Dual-wavelength efficient two-photon photorelease of glycine by π-extended dipolar coumarins.

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SUPPORTING INFORMATION

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I. Synthesis and characterization

1. Materials and methods

All air and moisture sensitive manipulations were carried out using standard techniques, with flame-dried reaction vessels, anhydrous solvents, and under argon atmosphere. CH$_2$Cl$_2$, and THF were dried over two columns of alumina and degassed in an Innovative Technologies Pure Solve solvent purification system. N,N-dimethylformamide and Et$_3$N were freshly distilled over CaH$_2$ before use. Alternately, THF was freshly distilled over sodium/benzophenone before being used. Extraction and column chromatography solvents were purchased in anhydrous form, and used as received. Palladium (II) acetate was recrystallized from chloroform before being used. All reagents were purchased from Fisher Scientific, Aldrich, TCI, Fluorochem or Strem and used without further purification unless indicated otherwise. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminum plaques, and column chromatography was performed on Macherey-Nagel silica gel 60 (40-63 µm).

$^1$H and $^{13}$C spectra were recorded on a Bruker Advance III 200 spectrometer at 200 MHz and 50 MHz, on a Bruker Advance I 300 spectrometer at 300 MHz and 75 MHz on a Bruker Advance II 400 spectrometer at 400 MHz and 100 MHz, or on a Bruker Advance III 600 spectrometer at 600 MHz and 150 MHz respectively. Shifts (δ) are given in parts per million with respect to solvent residual peak, and coupling constant (J) are given in Hertz.

Melting points were measured on a Stuart SMP 10 instrument.

HRMS-ESI spectra were recorded by the CESAMO (Bordeaux, France) on a QStar Elite mass spectrometer (Applied Biosystems). The instrument is equipped with an ESI source and spectra were recorded in the negative/positive mode. The electrospray needle was maintained at 4500 V and operated at room temperature. Samples were introduced by injection through a 20 µL sample loop into a 400 µL/min flow of methanol from the LC pump. HRMS-FD spectra were performed on a TOF mass spectrometer AccuTOF GCv using an FD emitter with a voltage of 10 kV. One to two microliters solution of the compound is deposited on a 13µm emitter wire.

Infrared spectra were recorded using a FT-IR Perkin Elmer Spectrum 100 Optica.

2. Synthesis of the intermediate 3-iodocoumarin

The formation of the alcohol 1 has been extensively described in literature using selenium dioxide as oxidizing agent. This method proved somewhat unreliable in our hands, affording poor yields and reproducibility, and remnant traces of toxic selenium salts in the product. Consequently, an
original alternative pathway was implemented (Scheme S1). Starting from the commercially available 7-diethylamino-4-methylcoumarin, a double elimination reaction with DMFDMA allowed the formation of the enamine 20. The resulting double bond was cleaved in mild conditions with sodium periodate, thus yielding the key aldehyde 21, which was reduced to alcohol 1 following a standard procedure. This three-step sequence was found to be much cleaner, more efficient and convenient, as it afforded alcohol 1 in fairly high yield, on large scale, and without purification on the intermediates. After trying several mild conditions, alcohol 1 could eventually be iodinated using a highly activated source of electrophilic iodine, with N-iodosuccinimide in presence of a strong Lewis acid. This yielded iodinated derivative 2 on a twenty-gram scale.

![Scheme S1. Synthesis of the iodinated intermediate.](image)

7-(diethylamino)-4-(2-dimethylamino)vinylcoumarin (20):

To a stirred solution of 7-(diethylamino)-4-methylcoumarin (20 g, 86.5 mmol, 1 eq.) in dry DMF (10 mL) under argon atmosphere was added DMFDMA (11.7 mL, 86.7 mmol, 1 eq.). The mixture was heated to 140 °C and heavily stirred for 8 h, then more DMFDMA (0.46 mL, 34.6 mmol, 0.4 eq.). Heating was maintained for 8 h, then the solvent was removed under vacuum. The residue was then suspended in cyclohexane (40 mL), filtered, washed with Et₂O (40 mL) and dried under vacuum to afford 20 as a bright yellow powder. (24.6 g, quant.).

**1H NMR (CDCl₃, 300 MHz), δ (ppm):** 7.52 (d, J = 9.0 Hz, 1H), 7.21 (d, J = 13.0 Hz, 1H), 6.54 (dd, J = 9.0, 2.7 Hz, 1H), 6.48 (d, J = 2.7 Hz, 2H), 5.84 (s, 1H), 5.21 (d, J = 13.0 Hz, 1H), 3.39 (q, J = 7.1 Hz, 4H), 2.99 (s, 6H), 1.19 (t, J = 7.1 Hz, 6H). **13C NMR (CDCl₃, 75 MHz), δ (ppm):** 163.6, 156.5, 152.5, 150.2, 146.7, 124.9, 108.2, 108.0, 98.2, 93.5, 87.5, 44.8, 40.9, 12.6. **HRMS (ESI):** m/z = 287.1760, calcd for C₁₇H₂₃N₂O₂ (M+H)⁺: 287.1754. **FTIR ν (cm⁻¹):** 2970.3, 2929.0, 2867.0, 1671.9, 1599.6, 1565.1, 1375.7, 1358.5, 1231.1, 1110.5, 814.3, 766.1. **Melting point:** 145-153 °C.
7-diethylamino-4-formylcoumarin (21):

The enamine 20 (24.6 g, 85.9 mmol, 1 eq.) was dissolved in a mixture of THF and CH₂Cl₂ (300 mL, 5/1, v/v). A solution of NaIO₄ (55.1 g, 258 mmol, 3 eq.) in H₂O (250 mL) was then added, and the resulting mixture was stirred at room temperature for 2 h. Upon completion of the reaction, the mixture was filtered on Celite®, and the filtrate was partially concentrated under vacuum. The aqueous layer was then extracted with CH₂Cl₂ (5 x 100 mL), and the combined organic layers were washed with brine (200 mL), dried over Na₂SO₄, filtered and evaporated. Compound 21 was obtained as a dark red solid, and pure enough to be used without further purification. (21.0 g, quant.).

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 10.03 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 6.63 (dd, J = 9.2, 2.7 Hz, 1H), 6.52 (d, J = 2.7 Hz, 1H), 6.45 (s, 1H), 3.43 (q, J = 7.1 Hz, 4H), 1.22 (t, J = 7.1 Hz, 6H). ¹³C NMR (CDCl₃, 75 MHz), δ (ppm): 192.7, 162.0, 157.5, 151.1, 144.0, 127.2, 117.5, 109.6, 103.8, 97.7, 44.9, 12.6. MS (ESI): m/z = 278, calcd for C₁₅H₂₀NO₄ (M+H+MeOH)⁺: 278. FTIR ν (cm⁻¹): 3056.4, 2966.9, 2935.9, 2863.6, 1720.1, 1699.5, 1616.8, 1578.9, 1520.4, 1344.7, 1072.6, 797.1. Melting point: 70 ℃.
Sodium borohydride (3.90 g, 103 mmol, 1.2 eq.) was added portionwise to a stirred solution of the aldehyde 21 (21.0 g, 85.6 mmol, 1 eq.) in dry methanol (400 mL) at 0 °C and under argon atmosphere. The resulting mixture was allowed to warm up to room temperature and stirred for 16 h. The reaction was carefully quenched with an HCl solution (1M), and MeOH was partially evaporated. The pH was adjusted to 6 by addition of an aqueous saturated NaHCO₃ solution, then the aqueous layer was extracted with CH₂Cl₂ (4 x 100 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by filtration over a pad of silica gel (eluent: CH₂Cl₂, then CH₂Cl₂:acetone, 90:10), to give 1 as a pale greenish solid (15.0 g, 71%).

