2O$ in 0.4 mL of water. The solution was stirred vigorously at 70°C for 3 days and at room temperature for 2 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The newly formed percipitate was filtered and washed with diethyl-ether. The residue was recrystallized from a mixture of DMF/water. A compound was obtained as yellow solid (0.08 g, 20.6 % yield; m.p. 131-134°C). Spectroscopic analysis: FTIR ($\tilde{\nu}$, cm^{-1}): 3298, 1723, 1516, 1433, 1218; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.21 (s, 1H), 9.08 (t, J = 5.9 Hz, 1H), 8.87 (d, J = 6.2 Hz, 1H), 8.83 (s, 1H), 8.72 (s, 1H), 8.42 – 8.19 (m, 7H), 8.11 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 8.4 Hz, 2H), 4.68 (t, J = 5.9 Hz, 2H), 4.55 – 4.44 (m, 1H), 3.85 – 3.67 (m, 2H), 3.59 (s, 3H), 3.00 (d, J = 7.3 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.1, 159.8, 159.5, 156.0, 145.9, 130.9, 130.5, 130.4, 129.9, 128.0, 127.7, 127.5, 127.4, 127.0, 126.5, 125.5, 125.1, 124.9, 124.7, 124.3, 123.9, 115.1, 54.0, 52.0, 48.5, 35.1; ESI-MS (m/z): calculated for $C_{32}H_{27}N_5O_5H^+$ (M+H^+): 562.6, found: 562.4; Elemental analysis for

$C_{32}H_{27}N_5O_5$ (M_r = 561.59) theoretical (%): C 68.44; H 4.85; N 12.47; O 14.24, found (%): C 68.46; H 4.90; N 12.51; O 14.27.

Synthesis of Tyr-OTs-2-pyrene (4): In the flask were added **9d** (0.32 mmol) and 1-ethynylpyrene (0.40 mmol) in 15 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 0.8 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.048 mmol $CuSO_4 \bullet 5H_2O$ in 0.2 mL of water. The solution was stirred vigorously at 70°C for 3 days and at room temperature for 3 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The organic phase was dried ($MgSO_4$) and evaporated. The remaining solid was purified by column chromatography using CH_2Cl_2/MeOH (50:1). A compound was obtained as yellow solid (0.32 g, 60.73 % yield; m.p. 94-96°C). Spectroscopic analysis: FTIR ($\tilde{\nu}$, cm^{-1}): 3374, 2953, 1744, 1668, 1502, 1366, 1174; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.08 – 9.02 (m, 1H), 8.83 (d, J = 9.3 Hz, 1H), 8.70 (s, 1H), 8.37 (d, J = 8.0 Hz, 1H, Pir-CH), 8.35 – 8.29 (m, 3H) 8.26 – 8.21 (m, 1H), 8.11 (t, J = 7.6 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 8.6 Hz, 1H), 4.68 (t, J = 6.0 Hz, 1H), 4.60 – 4.47 (m, 1H), 3.81 – 3.68 (m, 1H), 3.57 (s, 3H), 3.13 – 3.03 (m, 2H), 2.36 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.80 (s), 159.67 (d, J = 11.2 Hz), 147.66 (s), 145.92 (s), 145.62 (s), 136.66 (s), 131.36 (s), 130.95 (s), 130.53 (d, J = 2.6 Hz), 130.38 (s), 130.09 (s), 128.10 (s), 127.96 (s), 127.66 (s), 127.47 (s), 127.36 (s), 126.98 (s), 126.47 (s), 125.49 (s), 125.49 (s), 125.14 (s), 125.10 (s), 124.89 (s), 124.72 (s), 124.29 (s), 123.93 (s), 64.90 (s), 53.46 (s), 52.09 (s), 48.56 (s), 34.97 (s), 21.11 (s), 15.52 – 14.91 (m); ESI-MS (m/z): calculated for $C_{39}H_{33}N_5O_7SH^+$ ($M+H^+$): 716.8, found: 716.4; Elemental analysis for $C_{39}H_{33}N_5O_7S$ (M_r = 715.77) theoretical (%): C 65.44; H 4.65; N 9.78; O 15.65; S 4.48, found (%): C 65.42; H 4.68; N 9.85; O 15.69; S 4.52.



Synthesis of Phe-*bis*azide (11b): The solution of **10b** (2.725 g, 7.65 mmol) in dry pyridine (65 mL), *p*-TsCl (4.375 g, 22.95 mmol) was added and the reaction mixture was stirred for 3.5 days at room temperature. When the reaction was complete (thin-layer chromatography), the solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O, HOAc (5%) and then again with H_2O. The organic phase was dried ($MgSO_4$) and evaporated. The crude tosylate was used in the next step without further purification. The tosylate was reacted with sodium azide (1.492 g, 22.95 mmol) in DMF (50 mL). The reaction mixture was heated for 1h at 100°C and stirred overnight at room temperature. The salt was removed by filtration and the filtrate was evaporated under reduced pressure. The remaining solid was purified by column chromatography using CH_2Cl_2/MeOH (100:1). A compound was obtained as white solid (0.972 g, 31.3 % yield; m.p. 118-119°C). Spectroscopic analysis: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (d, J = 9.3 Hz, 1H), 7.30 – 7.05 (m, 5H), 4.20 – 4.05 (m, 1H), 3.48 – 3.36 (m, 2H), 2.77 (d, J = 7.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.56 (s), 137.86 (s), 128.88 (s), 128.14 (s), 126.18 (s), 53.04 (s), 50.74 (s), 36.92 (s).

Synthesis of Tyr-OTs-*bis*azide (11c): The solution of **10c** (0.860 g, 2.21 mmol) in dry pyridine (20 mL), *p*-TsCl (1.053 g, 5.53 mmol) was added and the reaction mixture was stirred for 5 days at room temperature. When the reaction was complete (thin-layer chromatography), the solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O, HOAc (5%) and then again with H_2O. The organic phase was dried ($MgSO_4$) and evaporated. The crude tosylate was used in the next step without further purification. The tosylate was reacted with sodium azide (0.360 g, 5.53 mmol) in DMF (20 mL). The reaction mixture was heated for 1h at 100°C and stirred 7 days at room temperature. The salt was removed by filtration and the filtrate was evaporated under reduced pressure. The remaining solid was purified by column chromatography using petroleum ether/ethyl acetate (1:1). A compound was obtained as white solid (0.643 g, 39 % yield; m.p. 156-157°C). Spectroscopic analysis: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (d, J =

9.3 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.12 – 4.04 (m, 1H), 3.39 (ddd, *J* = 17.0, 12.4, 6.6 Hz, 10H), 2.79 – 2.69 (m, 2H), 2.40 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.55 (s), 147.46 (s), 145.61 (s), 137.32 (s), 131.42 (s), 130.32 (s), 130.09 (s), 128.11 (s), 121.65 (s), 52.96 (s), 50.67 (s), 36.15 (s), 21.12 (s).

Synthesis of Leu-*bis*pyrene (5): In the flask were added **11a** (0.3 mmol) and 1-ethynylpyrene (0.74 mmol) in 15 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 0.74 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.04 mmol $CuSO_4 \bullet 5H_2O$ in 0.2 mL of water. The solution was stirred vigorously at 70°C for 3 days and at room temperature for 2 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The layers were separated and the newly formed percipitate was filtered from the water-layer and washed with diethyl-ether. The residue was recrystallized from a mixture of DMF/H_2O. A compound was obtained as brown-gray solid (0.16 g, 71.3 % yield; m.p. 258-260°C).Spectroscopic analysis: FTIR ($\tilde{\nu}$, cm^{-1}): 3700, 3311, 2956, 1658, 1512; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (d, *J* = 9.3 Hz, 1H), 8.70 (d, *J* = 9.3 Hz, 1H), 8.56 (s, 1H), 8.26 (t, *J* = 7.2 Hz, 1H), 8.22 – 8.11 (m, 1H), 8.09 – 8.02 (m, 1H), 4.63 – 4.55 (m, 2H), 4.44 (s, 1H), 1.70 – 1.50 (m, 2H), 1.35 – 1.21 (m,1H), 0.85 (2 x d, *J* = 6.6 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.79 (s), 145.75 (s), 130.82 (s), 130.37 (s), 130.26 (s), 127.82 (s), 127.48 (s), 127.39 (s), 127.19 (s), 126.88 (s), 126.30 (s), 125.38 (s), 125.31 (s), 125.02 (s), 124.90 (s), 124.71 (s), 124.13 (s), 123.81 (s), 52.69 (s), 48.25 (s), 24.25 (s), 23.04 (s), 21.55 (s); ESI-MS (m/z): calculated for $C_{50}H_{46}N_8O_2H^+$ ($M+H^+$): 792.0, found: 791.5; Elemental analysis for $C_{50}H_{46}N_8O_2$ (M_r = 790.95) theoretical (%): C 75.93; H 5.86; N 14.17; O 4.05, found (%): C 75.91; H 5.82; N 14.19; O 4.03.

