## **Chemical Science**

### **ELECTRONIC SUPPORTING INFORMATION**

## Molecular engineering of chromophores for combined second-harmonic and two-photon fluorescence in cellular imaging

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### Addition to B Materials and Methods

#### A Synthesis

25 The data for compounds 1 – 11 are listed below.
Compound 1: 3,6-bis-[2-(pyridin-4-yl)-ethenyl]-9-pentyl-carbazole

To a  $N_2$ -protected three-neck flask (50 mL), 3,6-diiodo-9-pentylcarbazole (2.10 mg, 5.0 mmol), tri-*o*-tolylphosphine (150

- <sup>30</sup> mg, 0.49 mmol), Pd(OAc)<sub>2</sub> (15.5 mg, 0.06 mmol), 4vinylpyridine (2.2 mL, 20 mmol), dried triethyl amine (20 mL) were added. The resulting mixture was heated to reflux for 2 days. After cooling down, the solvent was removed. The residue was poured into water and extracted with chloroform ( $3 \times 30$
- $_{35}$  mL). Organic layers were combined, dried over MgSO<sub>4</sub>, concentrated and purified by flash column chromatography using chloroform / petroleum ether (V/V = 1/1) as eluant to give a yellow powder as product (1.04 g, yield 47%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>,  $\delta$  / ppm): 0.90 (t, *J* = 6.6 Hz, 3 H),

- <sup>40</sup> 1.38 (m, 4 H), 1.90 (m, 2 H), 4.32 (t, 2 H, J = 7.1 Hz), 7.12 (d, J = 16.4 Hz, 2 H), 7.42 (dd, J = 1.5 Hz, J = 6.2 Hz, 4 H), 7.48 (d, J = 8.8 Hz, 2 H), 7.55 (d, J = 16.4 Hz, 2 H), 7.75 (dd, J = 1.5 Hz, J = 8.8 Hz, 2 H), 8.30 (d, J = 1.5 Hz, 2 H), 8.61 (dd, J = 1.5 Hz, J = 6.2 Hz, 4 H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, δ / ppm): 13.9, 22.4,
- $\label{eq:stars} \begin{array}{l} {}_{45} \ 28.7, \ 29.4, \ 46.0, \ 109.4, \ 112.9, \ 122.5, \ 123.7, \ 124.5, \ 124.7, \ 130.1, \\ {}_{130.8, \ 131.4, \ 132.0, \ 141.0, \ 146.7; \ Anal. \ calcd \ for \ C_{31}H_{29}N_3: C, \\ {}_{83.94; \ H, \ 6.59; \ N, \ 9.47; \ Found: \ C, \ 83.58; \ H, \ 6.83; \ N, \ 9.59. \end{array}$

#### Compound 2: 3,6-bis-[2-(pyridin-4-yl)-ethynyl]-9-benzyl-50 carbazole

To a 50 mL three neck flask, 3,6-diethynyl-9-benzyl-carbazole (562 mg, 2.0 mmol), CuI (38 mg, 0.2 mmol), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (70 mg, 0.1 mmol), PPh<sub>3</sub> (52 mg, 0.2 mmol), 4-bromopyridinium hydrobromide (1.20 g, 6.0 mmol), dried triethyl amine (20 mL)

ss and dried toluene (20 mL) were added and bubbled with N<sub>2</sub> for 10 min. The resulting mixture was allowed to reflux for 6 h. After cooling down, the solvent was removed. The residue was poured into water and extracted with toluene ( $3 \times 30$  mL). Organic layers were combined, dried over MgSO<sub>4</sub>, concentrated and purified by

flash column chromatography using chloroform / petroleum ether (V/V = 1/1) as eluant to give a yellow powder as product (686 mg, yield 75%).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>,  $\delta$  / ppm): 5.76 (s, 2 H), 7.11 (m, 2H), 7.28 (m, 3H), 7.41 (m, 6 H), 7.67 (dd, J = 1.6 Hz, J = 8.8

<sup>65</sup> Hz, 2 H), 8.30 (d, J = 1.6 Hz, 2 H), 8.61 (d, J = 6.2 Hz, 4 H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, δ / ppm): 64.2, 87.4, 94.7, 109.4, 112.9, 122.5, 123.2, 124.1, 127.2, 129.5, 130.8, 131.4, 132.0, 137.7, 147.5; Anal. calcd for C<sub>33</sub>H<sub>21</sub>N<sub>3</sub> : C, 86.25; H, 4.61; N, 9.14; Found: C, 86.71; H, 4.23; N, 9.06.