\[
\begin{align*}
\text{1H NMR (CDCl₃, 300 MHz), } & \delta \text{ (ppm): } 7.30 (d, J = 9.0 \text{ Hz}, 1H), 6.54 (dd, J = 9.0, 2.6 \text{ Hz}, 1H), 6.45 (d, J = 2.6 \text{ Hz}, 1H), 6.27 (t, J = 1.1 \text{ Hz}, 1H), 4.82 (dd, J = 5.7, 1.1 \text{ Hz}, 2H), 3.38 (q, J = 7.1 \text{ Hz}, 4H), 2.96 (t, J = 5.7 \text{ Hz}, 1H), 1.18 (t, J = 7.1 \text{ Hz}, 6H). \\
\end{align*}
\]

\[
\begin{align*}
\text{13C NMR (CDCl₃, 50 MHz), } & \delta \text{ (ppm): } 163.1, 156.2, 155.3, 150.6, 124.5, 108.7, 106.4, 105.3, 97.8, 61.0, 44.8, 12.5. \\
\text{HRMS (ESI): } & m/z = 270.1109, \text{ calcd}
\end{align*}
\]

7-diethylamino-4-hydroxymethylcoumarin (1):
for C$_{14}$H$_{17}$NO$_3$Na (M+Na)$^+$: 270.1100. FTIR $\nu$ (cm$^{-1}$): 3435.3, 2970.3, 2929.0, 1678.8, 1599.6, 1523.8, 1324.1, 1086.4, 831.6. Melting point: 149 °C.

7-diethylamino-4-hydroxymethyl-3-iodocoumarin (2):

To a stirred solution of 1 (17.2 g, 69.6 mmol, 1 eq.) in CHCl$_3$ (350 mL) under argon atmosphere at 0 °C were added successively N-iodosuccinimide (17.2 g, 76.5 mmol, 1.1 eq.) and BF$_3$.Et$_2$O (48%, 9.2 mL, 34.8 mmol, 0.5 eq.). The resulting mixture was allowed to warm up to room temperature, and stirred in the dark for 2 h. The pink mixture was quenched with an aqueous Na$_2$S$_2$O$_3$ saturated solution, then extracted with AcOEt (3 x 75 mL). The combined organic layers were washed with water, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude was purified by a short column chromatography of silica gel (eluent: toluene:AcOEt, 80:20), to give 2 as a bright yellow solid (18.1 g, 70%).

$^1$H NMR (acetone-$d_6$, 300 MHz), $\delta$ (ppm): 7.85 (d, $J = 9.2$ Hz, 1H), 6.74 (dd, $J = 9.2$, 2.6 Hz, 1H), 6.52 (d, $J = 2.6$ Hz, 1H), 4.97 (d, $J = 5.9$ Hz, 2H), 4.67 (t, $J = 5.9$ Hz, 1H), 3.52 (q, $J = 7.1$ Hz, 4H), 1.22 (t, $J = 7.1$ Hz, 6H). $^{13}$C NMR (acetone-$d_6$, 75 MHz), $\delta$ (ppm): 159.0, 157.7, 156.6, 151.7, 128.3, 109.7, 108.6, 97.2, 83.4, 67.6, 45.3, 12.8. HRMS (ESI): m/z = 396.0069, calcd for C$_{14}$H$_{16}$INO$_3$Na
(M+Na): 396.0067. **FTIR ν (cm⁻¹):** 3379.5, 2969.9, 2928.0, 1677.6, 1611.7, 1561.3, 1503.7, 1414.5, 1356.2, 1140.9, 830.7, 801.5, 761.2. **Melting point:** 146 °C.

3. Synthesis of the vinyl moieties

![FTIR spectrum](image1)

**Scheme S2.** Synthesis of 2-vinylthiazole 3 by Wittig reaction.

2-vinylthiazole (3):

Argon was bubbled into a stirred suspension of methyltriphenylphosphonium bromide (3.47 g, 9.72 mmol, 1.1 eq.) in dry THF (20 mL) for 15 min, then potassium tert-butoxide (1.09 g, 9.72 mmol, 1.1 eq.) at 0 °C. The resulting yellow mixture was stirred at 0 °C for 1 h, then a solution of thiazole-2-carbaldehyde (1.0 g, 8.84 mmol, 1 eq.) in dry degassed THF (20 mL) was added dropwise. The resulting purple mixture was allowed to warm up to r.t. overnight, then it was
quenched with brine and extracted with Et₂O (3 x 20 mL). The combined organic layers were washed with water, then dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography of silicagel (gradient eluent: pentane:Et₂O, 95:5 to 80:20) to give 3 as a pale yellow oil (624 mg, 64%).

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 7.78 (d, J = 3.3 Hz, 1H), 7.24 (d, J = 3.3 Hz, 1H), 6.94 (ddd, J = 17.5, 10.9, 0.7 Hz, 1H), 6.06 (d, J = 17.5 Hz, 1H), 5.55 (d, J = 10.9 Hz, 1H). ¹³C NMR (CDCl₃, 75 MHz), δ (ppm): 167.4, 143.4, 130.6, 119.9, 118.6.
Scheme S3. Synthesis of electron-withdrawing intermediates by Suzuki coupling.

2-vinylbenzo[d]thiazole (4):

Compound 4 was synthesized according to a procedure described in literature.² Pale yellow oil which crystallized upon standing. (977 mg, 66%). Analytical data were in accordance with literature.

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 8.00 (dd, J = 7.9, 0.7 Hz, 1H), 7.84 (dd, J = 7.9, 0.7 Hz, 1H), 7.46 (td, J = 7.7, 1.2 Hz, 1H), 7.37 (td, J = 7.7, 1.2 Hz, 1H), 7.04 (dd, J = 17.5, 10.9 Hz, 1H), 6.18 (d, J = 17.5 Hz, 1H), 5.75 (d, J = 10.9 Hz, 1H).

2-vinylbenzo[d]oxazole (5):
Compound 5 was synthesized according to a procedure described in literature.² Pale yellow oil. (913 mg, 64%). Analytical data were in accordance with literature.

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 7.70 (ddd, J = 4.9, 2.4, 0.6 Hz, 1H), 7.49 (ddd, J = 4.9, 2.4, 0.6 Hz, 1H), 7.35 – 7.27 (m, 2H), 6.74 (dd, J = 17.6, 11.1 Hz, 1H), 6.46 (dd, J = 17.6, 1.1 Hz, 1H), 5.83 (dd, J = 11.1, 1.1 Hz, 1H).

4-vinylbenzo[c][1,2,5]thiadiazole (6):

A stirred suspension of 4-bromobenzo[c][1,2,5]thiadiazole (400 mg, 1.86 mmol, 1 eq.) and potassium vinyltrifluoroborate (299 mg, 2.23 mmol, 1.2 eq.) in iPrOH (19 mL) and Et₃N (0.26 mL, 1.86 mmol, 1 eq.) was degassed with argon for 20 min, then PdCl₂(dppf) (30.4 mg, 37 µmol, 2 mol%) was added. The resulting mixture refluxed overnight under argon atmosphere. Upon cooling down to room temperature, the mixture was filtered over Celite®, and solvents were removed under vacuum. The resulting yellow oil was used crude in next step (300 mg, quant.)

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 7.94 – 7.87 (m, 1H), 7.63 – 7.52 (m, 2H), 7.24 (dd, J = 17.7, 11.3 Hz, 1H), 6.54 (dd, J = 17.7, 1.3 Hz, 1H), 5.64 (dd, J = 11.3, 1.3 Hz, 1H).