Synthesis of Phe-*bis*pyrene (6): In the flask were added **11b** (1.23 mmol) and 1-ethynylpyrene (3.075 mmol) in 39 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 3.075 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.185 mmol $CuSO_4 \bullet 5H_2O$ in 0.6 mL of water. The solution was stirred vigorously at 70°C for 2 days and at room temperature for 2 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The newly formed percipitate was filtered and washed with diethyl-ether. The residue was recrystallized from a DMF. A compound was obtained as light brown solid (0.687 g, 65.0 %

yield; m.p. 296-298°C). Spectroscopic analysis: FTIR $\tilde{\nu}$, cm^{-1}): 3336, 3040, 1661, 1510, 1450, 1363; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.97 (d, J = 8.3 Hz, 1H), 8.66 (d, J = 9.4 Hz, 1H), 8.54 (s, 1H), 8.24 (dd, J = 7.5, 4.1 Hz, 2H), 8.11 (d, J = 12.4 Hz, 4H), 8.02 (dd, J = 8.2, 3.5 Hz, 2H), 7.33 – 7.10 (m, 5H), 4.79 – 4.45 (m, 3H), 2.94 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.95 (s), 146.24 (s), 138.11 (s), 131.27 (s), 130.80 (s), 130.72 (s), 129.51 (s), 128.65 (s), 128.27 (s), 127.92 (s), 127.82 (s), 127.63 (s), 127.33 (s), 126.75 (s), 125.82 (s), 125.73 (s), 125.49 (s), 125.34 (s), 125.17 (s), 124.55 (s), 124.25 (s), 52.72 (s), 51.80 (s), 37.54 (s); ESI-MS (m/z): calculated for $C_{56}H_{42}N_8O_2H^+$ (M+H^+): 860.0, found: 859.4; Elemental analysis for $C_{56}H_{42}N_8O_2$ (M_r = 858.99) theoretical (%): C 78.30; H 4.93; N 13.04; O 3.73, found (%): C 78.28; H 4.95; N 13.08; O 3.70.

Synthesis of Tyr-OTs-*bis*pyrene (7): In the flask were added **11c** (0.250 mmol) and 1-ethynylpyrene (0.625 mmol) in 15 mL of water/THF/ethanol mixture (1/1/1, v/v/v). 0.625 mL of freshly prepared 1M aqueous solution of sodium ascorbate was then injected and followed by the addition of 0.038 mmol $CuSO_4 \bullet 5H_2O$ in 0.1 mL of water. The solution was stirred vigorously at 70°C for 4 days and at room temperature for 2 days. Then, reaction mixture was evaporated to dryness. CH_2Cl_2 and 25% aqueous solution of NH_3 were added to the residue. The layers were separated and the newly formed percipitate was filtered from the water-layer and washed with diethyl-ether. The residue was recrystallized from a mixture of DMF/H_2O. A compound was obtained as light brown solid (0.1578 g, 66.7 % yield; m.p. 263-265°C). Spectroscopic analysis: FTIR ($\tilde{\nu}$, cm^{-1}): 3308, 1656, 1503, 1198, 1177; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.96 (d, J = 9.0 Hz, 1H), 8.63 (d, J = 9.3 Hz, 1H), 8.51 (s, 1H), 8.23 (dd, J = 11.3, 7.5 Hz, 2H), 8.14 – 8.08 (m, 4H), 8.06 – 7.99 (m, 2H), 7.66 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 4.69 – 4.55 (m, 4H), 2.94 – 2.85 (m, 3H), 2.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.43 (s), 147.61 (s), 145.78 (s), 145.60 (s), 137.09 (s), 131.43 (s), 130.78 (s), 130.46 (s), 130.32 (d, J = 3.3 Hz), 130.22 (s), 130.09 (s), 128.08 (s), 127.76 (s), 127.42 (s), 127.31 (s), 127.13 (s), 126.81 (s), 126.25 (s), 125.32 (s), 125.19 (s), 124.98 (s), 124.82 (s), 124.69 – 124.52 (m), 124.05 (s), 123.75 (s), 121.68 (s), 52.18 (s), 51.11 (s), 40.02 (s), 36.36 (s), 21.09 (s); ESI-MS (m/z): calculated for $C_{70}H_{54}N_8O_8S_2Na^+$ (M+Na^+): 1222.4, found: 1221.5; Elemental analysis for $C_{70}H_{54}N_8O_8S_2$ (M_r = 1199.36) theoretical (%): C 70.10; H 4.54; N 9.34; O 10.67; S 5.35, found (%): C 70.18; H 4.58; N 9.39; O 10.72; S 5.41.

UV spectra of 1 – 7

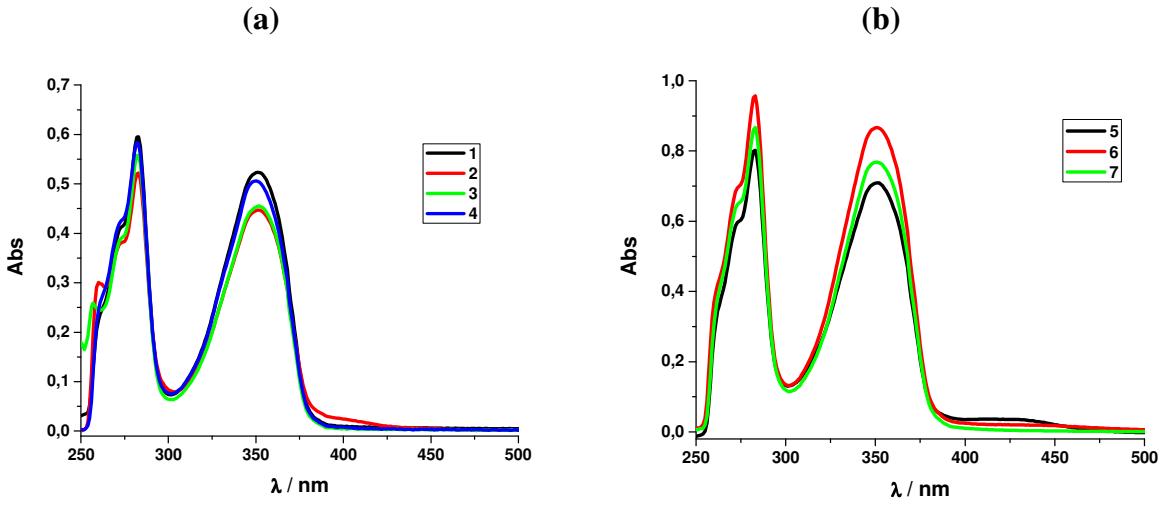


Figure S1. UV-Vis spectra of (a) mono-pyrenes **1 - 4** and (b) bis-pyrenes **5** – **7** in DMSO (c = 1.5×10^{-5} mol dm^{-3}).

