Synthesis and characterization of water-soluble two-photon excited blue fluorescent chromophores for bioimaging.

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Supplementary information

Synthesis of methyl 3,4,5-(1,4,7,10-tetraoxaundecyl)benzoate **2a** :

Tosylate $CH_3(OCH_2CH_2)_3OTs$ (52.80 g, 140 mmol) and methyl gallate **1** (7.70 g, 40 mmol) were dissolved in acetone (100 mL). A suspension of 57.00 g of potassium carbonate in acetone (100 mL) was then added. The mixture was refluxed under an argon atmosphere for two days. Carbonate was removed by celite filtration and the mother liquors were partitioned between water (50 mL) and dichloromethane (500 mL). The aqueous phase was extracted with dichloromethane (3 × 500 mL). The combined organic phases were washed with hydrochloric acid (20 mL), water (50 mL) and dried over magnesium sulfate. Evaporation of the solvents under vacuum led to a brown oil which was purified by flash chromatography (SiO_2, CH_2Cl_2-MeOH, 85-15 in vol.) to give **2a** as a yellow oil (60 %). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.28 (s, 2H), 4.23-4.17 (m, 6H), 3.88 (s, 3H), 3.86 (t, 4H, J=4.6 Hz), 3.79 (t, 2H, J=5.05 Hz), 3.73-3.69 (m. 6H), 3.67-3.60 (m, 12H), 3.57-3.47 (m, 6H), 3.37 (s, 9H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 166.57, 152.27, 142.54, 124.94, 109.01, 72.38, 71.90, 70.79, 70.66, 70.63, 70.56, 70.53, 70.49, 70.45, 69.60, 68.82, 58.98, 52.13. HRMS *m/e* calculated for $C_{29}H_{50}O_{14}$: 622.3206, found : 622.3200.

Synthesis of methyl 3,4,5-(1,4,7-trioxaoctyl)benzoate **2b**:

This compound was prepared using a similar procedure to that used for **2a** starting with tosylate $CH_3(OCH_2CH_2)_2OTs$ in 61 % yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.28 (s, 2H), 4.25-4.19 (m, 6H), 3.87 (s, 3H), 3.86 (t, 4H, J=4.82 Hz), 3.80 (t, 2H, J=4.82 Hz), 3.72-3.69 (m, 6H), 3.56-3.51 (m, 6H), 3.37 (s, 3 H), 3.36 (s, 6H) ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 166.57, 152.27, 124.97, 108.99, 72.47, 72.03, 71.98, 70.74, 70.58, 70.45, 69.62, 68.85, 59.03, 58.99.

Synthesis of methyl 3,4,5-(1,4-dioxapentyl)benzoate **2c**:

This compound was prepared using a similar procedure to that used for **2a** starting with tosylate $CH_3OCH_2CH_2OTs$ in 60 % yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.31 (s, 2H), 4.24-4.18 (m, 6H), 3.89 (s, 3H), 3.77 (t, 4H, J=4.81 Hz), 3.72 (t, 4H, J=4.82 Hz), 3.45 (s, 3H), 3.44 (s, 6H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 166.62, 152.41, 142.82, 125.04, 109.29, 72.30, 71.86, 70.97, 68.90, 59.16, 58.87, 52.18.

Synthesis of 3,4,5-(1,4,7,10-tetraoxaundecyl)phenylmethanol **3a**:

Under argon, the ester **2a** (5.00 g, 8 mmol) was dissolved in anhydrous THF (80 mL). The solution was cooled in ice bath and $LiAlH_4$ (0.25 g, 6 mmol) was added slowly. Resulting mixture was stirred at room temperature for one hour and then refluxed under argon overnight. The unreacted metal hydride was neutralized with ethyl acetate (1 mL), ethanol (1 mL) and water (10 mL). The mixture was filtered over Celite and extracted with dichloromethane. The combined organic phases were dried over $MgSO_4$ and evaporated under vacuum to yield **3a** as slightly yellow oil (99 %). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 6.62 (s, 2H), 4.57 (s, 2H), 4.18-4.11 (m, 6H), 3.83 (t, 4H, J=4.82 Hz), 3,78 (t, 2H, J=5.04 Hz), 3.74-3.67 (m, 6H), 3.66-3.57 (m, 12H), 3.56-3.52 (m, 6H), 3.37 (s, 9H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 152.62, 137.64, 136.83, 106.53, 72.23, 71.89, 70.73, 70.64, 70.53, 70.50, 70.45, 69.79, 68.78, 65.08, 58.96, 49.56. HRMS *m/e* calculated for $C_{28}H_{50}O_{13}$: 594.32514, found : 594.32568.