NB: Compound 6 was not further characterized due to its tendency to polymerize, and was used shortly after synthesis.
Scheme S4. Synthesis of the intermediate 9,9-dibutyl-7-vinyl-9H-fluorene-2-carbaldehyde 7.

7-bromo-9,9-dibutyl-9H-fluorene-2-carbaldehyde (23):
To a stirred solution of 2,7-dibromo-9,9-dibutyl-9H-fluorene\(^3\) \(22\) (5.0 g, 11.5 mmol, 1 eq.) in dry THF at -78 °C under argon atmosphere was added dropwise \(n\)-BuLi (5.5 mL, 2.3 M in hexane, 12.6 mmol, 1.1 eq.). After 1 h, DMF (2.3 mL, 30 mmol, 2.6 eq.) was added and the mixture was allowed to warm up to room temperature for 16 h. The mixture was then quenched with a saturated NH\(_4\)Cl solution, then extracted with CH\(_2\)Cl\(_2\). The combined organic layers were washed with brine, then dried over Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure. The residue was purified by column chromatography of silicagel (gradient eluent: petroleum ether:AcOEt, 10:0 to 9:1) to give \(23\) as a white solid (3.20 g, 73%).

\(^1\)H NMR (CDCl\(_3\), 300 MHz), \(\delta\) (ppm): 10.06 (s, 1H), 7.86 (dt, \(J = 4.1, 1.9\) Hz, 2H), 7.81 (d, \(J = 8.1\) Hz, 1H), 7.64 (d, \(J = 8.1\) Hz, 1H), 7.52 (dd, 2H), 2.11 – 1.89 (m, 4H), 1.07 (p, \(J = 7.2\) Hz, 4H), 0.66 (t, \(J = 7.3\) Hz, 6H), 0.62 – 0.46 (m, 4H). \(^{13}\)C NMR (CDCl\(_3\), 75 MHz), \(\delta\) (ppm): 192.3, 154.3, 151.1, 146.4, 138.6, 135.6, 130.6, 130.5, 126.5, 123.1, 122.3, 120.1, 55.6, 39.9, 25.9, 22.9, 13.7.

\(2\)-bromo-9,9-dibutyl-7-vinyl-9H-fluorene (24):

Argon was bubbled into a stirred suspension of methyltriphenylphosphonium bromide (3.06 g, 8.56 mmol, 1.1 eq.) in dry THF (25 mL) for 15 min, then
potassium tert-butoxide (960 mg, 8.56 mmol, 1.1 eq.) at 0 °C. The resulting yellow mixture was stirred at 0 °C for 1 h, then a solution of 23 (3.0 g, 7.79 mmol, 1 eq.) in dry degassed THF (25 mL) was added dropwise. The resulting mixture was allowed to warm up to r.t. overnight, then it was quenched with a saturated NH₄Cl solution and extracted with Et₂O (3 x 30 mL). The combined organic layers were washed with water, then dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography of silicagel (eluent: petroleum ether) to give 24 as a white powder (2.29 g, 77%).

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 7.64 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.52 – 7.38 (m, 4H), 6.83 (dd, J = 17.6, 10.9 Hz, 1H), 5.84 (dd, J = 17.6, 1.0 Hz, 1H), 5.30 (dd, J = 10.8, 0.9 Hz, 1H), 2.00 (ddd, J = 11.4, 6.3, 2.4 Hz, 4H), 1.11 (p, J = 7.2 Hz, 4H), 0.71 (t, J = 7.3 Hz, 6H), 0.68 – 0.57 (m, 4H). ¹³C NMR (CDCl₃, 75 MHz), δ (ppm): 153.3, 150.8, 140.0, 139.9, 137.3, 137.0, 130.0, 126.2, 125.4, 121.1, 121.1, 121.0, 119.9, 113.5, 55.3, 40.2, 25.9, 23.1, 13.9.

9,9-dibutyl-7-vinyl-9H-fluorene-2-carbaldehyde (7):

To a stirred solution of 24 (2.0 g, 5.22 mmol, 1 eq.) in dry THF (25 mL) at -78 °C under argon atmosphere was added dropwise n-BuLi (2.5 mL, 2.3 M in
hexane, 5.74 mmol, 1.1 eq.). After 1 h, DMF (1.2 mL, 15.7 mmol, 3 eq.) was added and the mixture was allowed to warm up to room temperature for 16 h. The mixture was then quenched with a saturated NH₄Cl solution, then extracted with CH₂Cl₂. The combined organic layers were washed with brine, then dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography of silicagel (gradient eluent: petroleum ether:AcOEt, 10:0 to 9:1) to give 7 as a pale yellow solid (830 mg, 48%).

**1H NMR (CDCl₃, 300 MHz), δ (ppm):** 10.06 (s, 1H), 7.88 – 7.78 (m, 3H), 7.75 – 7.71 (m, 1H), 7.48 – 7.38 (m, 2H), 6.82 (dd, J = 17.6, 10.9 Hz, 1H), 5.85 (dd, J = 17.6, 0.9 Hz, 1H), 5.33 (dd, J = 10.9, 0.8 Hz, 1H), 2.10 – 1.91 (m, 4H), 1.06 (p, J = 7.1 Hz, 4H), 0.66 (t, J = 7.3 Hz, 6H), 0.62 – 0.46 (m, 4H). **13C NMR (CDCl₃, 75 MHz), δ (ppm):** 192.4, 152.6, 151.7, 147.2, 139.4, 138.2, 137.1, 135.2, 130.6, 125.5, 123.0, 121.0, 120.8, 119.9, 114.3, 55.1, 40.1, 25.9, 23.0, 13.9. **HRMS (ESI):** m/z = 333.2202, calcd for C₂₄H₂₉O (M+H)⁺: 333.2212.
4. Synthesis of the new PPGs

Scheme S5. Synthesis of the \( \pi \)-extended coumarin cages. Reagents and conditions: (a) 3-7, \( \text{Pd(OAc)}_2 \), \( n\)-BuNCl, LiCl, \( \text{NaHCO}_3 \), DMF, 130°C (8, 90%; 9, 96%; 10, 92%; 11, 76%; 12, 89%). (b) Fmoc-Gly-OH, EDC.HCl, DMAP (cat.), \( \text{CH}_2\text{Cl}_2 \), r.t. (13, 52%; 14, 59%; 15, 69%; 16, 58%; 17, 84%, 19, 64%). (c) 2-Aminothiophenol hydrochloride, DMF, 120 °C. (42%).

General procedure for the Heck coupling between 2 and the vinyl moieties:
In a Schlenk tube were introduced under argon atmosphere lithium chloride (1.7 eq.), tetra-\( n \)-butylammonium chloride (1.1 eq.), \( \text{NaHCO}_3 \) (3 eq.), the vinyl synthon (if solid), and 2 (1 eq.). The powders were freeze-dried in the Schlenk for 2 h. The flask was then immediately connected to a Schlenk line, purged and refilled with argon 3 times, then dry DMF was added and argon was bubbled into the mixture for 15 minutes.

\textit{NB: If liquid, the vinyl synthon was added at this stage as a solution in DMF.}

Finally, \( \text{Pd(OAc)}_2 \) (5 mol%) was added, the tube was sealed with a screw cap, and the mixture heated to 130 °C until completion of the reaction. Upon cooling down to room temperature, the reaction was quenched with water, and appropriate work-up and purification were carried out.
(E)-7-(diethylamino)-4-(hydroxymethyl)-3-(2-(thiazol-2-yl)vinyl)coumarin (8):

Following the general procedure for Heck coupling, with 2 (200 mg, 0.536 mmol, 1 eq.) and 3 (89.4 mg, 0.804 mmol, 1.5 eq.). Upon cooling down to room temperature, the reaction was quenched with water, and evaporated.