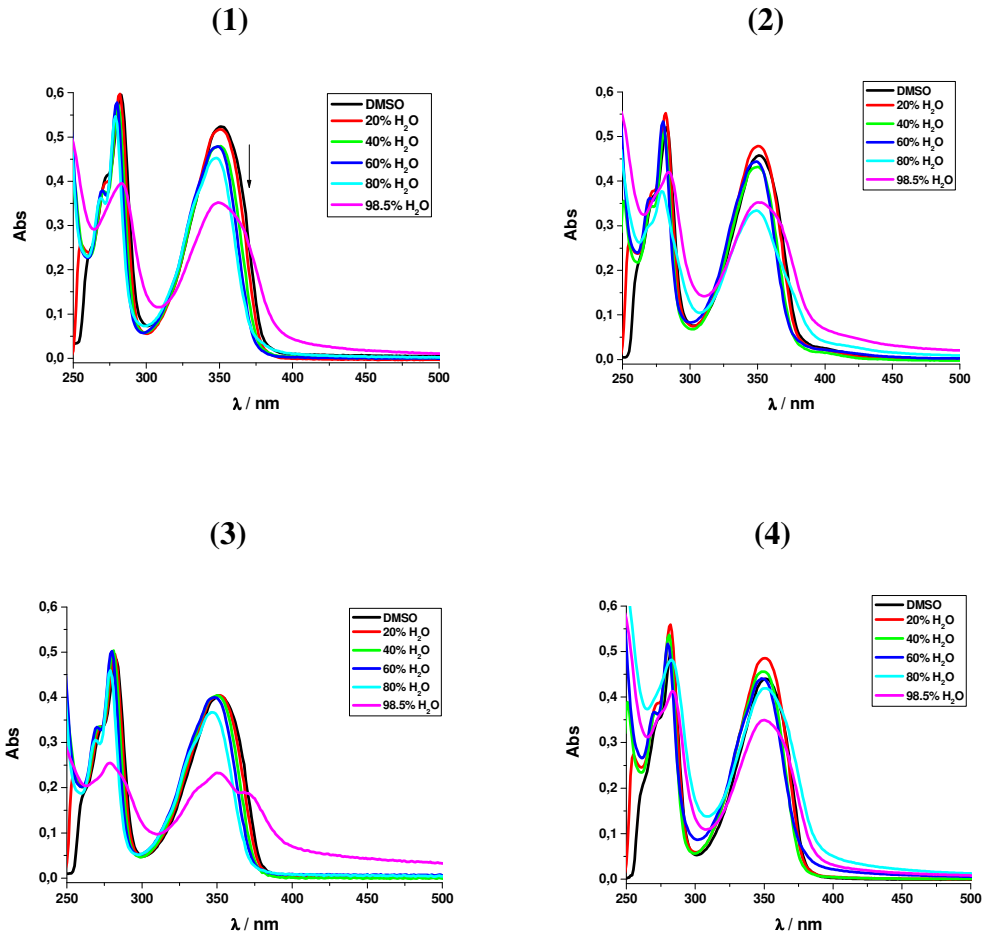


Figure S2. Solvent-dependent UV-Vis spectra of compounds **1 - 4** in DMSO and DMSO/water solutions ($c = 1.5\times10^{-5}$ mol dm^{-3}).

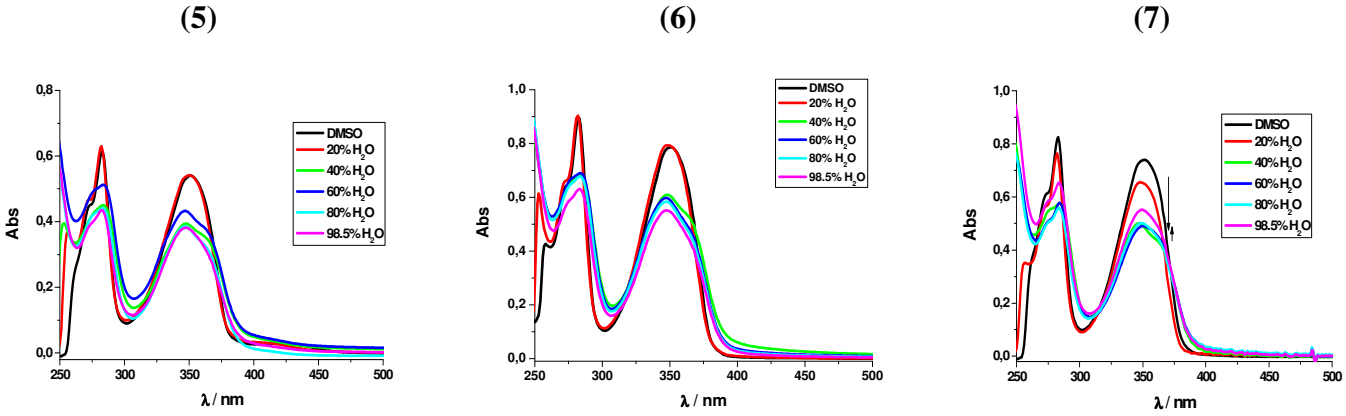


Figure S3. Solvent-dependent UV-Vis spectra of compounds **5 - 7** in DMSO and DMSO/water solutions ($c = 1.5\times10^{-5}$ mol dm^{-3}).

Fluorescence spectra of 1 – 7

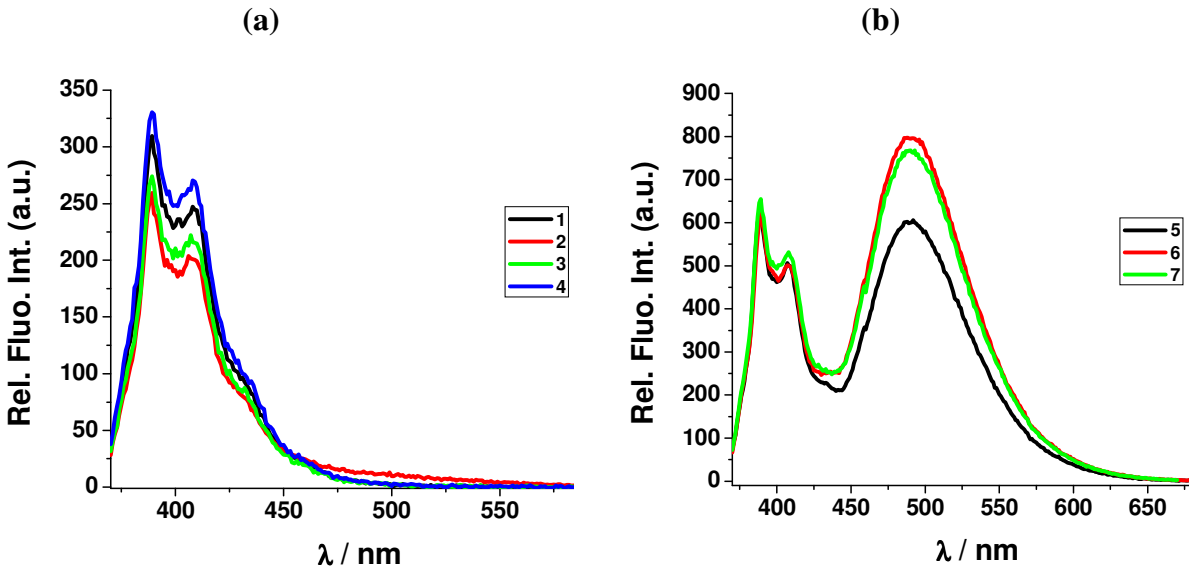


Figure S4. Fluorescence spectra of (a) mono-pyrenes **1 - 4** (λ_{ex} = 351 nm, slit 2.5-5) and (b) bis-pyrenes **5** – **7** (λ_{ex} = 351 nm, slit 5-5) in DMSO (c = 1×10^{-6} mol dm^{-3}).

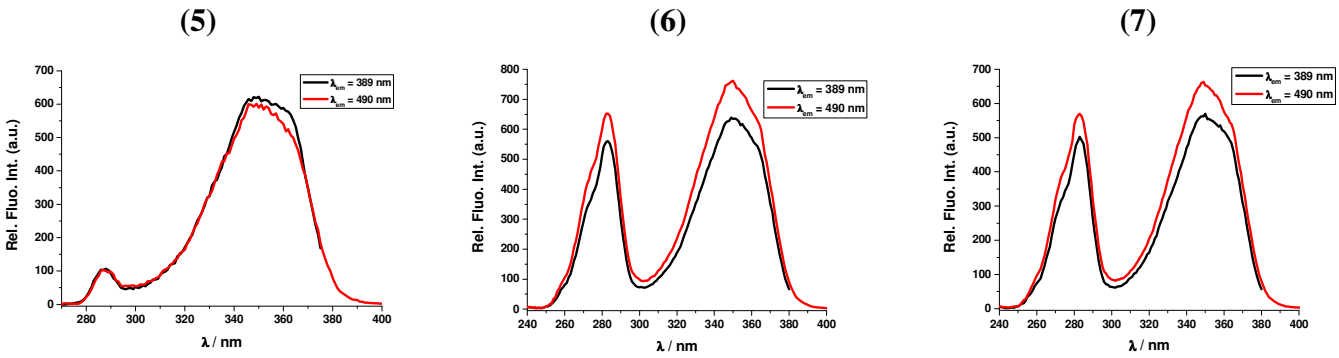


Figure S5. Excitation spectra of **5** – **7** (c = 1×10^{-6} mol dm^{-3} in DMSO) recorded at 389 nm (monomer emission) and 490 nm (excimer emission).