#### Compound 3: 3,6-bis-[2-(4-nitro-phenyl)-ethynyl]-9-pentylcarbazole

Yield: 68%. m.p.202-204 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>,  $\delta$  / ppm): 0.90 (t, *J* = 6.6 Hz, 3 H), 1.38 (m, 4 H), 1.90 (m, 2 H), 4.32

(0.4 ppm).

(t, 2 H, J = 7.1 Hz), 7.41 (d, J = 8.7 Hz, 2 H), 7.67 (d, J = 9.0 Hz, 6 H), 8.22 (d, J = 9.0 Hz, 4 H), 8.32 (s, 2 H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>,  $\delta$  / ppm): 13.9, 22.4, 28.7, 29.4, 46.0, 86.7, 96.3, 109.4, 112.9, 122.5, 123.7, 124.7, 130.1, 130.8, 132.0, 141.0, 146.7; IR  $\varsigma$  (KBr, cm<sup>-1</sup>): 2962, 2922, 2201, 1585, 1512, 1339, 1105, 854, 749; HRMS (C<sub>27</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>): Calcd.: 527.18451; Obsd.: 527.18474

#### Compound 4: 4-[2-(9-methyl-carbazol-3-yl)vinyl]-1-10 methylpyridinium 4-aminobenzolsulfonate

- 1,4-dimethylpyridinium 4-aminobenzolsulfonate (10 mmol) and 9-pentyl-carbazol-3-aldehyde (10 mmol) were added to 50 mL flask with 25 mL ethanol, followed with catalytic piperidine (1.0 mL). The resulting mixture was allowed to stir overnight. After 15 concentration, the residue was recrystallized from acetonitrile /
- diethyl ether, further recrystallization gave desirable product. Yield: 55%. m.p.270-271 °C. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD,  $\delta$  / ppm): 8.53 (d, 2 H, *J* = 6.8 Hz), 8.44 (s, 1 H), 8.16 (d, 1 H, *J* = 7.9 Hz), 8.05 (d, 1 H, *J* = 16.5 Hz), 8.01 (d, 2 H, *J* = 6.8 Hz),
- <sup>20</sup> 7.86 (d, 1 H, J = 7.9 Hz), 7.73 (d, 2 H, J = 7.9 Hz), 7.57 (d, 1H, J = 8.6 Hz), 7.51 (m, 2 H), 7.32 (d, 1 H, J = 16.5 Hz), 7.28 (t, 1 H, J = 8.6 Hz), 7.21 (d, 2 H, J = 7.9 Hz), 5.15 (s, 2 H), 4.49 (s, 3 H), 4.18 (s, 3 H). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD,  $\delta$  / ppm): 37.8, 43.2, 102.4, 110.8, 111.2, 115.3, 117.3,119.2, 119.8, 120.5, 121.5,
- $_{25}$  124.5, 127.5, 127.8, 128.4, 129.1, 131.2, 142.9, 145.3, 148.3; Anal. calcd for  $C_{27}H_{25}N_3O_3S\cdot H_2O$ : C, 66.24; H, 5.56; N, 8.58; Found: C, 66.73; H, 5.85; N, 8.32.