The resulting precipitate was collected by filtration, washed with a large amount of water, then with Et₂O (5 mL). The resulting powder was dried under vacuum to give 8 as an orange solid (171 mg, 90%).

**¹H NMR (CDCl₃, 300 MHz), δ (ppm):** 7.82 (d, J = 9.1 Hz, 1H), 7.65 (d, J = 3.3 Hz, 1H), 7.29 (d, J = 3.3 Hz, 1H), 7.00 (d, J = 12.3 Hz, 1H), 6.67 (dd, J = 9.1, 2.6 Hz, 1H), 6.61 (d, J = 12.3 Hz, 1H), 6.51 (d, J = 2.6 Hz, 1H), 4.76 (s, 2H), 3.43 (q, J = 7.1 Hz, 4H), 1.22 (t, J = 7.1 Hz, 6H).

**¹³C NMR (CDCl₃, 101 MHz), δ (ppm):** 143.1, 142.5, 128.1, 127.6, 125.6, 125.4, 120.5, 119.1, 109.2, 109.0, 97.9, 97.5, 83.6, 72.7, 59.9, 44.9, 12.6. **FTIR ν (cm⁻¹):** 3437.5, 2961.3, 2922.6, 1700.8, 1618.8, 1595.7, 1568.2, 1415.4, 1354.1, 1282.4, 1145.8, 778.2.
(E)-3-(2-(benzo[d]thiazol-2-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)coumarin (9):

Following the general procedure for Heck coupling, with 2 (120 mg, 0.322 mmol, 1 eq.) and 4 (51.8 mg, 0.322 mmol, 1 eq.) The crude residue was precipitated in H$_2$O. The precipitate was collected by filtration, washed with a large amount of water, then with Et$_2$O (5 mL) and dried under vacuum. Dark purple solid (125 mg, 96%).

$^1$H NMR (DMSO-$d_6$, 600 MHz), $\delta$ (ppm): 8.08 (ddd, $J = 8.1, 1.2, 0.6$ Hz, 1H), 7.97 (d, $J = 15.8$ Hz, 1H), 7.97 (ddd, $J = 8.1, 1.2, 0.6$ Hz, 1H), 7.85 (d, $J = 15.8$ Hz, 1H), 7.80 (d, $J = 9.3$ Hz, 1H), 7.51 (ddd, $J = 8.1, 7.2, 1.2$ Hz, 1H), 7.42 (ddd, $J = 8.1, 7.2, 1.2$ Hz, 1H), 6.80 (dd, $J = 9.3, 2.6$ Hz, 1H), 6.57 (d, $J = 2.6$ Hz, 1H), 5.69 (t, $J = 5.6$ Hz, 1H), 4.81 (d, $J = 5.6$ Hz, 2H), 3.48 (q, $J = 7.1$ Hz, 4H), 1.15 (t, $J = 7.1$ Hz, 6H).

$^{13}$C NMR (DMSO-$d_6$, 151 MHz), $\delta$ (ppm): 167.4, 159.9, 155.3, 153.6, 152.7, 151.0, 134.0, 130.4, 127.9, 126.5, 125.3, 124.2, 122.5, 122.2, 112.4, 109.6, 107.7, 96.4, 55.4, 44.2, 12.4.

HRMS (ESI): m/z = 429.1244, calcld for C$_{23}$H$_{22}$N$_2$O$_3$NaS (M+Na)$^+$: 429.1243. FTIR v (cm$^{-1}$): 3452.2, 2969.6, 1707.0, 1620.4, 1589.4, 1567.0, 1417.4, 1353.3, 1281.1, 1146.0, 752.0.
(E)-3-(2-(benzo[d]oxazol-2-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)coumarin (10):

Following the general procedure for Heck coupling, with 2 (120 mg, 0.322 mmol, 1 eq.) and 5 (46.7 mg, 0.322 mmol, 1 eq.) The crude residue was precipitated in H₂O. The precipitate was collected by filtration, washed with a large amount of water, then with Et₂O (5 mL) and dried under vacuum. Orange solid (116 mg, 92%).

¹H NMR (DMSO-d₆, 600 MHz), δ (ppm): 8.03 (d, J = 15.9 Hz, 1H), 7.80 (d, J = 9.3 Hz, 1H), 7.73 – 7.72 (m, 2H), 7.71 (d, J = 15.7 Hz, 1H), 7.43 – 7.30 (m, 2H), 6.80 (dd, J = 9.3, 2.4 Hz, 1H), 6.57 (d, J = 2.4 Hz, 1H), 5.71 (t, J = 5.5 Hz, 1H), 4.81 (d, J = 5.5 Hz, 2H), 3.47 (q, J = 7.0 Hz, 2H), 1.14 (t, J = 7.0 Hz, 6H).¹³C NMR (DMSO-d₆, 151 MHz), δ (ppm): 163.2, 159.9, 155.5, 153.1, 151.2, 149.9, 141.9, 132.2, 128.1, 125.3, 124.7, 119.5, 115.6, 112.0, 110.6, 109.7, 107.7, 96.4, 55.2, 44.2, 12.4. HRMS (ESI): m/z = 413.1455, calcd for C₂₃H₂₂N₂O₄Na (M+Na)+: 413.1471. FTIR ν (cm⁻¹): 3445.2, 2969.2, 1711.0, 1620.2, 1497.1, 1418.4, 1353.5, 1249.2, 1145.5, 738.3.
(E)-3-((2-(benzo[c][1,2,5]thiadiazol-4-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)coumarin (11):

Following the general procedure for Heck coupling, with 2 (300 mg, 0.804 mmol, 1 eq.) and 6 (260.8 mg, 1.61 mmol, 2 eq.) The crude residue was purified by column chromatography of silicagel (eluent: CH$_2$Cl$_2$). Red solid (248 mg, 76%).

$^1$H NMR (CDCl$_3$, 300 MHz), $\delta$ (ppm): 8.47 (d, $J = 16.0$ Hz, 1H), 8.13 (d, $J = 16.0$ Hz, 1H), 7.88 (dd, $J = 8.3$, 1.4 Hz, 1H), 7.70 (d, $J = 9.2$ Hz, 1H), 7.65 – 7.52 (m, 2H), 6.65 (dd, $J = 9.2$, 2.6 Hz, 1H), 6.50 (d, $J = 2.6$ Hz, 1H), 5.10 (s, 2H), 3.43 (q, $J = 7.1$ Hz, 4H), 1.23 (t, $J = 7.1$ Hz, 6H). $^{13}$C NMR (CDCl$_3$, 101 MHz), $\delta$ (ppm): 161.3, 155.8, 155.2, 153.1, 150.4, 148.0, 131.2, 130.9, 129.9, 129.8, 128.8, 126.9, 126.4, 120.3, 116.9, 109.8, 97.8, 57.7, 45.3, 12.6. HRMS (FD): m/z = 407.13084, calc for C$_{22}$H$_{21}$N$_3$O$_3$S (M$^+$): 407.13036. FTIR v (cm$^{-1}$): 3443.4, 2968.6, 2928.1, 1695.4, 1618.4, 1603.4, 1509.8, 1413.4, 1352.3, 1263.1, 1142.9, 754.4.
(E)-9,9-dibutyl-7-(2-(diethylamino)-4-(hydroxymethyl)-coumarin-3-yl)vinyl)-9H-fluorene-2-carbaldehyde (12):

Following the general procedure for Heck coupling, with 2 (745 mg, 2.0 mmol, 1 eq.) and 7 (830 mg, 2.5 mmol, 1.25 eq.)
The crude residue was precipitated in H₂O. The precipitate was collected by filtration, washed with a large amount of water, then with Et₂O (5 mL) and dried under vacuum. Brown solid (1.0 g, 89%).