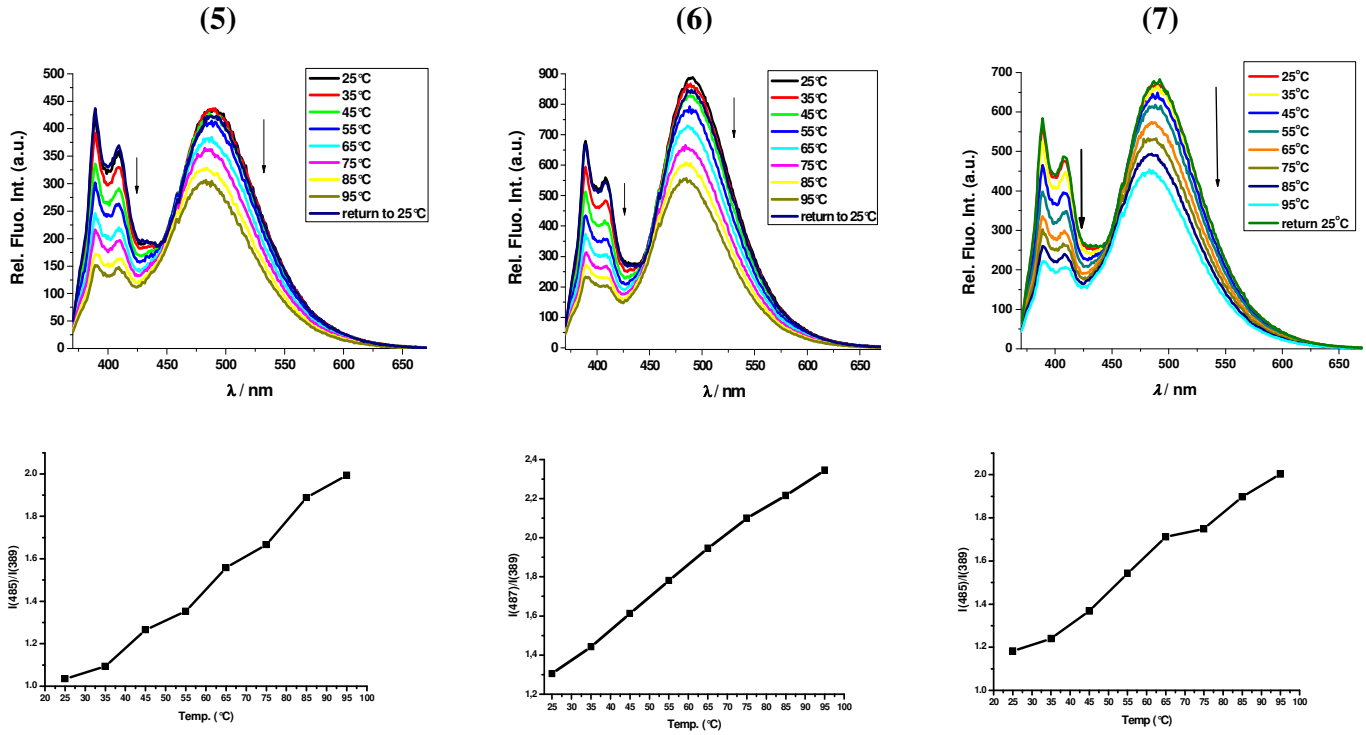


Figure S6. Temperature-dependence of fluorescence and excimer-to-monomer ratio (I_E/I_M) of compounds **5 - 7** in DMSO solutions ($c = 1\times10^{-6}$ mol dm^{-3}, $\lambda_{ex} = 351$ nm, slit 5-5).

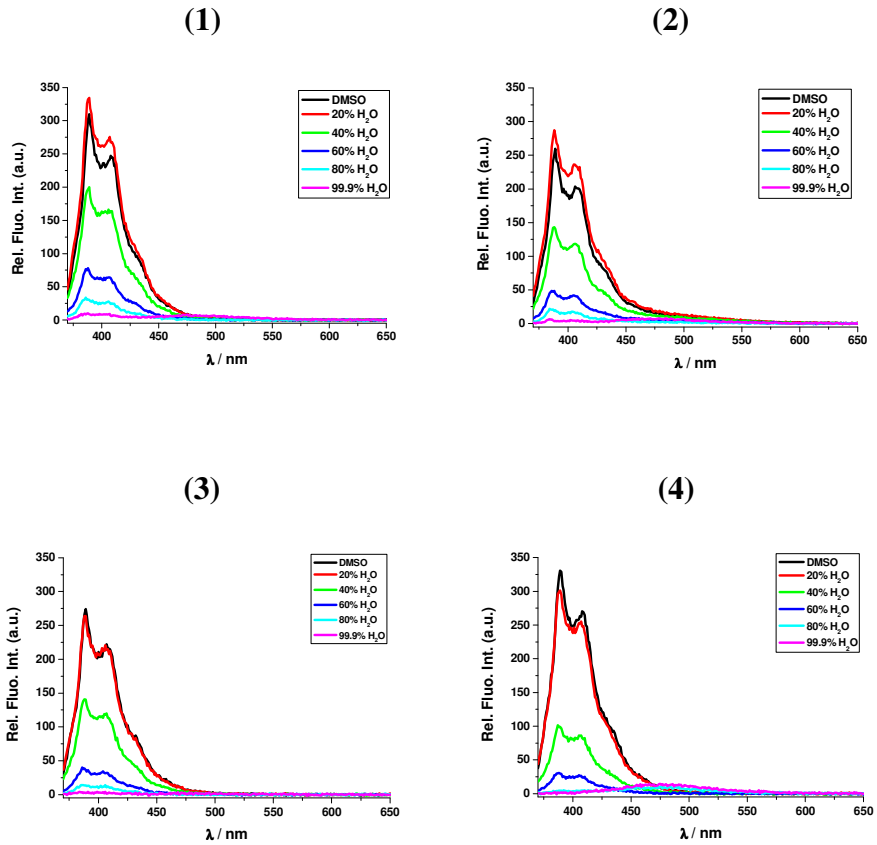


Figure S7. Solvent-dependent fluorescence spectra of compounds **1** - **4** in DMSO and DMSO/water solutions ($c = 1\times10^{-6}$ mol dm^{-3}, $\lambda_{ex} = 351$ nm, slit 2.5-5).

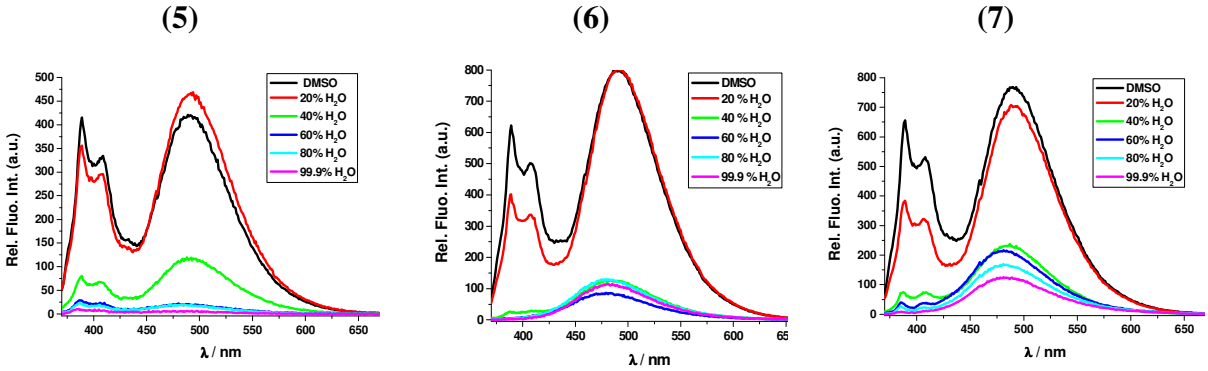
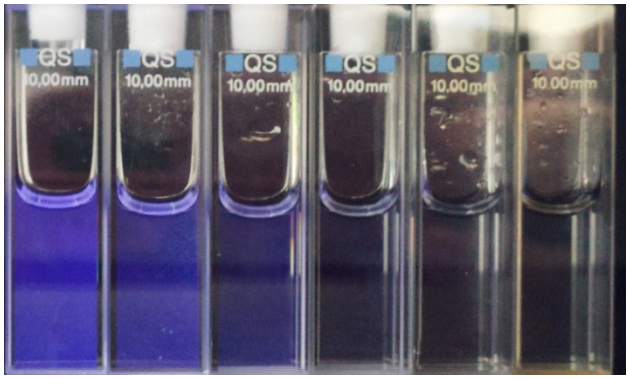


Figure S8. Solvent-dependent fluorescence spectra of compounds **5** - **7** in DMSO and DMSO/water solutions ($c = 1\times10^{-6}$ mol dm^{-3}, $\lambda_{ex} = 351$ nm, slit 5-5).