Synthesis of 3,4,5-(1,4,7-trioxaoctyl)phenylmethanol **3b**:

This compound was prepared using a similar procedure to that used for **3a** starting with the ester **2b** in 85 % yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 6.61 (s, 2H), 4.57 (s, 2H), 4.18-4.12 (m, 6H), 3.84 (t, 4H, J=5.26 Hz), 3.80 (t, 2H, J=4.83 Hz), 3.73-3.69 (m, 6H), 3.57-3.54 (m, 6H), 3.37 (s, 9H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 152.65, 137.70, 136.56, 106.42, 72.31, 72.00, 71.95, 70.62, 70.49, 70.35, 69.78, 68.79, 65.23, 58.96.

Synthesis of 3,4,5-(1,4-dioxapentyl)phenylmethanol **3c**:

This compound was prepared using a similar procedure to that used for **2a** starting with the ester **2c** in 97 % yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 6.62 (s, 2H), 4.60 (s, 2H), 4.21-4.12 (m, 6H), 3.79-3.69 (m, 6H), 3.44 (s, 9H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 145.30, 133.72, 129.11, 105.20, 73.01, 72.75, 69.14 53.96.

Synthesis of 3,4,5-(1,4,7,10-tetraoxaundecyl)benzaldehyde **4a**:

The alcohol **3a** (4.61 g, 7.7 mmol) is dissolved in dichloromethane (100 mL), and 7.00 g of MnO_2 (large excess) were added. The black mixture was stirred overnight at room temperature. The solids were removed by Celite filtration, and the solvent was removed by vacuum distillation to yield 4.60 g of a slightly yellow oil (96 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 9.81 (s, 1H), 7.13 (s, 2H), 4.28-4.2 (m, 6H), 3.87 (t, 4H, J=4.61 Hz), 3.80 (t, 2H, J=4.82 Hz), 3.75-3.69 (m, 6H), 3.67-3.61 (m, 12H), 3.61-3.51 (m, 6H), 3.38 (s, 9H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 190.96, 153.04, 144,14, 131.58, 109.00, 72.53, 71.94, 70.85, 70.70, 70.68, 70.60, 70.58, 70.56, 70.53, 69.62, 68,98. HRMS *m/e* calculated for $C_{28}H_{48}O_{13}$: 592,30949, found 592,30888.

Synthesis of 3,4,5-(1,4,7-trioxaoctyl)benzaldehyde **4b**:

This compound was prepared using a similar procedure to that used for **4a** starting the benzyl alcohol **3b** in 99 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 9.82 (s, 1H), 7.14 (s, 2H), 4.18-4.12 (m, 6H), 3.88 (t, 4H, J=5.26 Hz), 3.82 (t, 2H, J=5.04 Hz), 3.74-3.70 (m, 6H), 3.58-3.52 (m, 6H), 3.38 (s, 6H), 3.37 (s, 3H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm) : 190.95 ; 153.01, 144.05, 131.60, 108.91, 72.60, 72.02, 71.97, 70.76, 70.61, 70.47, 69.62, 68.95, 59.05, 59.01. HRMS *m/e* calculated for $C_{22}H_{36}O_{10}$: 460,23085, found : 460.23048.

Synthesis of 3,4,5-(1,4-dioxapentyl)benzaldehyde **4c**:

This compound was prepared using a similar procedure to that used for **4a** starting with the benzyl alcohol **3c** in 99 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 9.83 (s, 1H), 7.15 (s, 2H), 4.27 (t, 4H, J=4.6 Hz), 4.22 (t, 2H, J=4.6 Hz), 3.78 (t, 4H, J=4.82 Hz), 3.73 (t, 2H, J=4.82 Hz), 3.45 (s, 6H), 3.44 (s, 3H). ^{13}C-NMR (75.75 MHz, $CDCl_3$) δ (ppm); 190.95,153.13, 144.29, 131.66, 109.15, 72.43, 71.86, 70.96, 70.94, 68.99, 68.90, 59.14, 58.87. HRMS *m/e* calculated for $C_{16}H_{24}O_7$: 328.15220, found : 328.15251.