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 10.03 (s, 1H), 7.87 – 7.74 (m, 4H), 7.69 (d, J = 16.1 Hz, 1H), 7.69 (d, J = 9.3 Hz, 1H), 7.58 – 7.48 (m, 2H), 7.35 (d, J = 16.2 Hz, 1H), 6.61 (dd, J = 9.1, 2.6 Hz, 1H), 6.46 (d, J = 2.6 Hz, 1H), 5.01 (s, 2H), 3.38 (q, J = 7.1 Hz, 4H), 2.11 – 1.89 (m, 4H), 1.18 (t, J = 7.0 Hz, 6H), 1.05 (p, 4H), 0.64 (t, J = 7.3 Hz, 6H), 0.58 – 0.45 (m, 4H). ¹³C NMR (CDCl₃, 75 MHz), δ (ppm): 192.5, 161.8, 155.1, 152.7, 151.8, 150.3, 147.3, 146.9, 139.3, 138.6, 135.1, 134.9, 130.6, 126.5, 125.7, 123.1, 121.8, 121.2, 121.1, 120.0, 117.0, 109.3, 108.5, 97.4, 57.7, 55.2, 44.8, 40.1, 26.0, 23.0, 13.8, 12.6. HRMS (ESI): m/z = 600.3107, calcd for C₃₈H₄₃NO₄Na (M+Na)⁺: 600.3084.
(E)-3-(2-(7-(benzo[d]thiazol-2-yl)-9,9-dibutyl-9H-fluoren-2-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)-coumarin (18):

2-aminobenzenethiol hydrochloride (56 mg, 0.346 mmol, 2 eq.) was added to a solution of 12 (100 mg, 0.173 mmol, 1 eq.) in dry DMF. The mixture was stirred for 16 h at 130 °C. Upon cooling down to r.t., water was added and the mixture was concentrated. The residue was dissolved in AcOEt, washed with an aqueous LiCl solution (10% w/w) and with water. The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography of silicagel (eluent: toluene:AcOEt, 9:1) to give 18 as an orange solid (49 mg, 42%).

¹H NMR (CDCl₃, 300 MHz), δ (ppm): 8.13 (d, J = 1.6 Hz, 1H), 8.11 (d, J = 7.6 Hz, 2H), 8.05 (dd, J = 7.9, 1.6 Hz, 1H), 7.92 (d, J = 9.3 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.69 (d, J = 16.0 Hz, 1H), 7.58 (dd, J = 7.9, 1.6 Hz, 1H), 7.51 (d, J = 1.6 Hz, 1H), 7.50 (ddd, J = 8.3, 7.6, 1.6 Hz, 1H), 7.39 (td, J = 8.3, 7.6, 1.6 Hz, 1H), 7.33 (d, J = 16.1 Hz, 1H), 6.76 (dd, J = 9.3, 2.6 Hz, 1H), 6.61 (d, J = 2.6 Hz, 1H), 5.05 (s, 2H), 3.44 (q, J = 7.0 Hz, 4H), 2.08 (hept, J = 7.6, 6.6 Hz, 4H), 1.23 (t, J = 7.0 Hz, 6H), 1.10 (p, J = 7.3 Hz, 4H), 0.67 (t, J = 7.3 Hz, 6H), 0.63 – 0.55 (m, 4H). ¹³C NMR (CDCl₃, 75 MHz), δ (ppm): 168.9, 161.6, 155.0, 154.0, 152.1, 152.0, 146.0, 144.0, 140.2, 137.6, 134.9, 132.1, 127.2, 126.5, 126.4, 125.7, 125.1, 122.9, 121.7, 121.6, 120.5, 120.3, 120.2, 57.9, 55.4, 40.3, 26.0, 23.1, 13.9, 12.4. HRMS (ESI): m/z = 705.3107, calcd for C₄₄H₄₆N₂O₃NaS (M+Na)+ : 705.3121.
General procedure for the preparation of caged Fmoc-protected glycin (esterification):

To a stirred mixture of the acid (1.1 eq.) and \(N\)-(3-Dimethylaminopropyl)-\(N\)'-ethylcarbodiimide hydrochloride (1.1 eq.) under argon atmosphere in dry \(\text{CH}_2\text{Cl}_2\) (0.1 M) were added successively the alcohol (1 eq.) and catalytic DMAP (one crystal). The resulting mixture was stirred at r.t. overnight in the dark, then it was directly purified by column chromatography of silicagel.

\((E)-(7-(\text{diethylamino})-2-\text{oxo}-3-(2-(\text{thiazol-2-yl})\text{vinyl})\text{-coumarin-4-yl})\text{methyl} \quad ((9\text{H-fluoren-9-yl})\text{methoxy})\text{carbonyl} \text{glycinate (13)}:\)

Following the general procedure for esterification, with 8 (35 mg, 98.2 \(\mu\)mol, 1 eq.) and Fmoc-Gly-OH (32.1 mg, 108 \(\mu\)mol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: \(\text{CH}_2\text{Cl}_2\):acetone, 1:0 to 7:3) to give 13 as an orange powder (33 mg, 52\%).

\(^1\text{H} \text{NMR (DMSO-}\text{d}_6, \text{600 MHz}), \delta (\text{ppm}):\) 7.89 – 7.83 (m, 4H), 7.81 (t, \(J = 6.0 \text{ Hz}, 1\text{H}), 7.69 (d, \(J = 3.2 \text{ Hz}, 1\text{H}), 7.63 – 7.56 (m, 4\text{H}), 7.39 (t, \(J = 7.3 \text{ Hz}, 2\text{H}), 7.27 (t, \(J = 7.3 \text{ Hz}, 2\text{H}), 6.69 (\text{dd}, \(J = 9.2, 2.5 \text{ Hz}, 1\text{H}), 6.47 (d, \(J = 2.5 \text{ Hz}, 1\text{H}), 5.48 (s, 2\text{H}), 4.21 (d, \(J = 7.3 \text{ Hz}, 2\text{H}), 4.11 (t, \(J = 7.3 \text{ Hz}, 1\text{H}), 3.82 (d, \(J = 6.0 \text{ Hz}, 2\text{H}), 3.38 (q, \(J = 7.1 \text{ Hz}, 4\text{H}), 1.08 (t, \(J = 7.1 \text{ Hz}, 6\text{H}). \text{\(^{13}\text{C} \text{NMR (CDCl}_3, \text{101 MHz}), \delta (\text{ppm}):\) 169.9, 167.7, 163.6, 160.4, 156.5, 155.4, 151.1, 143.9, 143.3, 141.4, 128.6, 127.9, 127.2, 126.5, 125.2, 120.1, 119.1, 109.7, 109.2, 108.2, 97.7, 97.4, 67.4, 59.1, 47.1, 45.0, 43.1, 12.6. \text{FTIR } \nu (\text{cm}^{-1}):\) 3448.2, 3065.9, 2961.5, 2917.6, 2846.2, 1724.1, 1687.3, 1621.0, 1508.9, 1419.5, 1352.6, 1262.1, 1144.1, 1074.2, 754.5. \text{Melting point:} \text{170 \text{°C.}} \)
(E)-(3-(2-(benzo[d]thiazol-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl (((9H-fluoren-9-yl)methoxy)carbonyl)glycinate (14):

Following the general procedure for esterification, with 9 (60 mg, 147.6 µmol, 1 eq.) and Fmoc-Gly-OH (48.3 mg, 162 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH₂Cl₂:acetone, 100:0 to 94:6), to give 14 as an orange powder (60 mg, 59%).