(a)



(b)

(c)

Figure S9. Photographs of (a) **1**, (b) **4** and (c) **5** in DMSO/water mixtures with various water fractions. From left to right: the water fraction is 0%, 20%, 40%, 60%, 80%, and 99.9%.

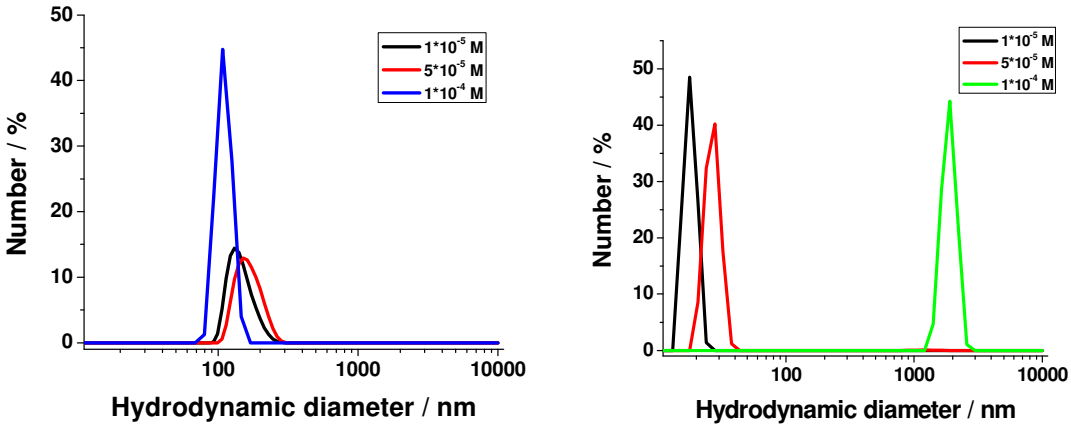


Figure S10. Dynamic light scattering (DLS) analysis of (a) **1** and (b) **3** at different concentrations measured in 99% water/DMSO.

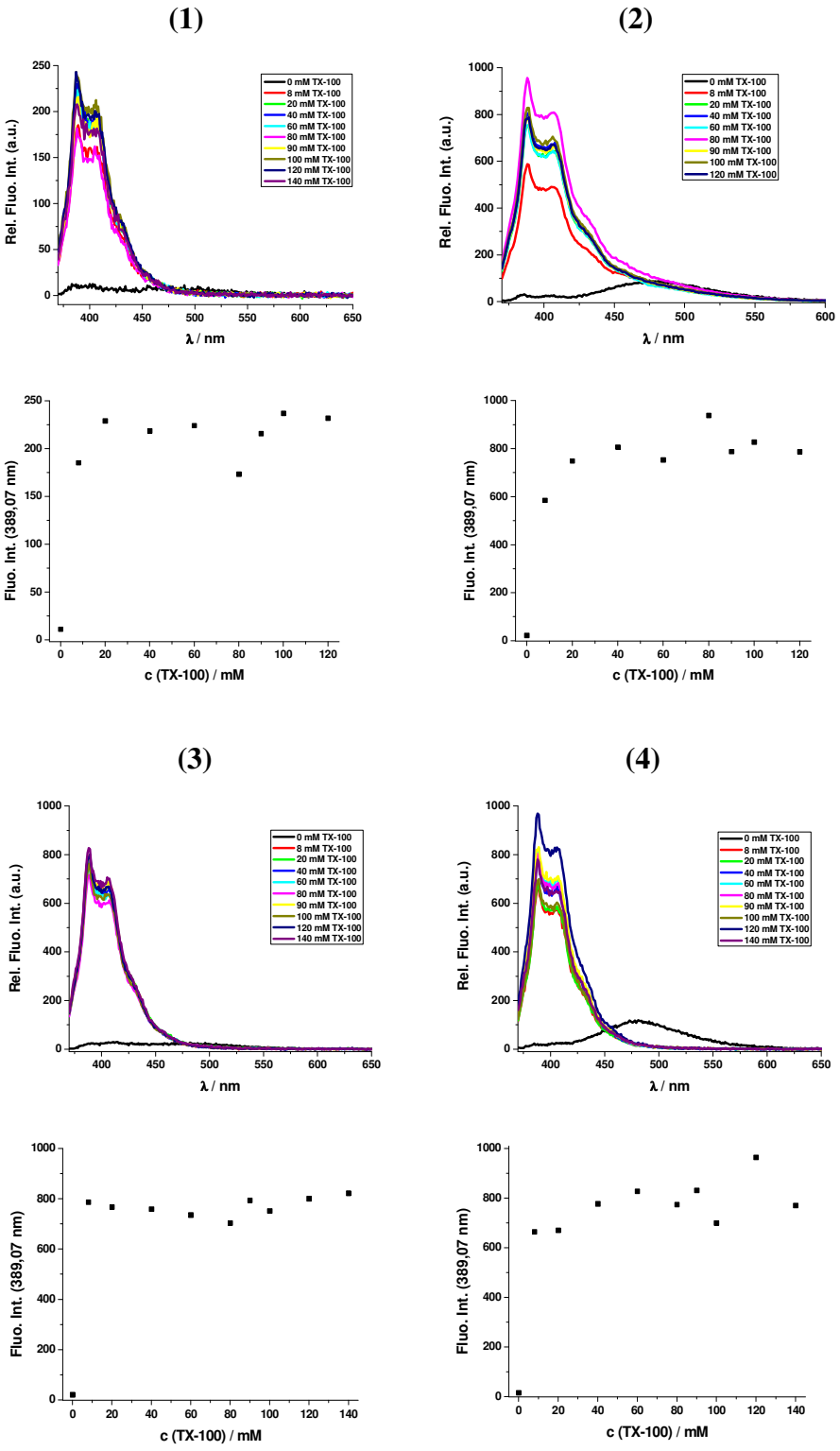


Figure S11. Fluorescence emission spectra of **1 - 4** (c = 1×10^{-6} mol dm^{-3}, λ_{ex} = 351 nm, slit 2.5-5 for **1**, and 5-5 for **2** – **4**, respectively) treated with different concentrations of TX-100 in H_2O [<1% DMSO] and dependence of the maximum fluorescence intensity (389 nm) on the concentration of TX-100 in the sample.