Synthesis of 4,4'-Bis-{2-[3,4,5-tris-(1,4,7,10-tetraoxaundecyl)phenyl]ethenyl}-biphenyl **7** :

To a solution of **5** (0.51 g, 1.1 mmol) in anhydrous THF (75 mL) were added sodium hydride (0.60 g, 25 mmol) portion wise. The suspension was stirred under argon at 50 °C for 1 hour. The aldehyde **4a** (1.32 g, 2.2 mmol) in anhydrous THF was added slowly. The slurry was then refluxed for 2 hours. After cooling, the mixture was quenched by ethanol. Solvents were removed under reduced pressure. The resulting oil was dissolved in dichloromethane and washed with brine (2 mL), water (2 mL) and dried over magnesium sulfate. The product was purified by column chromatography (silica, CH_2Cl_2/MeOH 97-3 to 80-20 in vol.) to afford **7** (75 %). UV-vis : λ_{max}=357 nm, ε = 6.2x10^4 mol^{-1}.L.cm^{-1} (water); λ_{max}= 362 nm, ε = 7.3x10^4 mol^{-1}.L.cm^{-1} (CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.65-7.56 (AA'BB', 8H, J=8.34 Hz), 7.02 (s, 4H), 6.79 (s, 4H), 4.23 (t, 8H, J=4.8 Hz), 4.18 (t, 4H, J=5.7 Hz), 3,89 (t, 8H, J=5.27 Hz), 3.81 (t, 4H, J=4.83 Hz), 3.78-3.72 (m, 12H), 3.70-3.64 (m, 24H), 3.57-3.54 (m, 12H), 3,38 (s, 18 H) ; ^{13}C-NMR (75.75MHz, $CDCl_3$) δ (ppm) : 151.79. 138.62, 137.52, 135.33, 131.89, 127.51, 126.61, 126.05, 125.87, 105.38, 71.40, 70.94, 70.92, 69.83, 69.70, 69.68, 69.56, 69.53, 69.51, 68.78, 67.91, 58.02. HRMS *m/e* calculated for $C_{70}H_{106}O_{24}$: 1330.70741 ($MH^{+\cdot}$), found : 1330.70745.

Synthesis of 4,4'-Bis-{2-[3,4,5-tris-(1,4,7-trioxaoctyl)phenyl]ethenyl}-biphenyl **8** :

This compound was prepared in 77 % yield as described for **7**, starting from 0.43 g (0.95 mmol) of bisphosphonate **5** and 0.88 g (1.9 mmol) of aldehyde **4b**. UV-vis : λ_{max}=357 nm, ε=5.9x10^4 mol^{-1}.L.cm^{-1} (water); λ_{max}=363 nm, ε=7.1x10^4 mol^{-1}.L.cm^{-1} (CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.65-7.55 (AA'BB', 8H, J=8.55 Hz), 7.02 (s, 4H), 6.79 (s, 4H), 4.25 (t, 8H, J=4.82 Hz), 4.20 (t, 4H, J=4.82 Hz), 3.90 (t, 8H, J=5.26 Hz), 3.83 (t, 4H, J=5.26 Hz), 3.76-3.72 (m, 12H), 3.60-3.56 (m, 12H), 3.40 (s, 12H), 3.39 (s, 6H) . ^{13}C-NMR (75.75MHz, $CDCl_3$) δ (ppm) 152.8, 138.46, 136.35, 132.94, 128.52, 127.63, 127.07, 126.88, 106.32, 96.12, 72.50, 72.07, 72.02, 70.77, 70.58, 70.45, 69.81, 68.91, 59.09, 59.04. HRMS *m/e* calculated for $C_{58}H_{82}O_{18}$: 1066.5501, found : 1066.55171.