**¹H NMR (DMSO-d₆, 600 MHz), δ (ppm):** 8.04 (d, J = 7.9 Hz, 1H), 7.99 – 7.95 (m, 2H), 7.97 (d, J = 7.7 Hz, 1H), 7.86 (d, J = 7.6 Hz, 2H), 7.82 (t, J = 6.1 Hz, 1H), 7.72 (d, J = 15.7 Hz, 1H), 7.65 (d, J = 9.3 Hz, 1H), 7.58 (dd, J = 7.5, 0.6 Hz, 2H), 7.52 – 7.48 (m, 1H), 7.43 – 7.40 (m, 1H), 7.38 (t, J = 7.5 Hz, 2H), 7.25 (td, J = 7.4, 1.1 Hz, 2H), 6.71 (dd, J = 9.2, 2.5 Hz, 1H), 6.49 (d, J = 2.5 Hz, 1H), 5.54 (s, 2H), 4.20 (d, J = 7.3 Hz, 2H), 4.11 (t, J = 7.2 Hz, 1H), 3.84 (d, J = 6.1 Hz, 2H), 3.40 (q, J = 7.0 Hz, 4H), 1.09 (t, J = 7.1 Hz, 6H).

**¹³C NMR (DMSO-d₆, 151 MHz), δ (ppm):** 170.0, 167.2, 159.4, 156.4, 155.0, 153.6, 151.0, 146.1, 143.7, 140.7, 134.2, 129.6, 127.6, 127.5, 126.9, 126.5, 125.4, 125.3, 125.1, 122.6, 122.1, 120.1, 113.7, 109.7, 107.5, 96.4, 65.8, 58.1, 46.4, 44.1, 42.3, 30.7, 12.4. **HRMS (ESI):** m/z = 708.2108, calcd for C₄₀H₃₅N₃O₆NaS (M+Na)⁺: 708.2138. **FTIR ν (cm⁻¹):** 3420.7, 3060.4,
Melting point: 142 °C.

(E)-(3-(2-(benzo[d]oxazol-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl ((9H-fluoren-9-yl)methoxy)carbonyl glycinate (15):

Following the general procedure for esterification, with 10 (60 mg, 153.6 μmol, 1 eq.) and Fmoc-Gly-OH (50.3 mg, 169 μmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH₂Cl₂:acetone, 1:0 to 9:1). to give 15 as an orange powder (71 mg, 69%).

¹H NMR (CDCl₃, 600 MHz), δ (ppm): 7.96 (d, J = 15.8 Hz, 1H), 7.90 (d, J = 15.8 Hz, 1H), 7.74 (d, J = 7.6 Hz, 2H), 7.73 – 7.70 (m, 1H), 7.54 – 7.51 (m, 2H), 7.51 (d, J = 8.3 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.34 – 7.30 (m, 1H), 7.32 (d, J = 9.2 Hz, 1H), 7.26 (t, J = 7.5 Hz, 2H), 6.61 (dd, J = 9.2, 2.2 Hz, 1H), 6.39 (d, J = 2.4 Hz, 1H), 5.56 (s, 2H), 5.41 (t, J = 5.8 Hz, 1H), 4.34 (d, J = 7.3 Hz, 2H), 4.12 (t, J = 7.3 Hz, 1H), 4.09 (d, J = 5.8 Hz, 2H), 3.36 (q, J = 7.2 Hz, 4H), 1.19 (t, J = 7.2 Hz, 6H). ¹³C NMR (CDCl₃, 151 MHz), δ (ppm): 169.9, 163.6, 160.1, 156.5, 155.7, 151.4, 150.7, 144.6, 143.9, 142.5,
141.4, 130.4, 127.9, 127.2, 126.7, 125.4, 125.2, 124.6, 120.1, 120.1, 119.4, 115.8, 110.6, 109.8, 108.0, 97.4, 67.5, 58.9, 47.1, 45.0, 43.0, 12.6.

**HRMS (ESI):** m/z = 692.2340, calcd for C_{40}H_{35}N_{3}O_{7}Na (M+Na)^+: 692.2367.

**FTIR ν (cm\(^{-1}\)):** 3446.0, 3324.2, 3065.9, 2972.5, 2923.1, 1721.7, 1694.5, 1615.3, 1542.8, 1507.9, 1419.8, 1351.6, 1274.4, 1151.1, 1059.4, 739.8.

**Melting point:** 226 °C.

(E)-(3-(2-(benzo[c][1,2,5]thiadiazol-4-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl ((9H-fluoren-9-yl)methoxy)carbonyl)glycinate (16):

Following the general procedure for esterification, with 11 (76 mg, 186.5 µmol, 1 eq.) and Fmoc-Gly-OH (61 mg, 205 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH\(_2\)Cl\(_2\):acetone, 100:0 to 98:2) to give 16 as a red powder (74 mg, 58%).

**\(^1\)H NMR (CDCl\(_3\), 600 MHz), δ (ppm):** 8.32 (d, J = 16.0 Hz, 1H), 8.09 (d, J = 16.0 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.3 Hz, 2H), 7.64 (d, J = 6.7 Hz, 1H), 7.58 – 7.52 (m, 1H), 7.51 (d, J = 7.3 Hz, 2H), 7.46 (d, J = 8.9 Hz, 1H), 7.36 (t, J = 7.3 Hz, 2H), 7.25 (t, J = 7.3 Hz, 2H), 6.56 (d, J = 8.4
Hz, 1H), 6.39 (d, J = 2.0 Hz, 1H), 5.55 (s, 2H), 5.52 (t, J = 5.5 Hz, 1H), 4.33 (d, J = 7.2 Hz, 2H), 4.13 (d, J = 5.5 Hz, 2H), 4.11 (t, J = 7.2 Hz, 1H), 3.34 (q, J = 6.9 Hz, 4H), 1.17 (t, J = 6.9 Hz, 6H).  

$^{13}$C NMR (CDCl$_3$, 151 MHz), $\delta$ (ppm): 169.9, 160.8, 156.4, 155.8, 155.1, 153.2, 150.5, 143.8, 142.3, 141.3, 131.0, 129.8, 128.3, 127.8, 127.1, 126.2, 126.1, 125.1, 120.5, 120.1, 118.7, 109.4, 108.4, 97.4, 67.4, 59.7, 47.1, 44.9, 43.1, 12.6.  

HRMS (FD): m/z = 686.21931, calcd for C$_{39}$H$_{34}$N$_4$O$_6$S (M$^+$): 686.21990.  

FTIR $\nu$ (cm$^{-1}$): 3445.9, 3060.4, 2969.6, 2923.1, 2862.6, 1715.9, 1703.4, 1618.8, 1602.2, 1512.0, 1416.2, 1355.0, 1265.1, 1146.0, 1078.7, 803.9, 748.9.  

Melting point: 116 °C.
(E)-(3-(2-(9,9-dibutyl-7-formyl-9H-fluoren-2-yl)vinyl)-7-(diethylamino)coumarin-4-yl)methyl (((9H-fluoren-9-yl)methoxy)carbonyl)glycinate (17):

Following the general procedure for esterification, with 12 (200 mg, 348 µmol, 1 eq.) and Fmoc-Gly-OH (113.5 mg, 381 µmol, 1.1 eq.), the product was purified by a column chromatography of neutral alumina (gradient eluent: CH$_2$Cl$_2$:AcOEt, 100:0 to 95:5) to give 17 as an orange powder (250 mg, 84%).