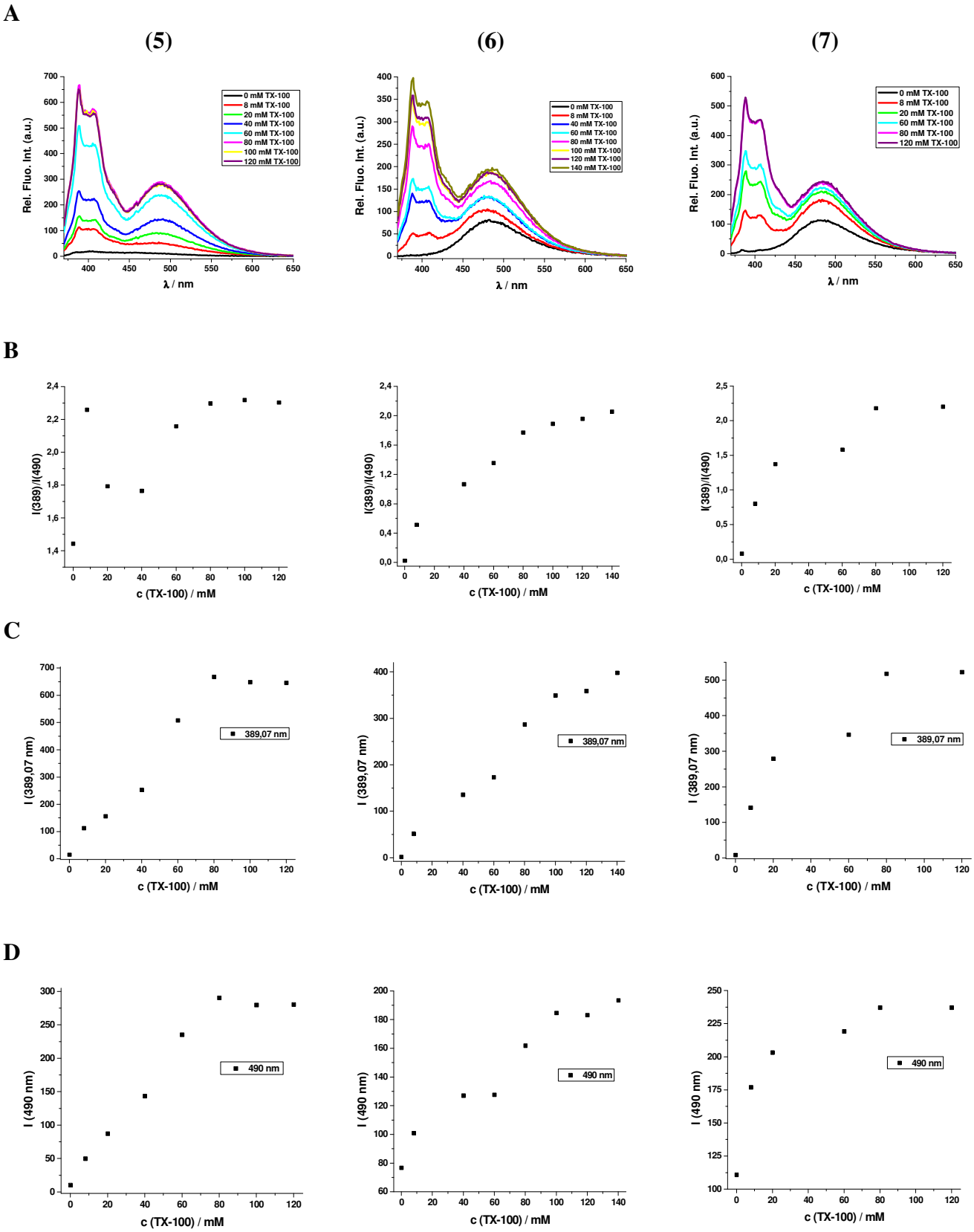


Figure S12. (A) Fluorescence emission spectra and (B) monomer-to-excimer ratio (I_M/I_E) of **5** - **7** ($c = 1 \cdot 10^{-6}$ mol/dm^3, λ_{ex} = 351 nm, slit 5-5) treated with different concentrations of TX-100 in H_2O (pH = 7.4, c = 10 mM) [<1% DMSO]. Dependence of the maximum fluorescence intensity 389 nm (C) and 490 nm (D) on the concentration of TX-100 in **5** - **7**.

Figure S13. Photographs showing fluorescence emission changes of **1** (left) and **5** (right); from left to right: solution in DMSO, fluorescence quenching after addition of water and fluorescence enhancement upon addition of TX-100.

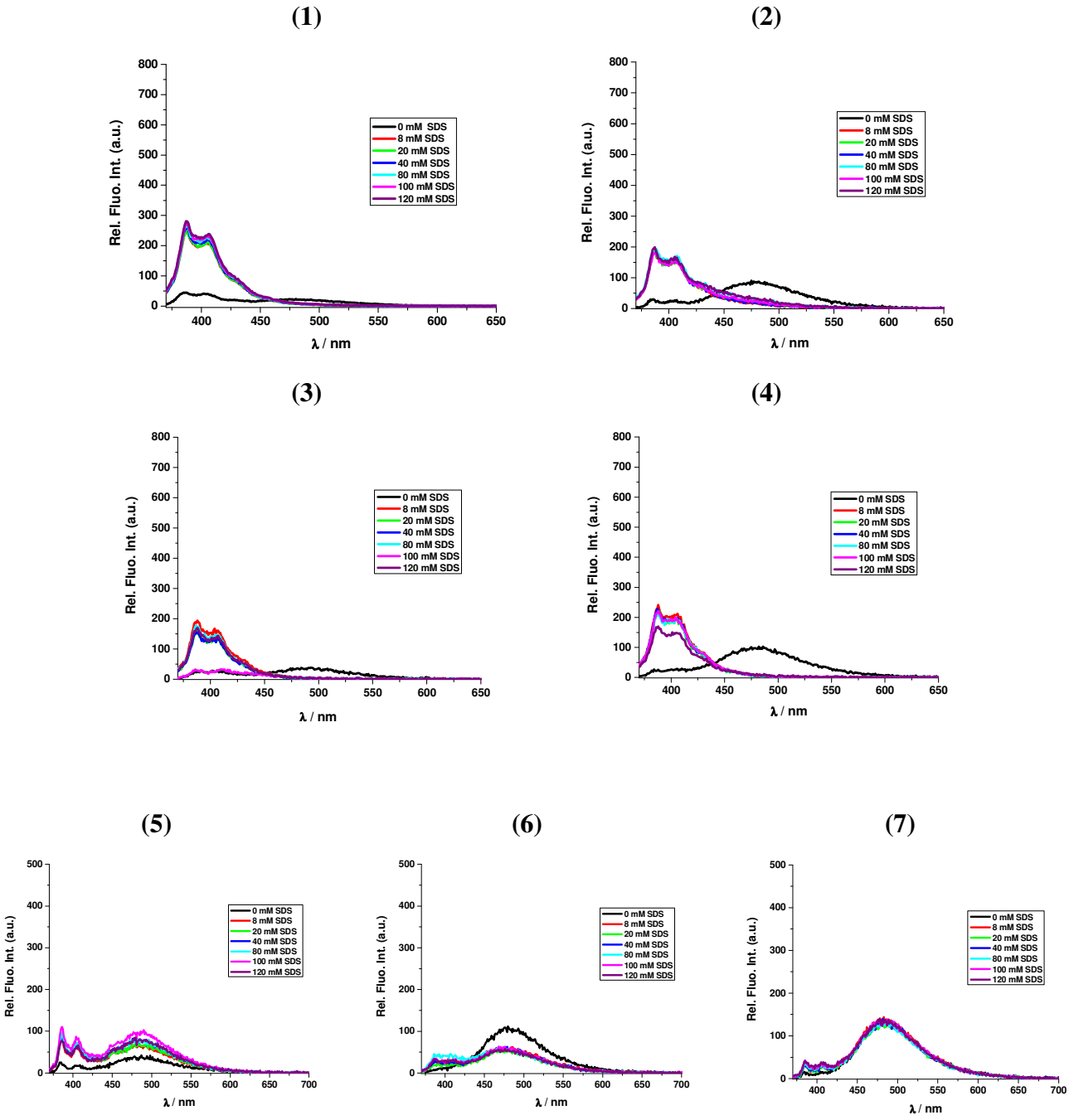


Figure S14. Fluorescence emission spectra of **1 - 7** ($c = 1\times10^{-6}$ mol dm^{-3}, $\lambda_{ex} = 351$ nm, slit 5-5) treated with different concentrations of SDS in H_2O [<1% DMSO].