#### Compound 5: 3-[2-(1-methylpyridinium)vinyl]-9-pentyl-30 carbazole tosylate

Yield: 64%. m.p. 241-242 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD,  $\delta$  / ppm): 8.52 (d, 2 H, *J* = 6.8 Hz), 8.43 (s, 1 H), 8.14 (d, 1 H, *J* = 7.9 Hz), 8.03 (d, 1 H, *J* = 16.5 Hz), 8.00 (d, 2 H, *J* = 6.8 Hz), 7.87 (d, 1 H, *J* = 7.9 Hz), 7.71 (d, 2 H, *J* = 7.9 Hz), 7.56 (d, 1H, *J* = 8.6 Hz), 7.52 (d) = 7.9 Hz), 7.57 (d) = 7.9 Hz)

<sup>35</sup> (m, 2 H), 7.30 (d, 1 H, J = 16.5 Hz), 7.27 (t, 1 H, J = 8.6 Hz), 7.22 (d, 2 H, J = 7.9 Hz), 4.46 (t, 2 H), 4.18 (s, 3 H), 2.34 (s, 3 H), 1.88 (m, 2 H), 1.37 (m, 4 H), 0.88 (t, 3 H). Anal. calcd for  $C_{32}H_{34}N_2O_3S \cdot H_2O : C$ , 70.56; H, 6.66; N, 5.14; Found: C, 70.90; H, 6.85; N, 4.92.

#### Compound 6: 3,6-bis[2-(1-methylpyridinium)vinyl]-9-pentylcarbazole diiodide

Yield: 46%. m.p. 256 - 259 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD, δ / ppm): 8.61 (d, 4 H, *J* = 6.5 Hz), 8.58 (s, 2 H), 8.12 (d, 2 H, *J* = 15.9 Hz), 8.09

<sup>45</sup> (d, 4 H, J = 6.5 Hz), 7.91 (d, 2 H, J = 8.6 Hz), 7.71 (d, 4 H, J = 8.6 Hz), 7.62 (d, 2 H, J = 8.6 Hz), 7.40 (d, 2 H, J = 15.9 Hz), 7.21 (d, 4 H, J = 8.6 Hz), 4.43 (t, 2 H), 4.25 (s, 6 H), 2.31 (s, 6 H), 1.87 (m, 2 H), 1.37 (m, 4 H), 0.87 (t, 3 H). Anal. calcd for C<sub>33</sub>H<sub>35</sub>I<sub>2</sub>N<sub>3</sub>·(H<sub>2</sub>O)<sub>2</sub>: C,51.91; H, 5.15; N, 5.50; found C, 51.76; H, <sup>50</sup> 4.97; N, 5.72.

#### Compound 7: 3-[2-(1',3',3'-trimethyl-indolium-2'-yl)vinyl]-9pentyl- carbazole iodide

Yield: 45%. m.p. 119-122 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD,  $\delta$  / ppm): 0.89 55 (m, 3 H), 1.39 (m, 4 H), 1.61 (m, 2 H), 1.92 (s, 6 H), 4.18 (m, 2 H), 4.49 (s, 3 H), 7.37 (m, 1 H), 7.68 (m, 8 H), 8.22 (d, *J* = 8.7 Hz, 1 H), 8.28 (d, *J* = 7.6 Hz, 1 H), 8.67 (d, *J* = 15.9 Hz, 1 H), 8.94 (s, 1 H); MS (m / z): 421.6 [M-I]<sup>+</sup>; Anal. calcd for C<sub>30</sub>H<sub>33</sub>IN<sub>2</sub>·H<sub>2</sub>O: C, 63.60; H, 6.23; N, 4.94. Found: C, 63.44; H,

60 6.10; N, 5.00.

#### Compound 8: 3,6-bis[2-(1',3',3'-thimethyl-indolium-2'yl)vinyl]-9-pentyl-carbazole diiodide

Yield: 72%. m.p. >300 °C. <sup>1</sup>H NMR(CD<sub>3</sub>OD,  $\delta$  / ppm): 0.91 (m, 65 3 H), 1.41(m, 4 H), 1.61 (m, 2 H), 1.94 (s, 12 H), 4.31 (m, 2H),

4.57 (s, 6H), 7.65 (m, 6 H), 7.84 (m, 6 H), 8.25 (d, J = 8.7 Hz, 2 H), 8.67 (d, J = 15.7 Hz, 2 H), 9.49 (s, 2 H). MS (m / z): 605.6 [M-2I]<sup>+</sup>, 732.6 [M-I]<sup>+</sup>. Anal. calcd for C<sub>43</sub>H<sub>47</sub>I<sub>2</sub>N<sub>3</sub>: C, 60.08; H, 5.51; N, 4.89. Found: C, 60.85; H, 5.65; N, 4.88.