$^1$H NMR (DMSO-$d_6$, 300 MHz), δ (ppm): 10.05 (s, 1H), 8.01 (d, $J = 7.6$ Hz, 0H), 7.94 (d, $J = 7.6$ Hz, 1H), 7.97 – 7.88 (m, 2H), 7.87 (d, $J = 7.6$ Hz, 1H), 7.69 (d, $J = 16.0$ Hz, 1H), 7.73 – 7.64 (m, 4H), 7.61 – 7.57 (m, 2H), 7.38 (d, $J = 16.0$ Hz, 1H), 7.25 (td, $J = 7.5$, 1.2 Hz, 1H), 6.69 (dd, $J = 9.3$, 2.5 Hz, 1H), 6.49 (d, $J = 2.4$ Hz, 1H), 5.52 (s, 2H), 4.24 (d, $J = 7.0$ Hz, 2H), 3.86 (d, $J = 6.0$ Hz, 2H), 3.39 (q, $J = 7.0$ Hz, 4H), 2.13 – 1.97 (m, 3H), 1.10 (t, $J = 6.9$ Hz, 6H), 1.06 – 0.92 (m, 4H), 0.59 (t, $J = 7.3$ Hz, 6H), 0.47 (q, $J = 7.0$ Hz, 4H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz), δ (ppm): 193.2, 170.4, 160.3, 157.0, 154.9, 152.8, 151.7, 150.5, 149.6, 146.9, 144.1, 143.0, 141.1, 139.9, 139.5, 138.8, 137.9, 135.6, 129.4, 128.0, 127.7, 127.4, 125.5, 121.8, 120.5, 120.5, 117.2, 110.2, 108.1, 107.1, 96.9, 66.4, 59.2, 55.2, 55.0, 46.9, 44.5, 42.9, 39.1, 26.2, 22.8, 14.1, 12.8. HRMS (ESI): m/z = 879.3971, calcd for C$_{55}$H$_{56}$N$_2$O$_7$Na (M+Na)$^+$: 879.3979. FTIR ν (cm$^{-1}$): 3064.1, 3042.2, 2956.4, 2929.2, 2870.0, 2857.3, 1715.8, 1691.0, 1619.2, 1572.9, 1515.0, 1450.1, 1416.2, 1354.6, 1266.3, 1167.1, 1145.8, 1079.1, 734.7.

![Image of 1H NMR spectrum](image-url)
(E)-(3-(2-(9,9-dibutyl-7-formyl-9H-fluoren-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl
((9H-fluoren-9-yl)methoxy)carbonyl)glycinate (19): Following the general procedure for esterification, with 18 (32 mg, 46.9 µmol, 1 eq.) and Fmoc-Gly-OH (15.5 mg, 51.6 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: toluene:AcOEt, 9:1 to 8:2) to give 19 as an orange powder (28 mg, 64%).

\[
\begin{align*}
\text{H NMR (CDCl}_3, 400 MHz), \delta (ppm): & & 9.01 (s, 1H), 8.15 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 1.6 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 8.04 (dd, J = 8.0, 1.6 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 16.5 Hz, 1H), 7.60 (dd, J = 8.0, 1.6 Hz, 1H), 7.56 – 7.50 (m, 8H), 7.53 (d, J = 1.6 Hz, 1H), 7.52 (td, J = 8.1, 7.6, 1.6 Hz, 1H), 7.39 (td, J = 8.1, 7.6, 1.6 Hz, 1H), 7.33 (d, J = 16.5 Hz, 1H), 6.61 (dd, J = 9.1, 2.6 Hz, 1H), 6.46 (d, J = 2.6 Hz, 1H), 5.56 (s, 2H), 4.38 (d, J = 7.2 Hz, 2H), 4.16 (t, J = 6.6 Hz, 1H), 4.11 – 4.06 (m, 2H), 3.37 (d, J = 7.1 Hz, 4H), 2.18 – 2.00 (m, 4H), 1.67 – 1.56 (m, 4H), 1.19 (t, J = 7.1 Hz, 6H), 0.67 (t, J = 7.3 Hz, 6H), 0.65 – 0.56 (m, 4H).
\end{align*}
\]

\[
\begin{align*}
13\text{C NMR (DMSO-}d_6, 151 MHz), \delta (ppm): & & 170.4, 169.1, 168.6, 168.2, 160.3, 157.0, 156.5, 154.8, 154.1, 152.1, 152.0, 150.6, 144.2, 143.1, 141.1, 139.9, 138.1, 134.9, 132.1, 128.0, 127.4, 126.6, 125.9, 125.6, 123.5, 123.0, 121.4, 120.6, 117.3, 109.8, 108.2, 96.9, 78.1, 66.4, 65.7, 64.6, 59.2, 55.4, 46.9, 44.5, 42.9, 39.0, 30.5, 26.3, 22.9, 19.0, 14.2, 14.0, 12.8. \text{HRMS (FD): } m/z = 961.41500, \text{ calcd for } C_{61}H_{59}N_3O_6S (M)^+: 961.41246.
\end{align*}
\]
5. Synthesis of the reference compounds DEAC-Gly and DEAC450-Gly

The synthetic pathway implemented for the synthesis of compounds 13-16 and 17, 19 was also used for the synthesis of the DEAC450 reference compound (DEAC450-Gly): a pre-formed acrylamide synthon was coupled with the iodinated intermediate 2 by Heck reaction. With fewer steps and no protecting groups involved, this proved to be an interesting alternative to the synthetic approach previously described synthesis of DEAC450-Glu. In that particular case, it turned out to be the easiest way to shape the amide bond, other activation modes ending up being surprisingly challenging with this substrate.

Scheme S6. Synthesis of the reference cage DEAC450.
di-\textit{tert}-butyl acryloyl-L-aspartate (25):

A stirred solution of di-\textit{tert}-butyl L-aspartate (1.0 g, 3.55 mmol, 1 eq.) in dry CH$_2$Cl$_2$ (36 mL) was placed under argon at 0 °C. Triethylamine (1.05 mL, 7.45 mmol, 2.1 eq.) was added and the mixture was stirred for 10 min, then acryloyl chloride (0.29 mL, 3.55 mmol, 1 eq.) was added dropwise over 5 minutes. Upon completion of the addition, the resulting mixture was allowed to warm up to room temperature, and stirred for 4 h. The solvents were then removed under vacuum, and the residue was suspended in THF. The white precipitate was filtered off over a pad of Celite®, and the filtrate was evaporated. The resulting white solid was pure enough to be used without further purification (1.1 g, \textit{quant.}).

$^1$H NMR (CDCl$_3$, 300 MHz), $\delta$ (ppm): 6.60 (d, $J = 7.7$ Hz, 1H), 6.29 (dd, $J = 17.0$, 1.6 Hz, 1H), 6.13 (dd, $J = 17.0$, 10.1 Hz, 1H), 5.67 (dd, $J = 10.1$, 1.6 Hz, 1H), 4.81 – 4.69 (m, 1H), 2.84 (ddd, $J = 42.6$, 17.2, 4.3 Hz, 2H), 1.46 (s, 8H), 1.43 (s, 9H). $^{13}$C NMR (CDCl$_3$, 151 MHz), $\delta$ (ppm): 170.5, 169.9, 165.1, 130.7, 127.0, 82.6, 81.7, 49.2, 37.5, 28.2, 28.0. FTIR $\nu$ (cm$^{-1}$): 3426.9, 2982.8, 1732.3, 1665.9, 1525.2, 1369.9, 1155.2, 845.8.
Following the general procedure for Heck coupling, with 2 (500 mg, 1.34 mmol, 1 eq.) and 25 (441 mg, 1.47 mmol, 1.1 eq.). The reaction was quenched with H₂O, and solvents were removed under vacuum. The residue was extracted with AcOEt, dried and evaporated. The crude was then purified by column chromatography of silicagel (gradient eluent: CH₂Cl₂:acetone, 98:2 to 70:30). Brown solid (257 mg, 57%).