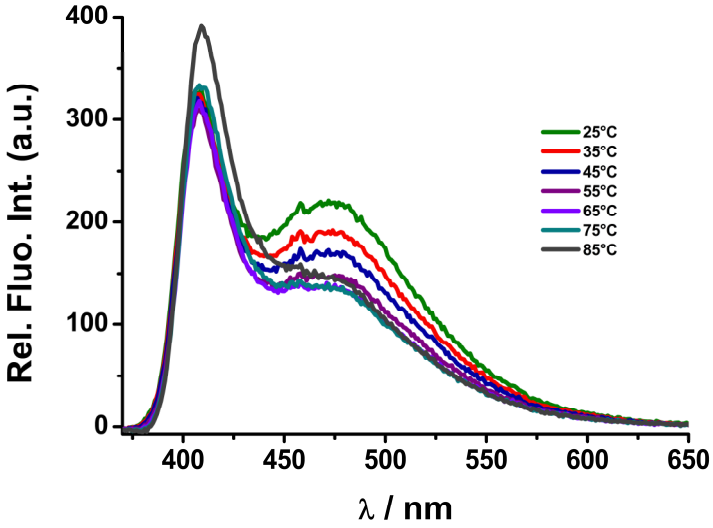


Figure S15. Fluorescence emission spectra at various temperature of **1-gel** in water/DMSO (4:1 v/v).

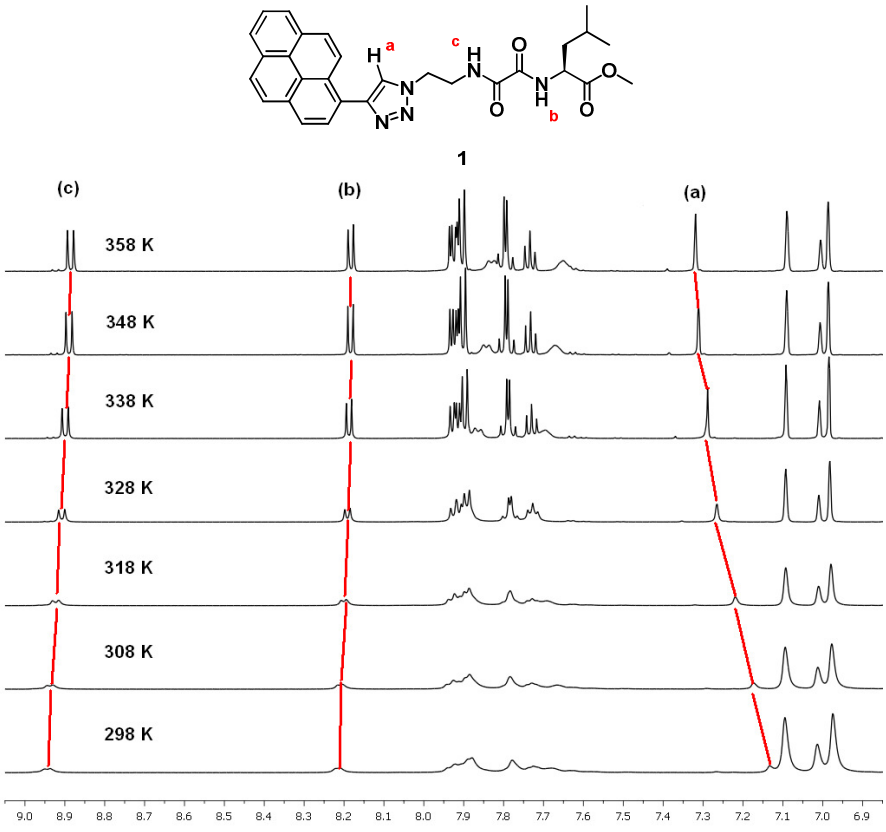


Figure S16. ^{1}H-NMR spectra of **1-gel** in toluene-d_8 in the 298-358 K range.

Biological studies:

Cells. Human lung carcinoma (A549; ATCC CCL-185) and human rhabdomyosarcoma (RD; ATCC CCL-136) cells were obtained from the ATCC Cell Biology Collection and were cultured according to the manufacturer's instructions. Cells were grown in Dulbecco Modified Eagle's Medium (DMEM, Sigma Aldrich, USA) supplemented with 10% of fetal bovine serum (FBS, Sigma Aldrich, USA) at 37 °C and 5 % CO_2 in a humified atmosphere.

Cytotoxicity assay – MTT. All studied compounds were dissolved in an appropriate volume of dimethyl sulfoxide solution (DMSO) in order to obtain the 1 mM stock solution. A549 or RD cells were seeded in 96-well tissue culture plates (7 x 10^3 cells/well), and 24 h later treated with compounds (concentration range of 10^{-8} M – 10^{-6} M, dilutions prepared in DMEM supplemented with 10% of FBS). Cells treated with the same dilutions of DMSO represented control sample. Plates were incubated (37 °C, 5 % CO_2) for 72 h. After that period, medium was removed from all samples, and 1x diluted MTT solution was added into each well. Plates were incubated (37 °C, 5 % CO_2) for 4 h allowing formazan crystals to form. The resulting MTT-formazan products were dissolved using DMSO, and their absorbance was measured using a microplate reader (Awareness Technology, Inc., United States) at 600 nm. Results are presented as cell survival representing absorbance mean ± SD made in four replicates, relative to the mean absorbance of control sample (DMSO).

Confocal microscopy. For live imaging, A549 cells were seeded in Ibidi imaging cell chambers at a density of 5 x 10^4 cells per well (Ibidi, Germany), and 48 h later incubated with compounds **1**, **2**, **5** and **7** (final concentration 10^{-5} M) for 90 min at 37 °C and then immediately monitored by confocal microscope. To check colocalization with mitochondria, cells were rinsed after incubation with compound **2**, and then incubated with a MitoTracker Deep Red solution (Invitrogen, Molecular Probes; final concentration 100 nM) for 10 min at 37 °C. Afterwards, cells were rinsed with DMEM supplemented with 10% of FBS and then immediately monitored by confocal microscope. To check uptake of compound **5**, cells were cooled on ice for 10 min 48 h after seeding. Cells were then treated with ice-cold solution of compound **5** (final concentration 10^{-4} M) and dextran (TRITC-dextran; Mr 65-85 kDa; Sigma-Aldrich, USA; final concentration 0.5 mg/mL) in DMEM supplemented with 10% of FBS for 15 min. Afterwards, cells were transferred to an incubator (37 °C and 5 % CO_2 in a humified atmosphere) for 1 h and then immediately observed by confocal microscope. For fixed cells imaging, A549 cells were seeded in 24-well plates on coverslips at a density of 2 x 10^4 cells per well and 48 h later fixed with 2% paraformaldehyde in PBS for 12 min at room temperature, three times washed with PBS, permeabilized with 0.1% Triton in PBS for 2 min at room temperature, washed two times with PBS and incubated with compounds for 15 min at room temperature. For titration of **1** - **7**, serial dilutions were prepared in DMSO (range 10^{-5} M to 10^{-3} M), and after incubation cells were washed two times with PBS and two times with mqH_2O and then slides were incorporated in mounting medium. Cells were then observed by confocal microscope. For fixed cells colocalization studies, cells were incubated in 10^{-3} M concentration of compound **5**, washed two times with PBS, blocked with 3% BSA/PBS for 30 min at room temperature and incubated with primary antibody against early endosome antigen 1 (EEA1, Cell Signaling Technology, USA, #2411, rabbit, 1:100 in 5% BSA/PBS), Lysosome Associated Membrane Protein 1 (LAMP-1, Abcam, UK, ab2417, rabbit, 1:250 in 5% BSA/PBS) or Golgi Apparatus (GM130, Cell Signaling Technology, USA, #12480, rabbit, 1:2500 in 5% BSA/PBS) for 1 h at room temperature. Cells were washed three times with PBS and then incubated with fluorescently labeled AF647 anti-rabbit secondary antibody (Cell Signaling Technology, USA, #4414, 1:1000 in 5% BSA/PBS) for 1 h at room

temperature. After washing cells two times with PBS and two times with mqH_2O, slides were incorporated in mounting medium. Cells were then observed by confocal microscope. Leica TCS SP8 X inverted confocal microscope (Leica Microsystems, Germany) with 63 x/1.40 oil-immersion objective was used. Colocalization was assessed by Pearson's correlation coefficient. Analysis was done by LAS X (Leica Microsystems, Germany), ImageJ (NIH, USA) software and appropriate JACoP plugin. To determine cell edges, Reflection Interference Contrast Microscopy (RICM) was used.