Synthesis of 4,4'-Bis-{2-[3,4,5-tris-(1,4-dioxaunpentyl)phenyl]ethenyl}-biphenyl **9** :

This compound was prepared in 75 % yield as described for **7**, starting from 0.43 g (0.95 mmol) of bisphosphonate **5** and 0.88 g (1.9 mmol) of aldehyde **4c**. UV-vis: λ_{max}=363 nm, ε=7.5x10^4 mol^{-1}.L.cm^{-1} (CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7,65-7,55 (AA'BB', 8H, J=8,55 Hz), 7,03 (s, 4H), 6,81 (s, 4H), 4,24-4,17 (m, 12H), 3,79 (t, 8H, J=5,04 Hz), 3,73 (t, 8H, J=4,82 Hz), 3,48 (s, 12 H), 3,46 (s, 6 H). ^{13}C-NMR (75.75MHz, $CDCl_3$) δ (ppm) : 152,93, 139,66, 138,94, 136,36, 132,99, 128,51, 127,68, 127,07, 127,02, 126,89, 106,91, 72,33, 71,87, 71,20, 71,19, 69,10, 59,22, 59,18, 59,18, 58,58. HRMS *m/e* calculated for $C_{46}H_{58}O_{12}$: 802,39374, found : 802,39283.

Synthesis of 4,4'-Bis-{2-[3,4,5-tris-(1,4,7,10-tetraoxaundecyl)phenyl]ethenyl}-7,7-di-n-propylfluorenyl **10**:
This compound was prepared in 72 % yield as described for **7**, starting from 1.10 g (2 mmol) of bisphosphonate **6** and 2.30 g (2 mmol) of aldehyde **4a**. UV-vis: λ_{max}=382 nm, ε = 5.8x10^4 mol^{-1}.L.cm^{-1} (water); λ_{max}=382 nm ε=8.1x10^4 mol^{-1}.L.cm^{-1} (CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.65 (d, 2H, J=8.33 Hz), 7.46 (m, 4H), 7.05 (s, 4H), 6.80 (s, 4H), 4.23 (t, 8H, J=4.92 Hz), 4.18 (t, 4H, J=4.82 Hz), 3.89 (t, 8H, J=5.02 Hz), 3.81 (t, 4H, J=4.82 Hz) 3.54-3.78 (m , 48H), 3.38 (s, 18 H), 2.01 (m, 4H), 0.70 (m, 10H). ^{13}C-NMR (75.75MHz, $CDCl_3$) δ (ppm) : 152.74, 151,51, 140.51, 138,32, 136.14, 133.09, 128.71, 127,71, 125.58, 120.62, 119.83, 106.20, 72.36, 71.90, 70.79, 70.67, 70.65, 70.53, 70.49, 69,75, 68.83, 58.97, 55.12, 42.83, 17.15, 14.44. HRMS *m/e* calculated for $C_{77}H_{118}O_{24}Na$ ($M^{+\cdot}Na$) : 1449.79053, found : 1449.79217.

Synthesis of 4,4'-Bis-{2-[3,4,5-tris-(1,4,7-trioxaoctyl)phenyl]ethenyl}-7,7-di-n-propylfluorenyl **11**
This compound was prepared in 60 % yield as described for **7**, starting from 0.55 g (1 mmol) of bisphosphonate **6** and 0.95 g (2 mmol) of aldehyde **4b**. UV-vis: λ_{max}=383 nm ε=8.6x10^4 mol^{-1}.L.cm^{-1} (CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.59 (d, 2H, J=8.44 Hz), 7.41 (m, 4H), 7.01 (s, 4H), 6.76 (s, 4H), 4.17 (m, 12H), 3.83 (t, 8H, J=5.26 Hz), 3.77 (t, 4H, J=5.37 Hz), 3.66-3.70 (m, 12H), 3.49-3.53 (m, 12 H), 3.33 (s, 12 H), 3.32 (s, 6H), 1.96 (m, 4H), 0.64 (m, 10H). ^{13}C-NMR (75.75MHz, $CDCl_3$) δ (ppm) 152.70, 151,43, 140.44, 138,20, 136.13, 133.05, 128.63, 127,70, 125.56, 120.57, 119.82, 106.08, 72.40, 71.96, 71.92, 70.64, 70.50, 70.33, 69,72, 68.81, 58.93, 58.89, 55.05, 42.79, 17.12, 14.41. HRMS *m/e* calculated for $C_{65}H_{98}NO_{18}$ ($M^{+\cdot}NH_4$) : 1180.67784, found : 1180.67672.