Compound 9: 2-[2-(9-methyl-carbazol-3-yl)vinyl]-3-methylbenzothiazolium iodide

Yield:65%. m.p. 258-260 °C. <sup>1</sup>H NMR(CD<sub>3</sub>OD,  $\delta$  / ppm): 4.40 (s, 3 H, N-CH<sub>3</sub>), 4.52 (s, 3 H, N-CH<sub>2</sub>), 7.32 (t, *J* = 7.4 Hz, 1 H, Ar-75 H), 7.54 (m, 2 H, Ar-H), 7.64 (d, *J* = 8.7 Hz, 1 H, Ar-H), 7.72 (m, 1 H, Ar-H), 7.85 (m, 1 H, Ar-H), 8.01 (d, *J* = 15.6 Hz, 1 H, CH=CH), 8.11 (d, *J* = 8.4 Hz, 1 H, Ar-H), 8.16 (d, *J* = 8.4 Hz, 2 H, Ar-H), 8.31 (d, *J* = 7.8 Hz, 1 H, Ar-H), 8.42 (d, *J* = 15.6 Hz, 1 H, CH=CH), 8.94 (s, 1 H, Ar-H). Anal. calcd for C<sub>23</sub>H<sub>19</sub>IN<sub>2</sub>S: C,

80 57.27; H, 3.97; N, 5.81. Found: C, 57.93; H, 4.13; N, 5.96.

# Compound 10: 2-[2-(9-pentyl-carbazol-3-yl)vinyl]-3-(4-sulfonatobutyl)benzothiazolium

90 % yield. m.p. 265-267 °C. <sup>1</sup>H NMR(CD<sub>3</sub>OD,  $\delta$  / ppm): 0.88 85 (m, 3 H, CH<sub>3</sub>), 1.17 (m, 2 H, CH<sub>2</sub>), 1.22 (m, 2 H, CH<sub>2</sub>), 1.24 (m, 2 H, CH<sub>2</sub>), 1.90 (m, 2 H, CH<sub>2</sub>), 2.25 (m, 2 H, CH<sub>2</sub>), 2.99 (m, 2 H, CH<sub>2</sub>), 4.09 (m, 2 H, CH<sub>2</sub>-SO<sub>3</sub><sup>-</sup>), 4.50 (m, 2 H, N-CH<sub>2</sub>), 7.33 (t, *J* = 7.4 Hz, 1 H, Ar-H), 7.56 (m, 2 H, Ar-H), 7.65 (d, *J* = 8.7 Hz, 1 H, Ar-H), 7.73 (m, 1 H, Ar-H), 7.84 (m, 1 H, Ar-H), 8.03 (d, *J* = 90 15.5 Hz, 1 H, CH=CH), 8.10 (d, *J* = 8.4 Hz, 1 H, Ar-H), 8.18 (d,

J = 8.4 Hz, 2 H, Ar-H), 8.30 (d, J = 7.8 Hz, 1 H, Ar-H), 8.40 (d, J = 15.5 Hz, 1 H, CH=CH), 8.90 (s, 1 H, Ar-H). Anal. calcd for  $C_{30}H_{32}N_2O_3S_2$ : C, 67.64; H, 6.05; N, 5.26. Found: C, 67.83; H, 5.82; N, 4.96.