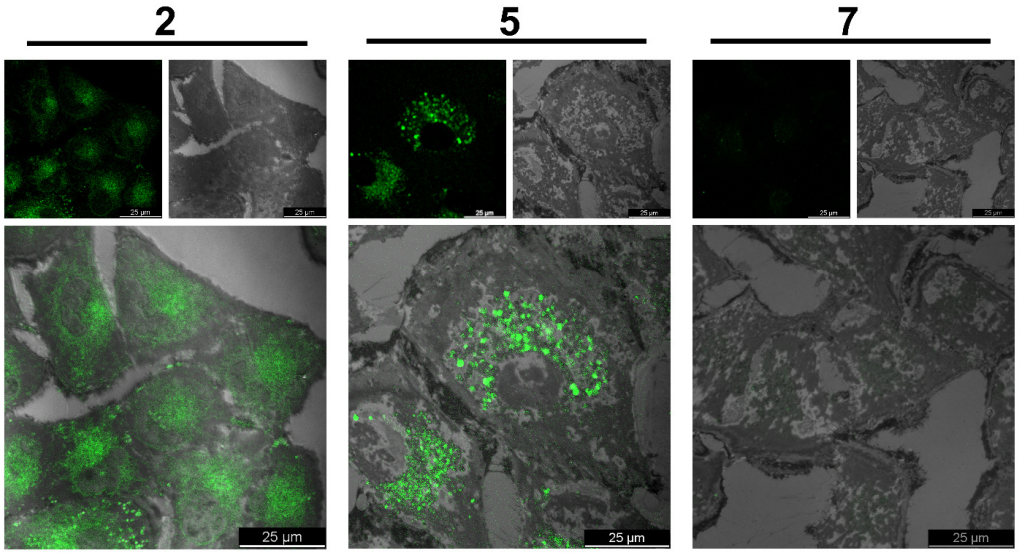


Figure S17. Fluorescent signal of 2, 5 and 7 in live A549 cells. Cells were incubated with 10^{-5} M of tested compounds (shown in green; λ_{exc} = 405 nm, λ_{em} = 410 - 510 nm) for 90 min at 37 °C and then monitored by confocal microscopy. Reflection Interference Contrast Microscopy (RICM) is shown in gray.

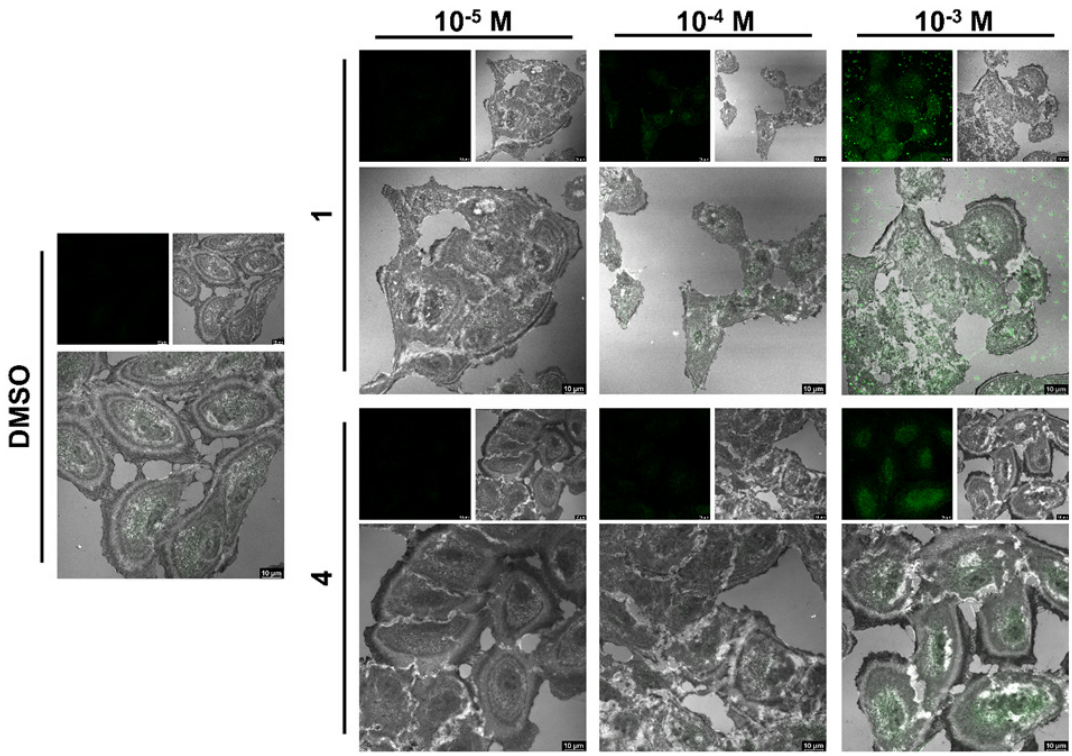


Figure S18. LCSM images of fixed A549 cells after incubation with pyrene derivatives **1** and **4** (range 10^{-5} M to 10^{-3} M; prepared in DMSO; shown in green; λ_{exc} = 405 nm, λ_{em} = 410 - 480 nm). Reflection Interference Contrast Microscopy (RICM) is shown in grey. Cells incubated with DMSO represented negative control.

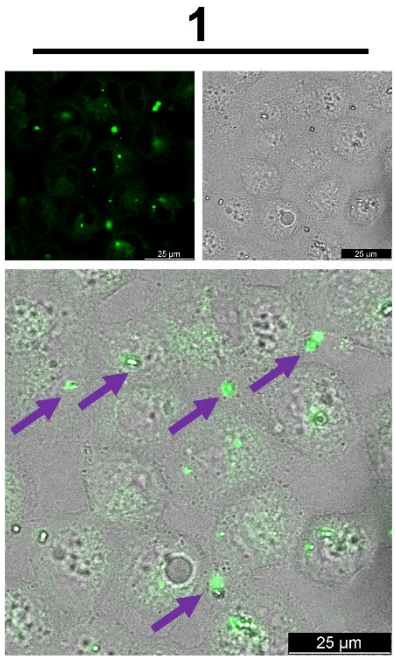


Figure S19. Fluorescent signal of 1 in live A549 cells. Cells were incubated with 10^{-5} M of **1** (shown in green; λ_{exc} = 405 nm, λ_{em} = 410 - 510 nm) for 90 min at 37 °C and then monitored by confocal microscopy. Bright field is shown in gray. Extracellular fluorescent signals of **1** are indicated by purple arrows.

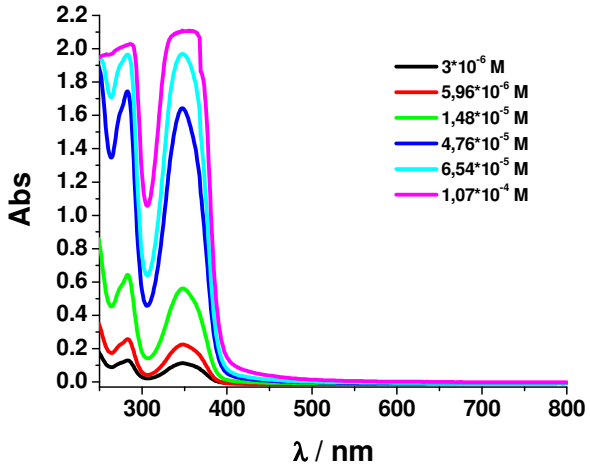


Figure S20. Absorption spectra of bis-pyrene **5** measured in water at different concentrations.

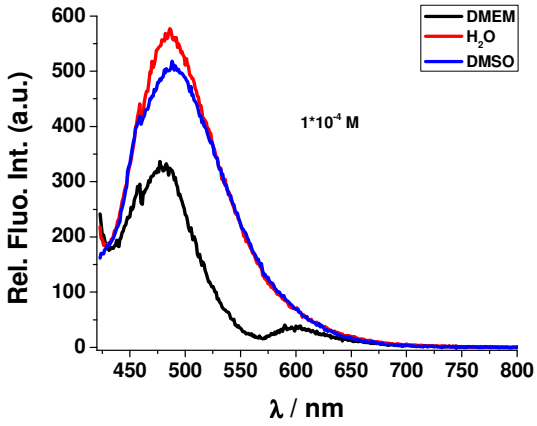


Figure S21. Fluorescence spectra (λ_{exc} = 405 nm, c = 1×10^{-4} mol/dm^3) of bis-pyrene **5** in water and Dulbecco's Modified Eagle Medium (DMEM) +10% FBS (slit 10-10) and DMSO (slit 5-10).