#### Compound 11: 3,6-bis{2-[3'-(4-sulfonatobutyl)benzothiazolium-2'-yl]vinyl}-9-pentyl-carbazole

41 % yield. m.p. 235-237 °C, <sup>1</sup>H NMR(CD<sub>3</sub>OD, δ / ppm): 0.88 (m, 6 H, CH<sub>3</sub>), 1.17 (m, 4 H, CH<sub>2</sub>), 1.24 (m, 2 H, CH<sub>2</sub>), 1.38 (m, <sup>100</sup> 4 H, CH<sub>2</sub>), 2.29 (m, 2 H, CH<sub>2</sub>), 3.00 (m, 3 H, CH<sub>2</sub>), 3.12(s, 2 H, CH<sub>2</sub>), 3.61 (m, 4 H, CH<sub>2</sub>-SO<sub>3</sub>), 7.33 (t, *J* = 7.4 Hz, 2 H, Ar-H), 7.72 (m, 1 H, Ar-H), 8.07 (m, 2 H, Ar-H), 8.39 (m, 4 H, Ar-H), 8.71 (m, 3 H, Ar-H), 8.90 (m, 4 H, Ar-H), 10.12 (ss, 2 H, Ar-H). Anal. calcd for C<sub>43</sub>H<sub>45</sub>N<sub>3</sub>O<sub>6</sub>S<sub>4</sub>: C, 62.37; H, 5.48; N, 5.07. Found: <sup>105</sup> C, 62.85; H, 5.35; N, 4.83.

#### C Nonlinear optical characterization

The reference at this wavelength is crystal violet with an octopolar hyperpolarizability tensor component  $\beta_{xxx}$  of 338 x 110  $10^{-30}$  esu in methanol. The differences in solvent and molecular symmetry between reference and unknowns were taken into account with the standard local-field correction factors at optical frequencies and the appropriate factors for the contributing tensor components, respectively. To this end, the HRS depolarization 115 ratio was also determined, corrected for the solvent contribution. For the octopolar reference, a depolarization ratio of  $1.4\pm0.1$  was obtained, in excellent agreement with the theoretical value of 1.5 and taking into account the finite experimental aperture slightly

lowering the ratio. This effect may be expected to become slightly more pronounced for the higher values, so the experimental depolarization ratios reported should be treated as estimates for their lower limit.

- s Based on the results for these depolarization ratios, an analysis in terms of diagonal and off-diagonal hyperpolarizability tensor elements has been performed. A sufficiently high value indicates a single major dipolar tensor element  $\beta_{zzz}$  dominating the response along the charge-transfer molecular *z*-axis. A low value
- <sup>10</sup> for the depolarization ratio of dipolar compounds indicates offdiagonal contributions in the *xz*-plane of the chromophore. The analysis was performed assuming  $C_{xv}$  symmetry for the monosubstituted and  $C_{2v}$  for the di-substituted derivates.

#### 15 D The multimodal (SHG and TPEF) microscopes

Further details on the microscope setup used for co-localization, FLIM and TSCPC-SHG imaging: Imaging was done through a 40x water immersion microscope objective with a NA of 1.1 (LD C-Apochromat, Zeiss). The CW excitation laser is an Ar-ion laser

- $_{20}$  at 488 nm (Lasos, Germany) with 87  $\mu$ W power on the stage. The excitation beam is reflected to the sample by a dichroic mirror (HFT-488, Zeiss) and the signal is detected in descanned mode. For multi-photon excitation a Mai-Tai DeepSee ultrafast tunable laser (Spectra Physics), delivering 150 fs pulses in the sample
- <sup>25</sup> plane at 80 MHz, was utilized. A central wavelength of 800 nm was chosen and the mean power at the stage was set to 10 mW. The beam was directed to the sample via a dichroic mirror (HFT KP 700/488) and the emission was detected in descanned mode. The excitation lasers are aligned for maximal image overlap. To
- <sup>30</sup> verify co-localization, entire spectral images (lambda-stack) were taken subsequently for both excitation wavelengths. The sample was within a temperature-controlled (Tempcontrol 37-2 digital, Pecon) incubator (XL LSM DARK S, Pecon) on the stage of the microscope.

#### 35

#### **Additional Figures**



Figure S1: Two-photon absorption cross section spectra for the compounds studied. Note the strong enhancement around 800 nm for compound 6, the blue-shifted spectrum for compound 3, spectral data only for shorter wavelengths for compound 2, and no data for compound 1. All the other compounds have weaker and red-shifted TPA spectra.