**1H NMR (CDCl₃, 600 MHz), δ (ppm):** 7.85 (d, J = 15.1 Hz, 1H), 7.72 (d, J = 9.2 Hz, 1H), 7.27 (d, J = 15.1 Hz, 1H), 6.78 (d, J = 8.1 Hz, 1H), 6.62 (dd, J = 9.2, 2.6 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 4.95 (s, 4H), 4.82 – 4.76 (m, 1H), 3.41 (q, J = 7.1 Hz, 4H), 2.90 (dd, J = 17.1, 4.4 Hz, 1H), 2.74 (dd, J = 17.1, 4.4 Hz, 1H), 1.46 (s, 9H), 1.43 (s, 9H), 1.20 (t, J = 7.1 Hz, 6H).

**13C NMR (CDCl₃, 151 MHz), δ (ppm):** 170.4, 169.9, 166.9, 160.8, 156.0, 152.0, 151.3, 133.0, 127.6, 124.0, 113.3, 109.6, 108.4, 97.2, 82.4, 81.6, 56.5, 49.5, 44.99, 49.53, 56.51. HRMS (FD): m/z = 544.27724, calcd for C₂₉H₄₀N₂O₈ (M⁺): 544.27846. **FTIR ν (cm⁻¹):** 3428.1, 2976.2, 2922.6, 1726.6, 1607.5, 1510.7, 1354.3, 1148.7, 845.1.
di-tert-butyl (E)-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)glycyl)oxy)methyl)-7-(diethylamino)-coumarin-3-yl)acryloyl)-L-aspartate (DEAC450-Gly):

Following the general procedure for esterification with 26 (26 mg, 47.7 µmol, 1 eq.) and Fmoc-Gly-OH (15.6 mg, 52.5 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH₂Cl₂:acetone, 1:0 to 8:2) to give DEAC450-Gly as a dark yellow powder (25 mg, 65%).

¹H NMR (CDCl₃, 600 MHz), δ (ppm): 7.80 (d, J = 15.1 Hz, 1H), 7.74 (d, J = 7.3 Hz, 2H), 7.52 (dd, J = 7.3, 1.5 Hz, 2H), 7.46 (d, J = 9.2 Hz, 1H), 7.38 (t, J = 7.3 Hz, 2H), 7.32 (d, J = 15.1 Hz, 1H), 7.27 (t, J = 7.3 Hz, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.58 (dd, J = 9.2, 2.4 Hz, 1H), 6.35 (d, J = 2.4 Hz, 1H), 5.52 (t, J = 5.8 Hz, 1H), 5.47 (s, 2H), 4.83 (dt, J = 8.5, 4.4 Hz, 1H), 4.36 – 4.27 (m, 2H), 4.10 (t, J = 7.3 Hz, 1H), 4.06 (d, J = 5.9 Hz, 2H), 3.33 (q, J = 7.1 Hz, 3H), 2.93 (dd, J = 17.1, 4.4 Hz, 1H), 2.77 (dd, J = 17.1, 4.5 Hz, 1H), 1.47 (s, 9H), 1.45 (s, 9H), 1.16 (t, J = 7.1 Hz, 5H).

¹³C NMR (CDCl₃, 151 MHz), δ (ppm): 170.4, 169.9, 169.7, 166.3, 160.2, 156.5, 155.7, 151.4, 145.9, 143.9, 141.3, 141.3, 132.3, 127.8, 127.1, 126.6, 125.3, 120.1, 115.0, 109.7, 107.9, 97.3, 82.5, 81.7, 67.5, 58.8, 49.5, 47.0, 45.0, 43.0, 37.8, 28.2, 28.1, 12.6. HRMS (ESI): m/z = 846.3561, calcd for C₄₆H₅₃N₃O₁₁Na (M+Na)⁺: 846.3572. Melting point: 132 °C.
Scheme S7. Synthesis of the reference cage DEAC.

(7-(diethylamino)-coumarin-4-yl)methyl ((9H-fluoren-9-yl)methoxy)carbonyl)glycinate (DEAC-Gly):

Following the general procedure for esterification, with 1 (400 mg, 1.62 mmol, 1 eq.) and Fmoc-Gly-OH (962 mg, 3.24 mmol, 2 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH$_2$Cl$_2$:acetone, 1:0 to 9:1), to give DEAC-Gly as a bright yellow powder (511 mg, 60%).

$^1$H NMR (DMSO-$d_6$, 600 MHz), $\delta$ (ppm): 7.89 (d, $J = 7.6$ Hz, 2H), 7.88 (t, $J = 6.2$ Hz, 1H), 7.69 (d, $J = 7.6$ Hz, 2H), 7.44 (d, $J = 9.0$ Hz, 1H), 7.41 (t, $J = 7.6$ Hz, 2H), 7.32 (td, $J = 7.6$, 1.1 Hz, 2H), 6.66 (dd, $J = 9.1$, 2.6 Hz, 1H), 6.52 (d, $J = 2.6$ Hz, 1H), 6.04 (t, $J = 0.9$ Hz, 1H), 5.34 (d, $J = 0.9$ Hz, 2H), 4.32 (d, $J = 7.1$ Hz, 2H), 4.23 (t, $J = 7.1$ Hz, 1H), 3.95 (d, $J = 6.2$ Hz, 2H), 3.40 (q, $J = 7.0$ Hz, 4H), 1.10 (t, $J = 7.0$ Hz, 6H).$^{13}$C NMR (DMSO-$d_6$, 151 MHz), $\delta$ (ppm): 169.8, 160.6, 156.6, 155.8, 150.4, 150.3, 143.8, 140.7, 127.6, 127.1, 125.5, 125.2, 120.1, 108.7, 105.2, 105.1, 96.8, 65.8, 61.6, 46.6, 44.0, 42.3, 12.3. HRMS (ESI): $m/z = 549.2013$, calcd for C$_{31}$H$_{30}$N$_2$O$_6$Na (M+Na)$^+$: 549.1996. FTIR $\nu$ (cm$^{-1}$): 3435.1, 3065.9, 2971.7, 2928.6, 2868.1, 1714.5, 1618.7, 1604.6, 1528.0, 1422.2, 1355.7, 1273.9, 1184.4, 1071.2, 760.9, 742.7. Melting point: 139 °C.
II. One- and two-photon photophysical studies

1. Materials and methods

All photophysical studies have been performed with freshly-prepared air-equilibrated solutions at room temperature (298 K). UV/Vis absorption spectra of \(\sim 10^{-5}\) M solutions were recorded on a Jasco V-670 spectrophotometer. Steady-state fluorescence measurements were performed on dilute solutions (ca. \(10^{-6}\) M, optical density \(\leq 0.1\)) contained in standard 1 cm quartz cuvettes using a Fluorolog spectrofluorometer. The emission spectra were corrected for the wavelength-sensitivity of the detection unit, obtained, for each compound, under excitation at the wavelength of the absorption maximum. Fluorescence quantum yields of these dilute chromophore solutions were measured according to literature procedures\(^5,6\) using Fluorescein (FLSCN, \(\Phi_f = 0.9\) in NaOH 0.1 M, \(\lambda_{exc} = 474\) nm), or Rhodamine-6G (R6G, \(\Phi_f = 0.94\) in EtOH, \(\lambda_{exc} = 488\) nm) depending on the emission range.\(^7\) The emission quantum yield values derived from these measurements were calculated with the following equation taking into account the refractive index \(n\), the absorbance \(A\), and the integral of the emission \(I_f(\lambda_{exc}, \lambda_f)\):

\[
\Phi_f = \frac{I_f(\lambda_{exc}, \lambda_f)}{k \cdot A \cdot \int I(\lambda) \cdot n^2 \cdot d\lambda}
\]
\[
\Phi_{\text{ech}} = \Phi_{\text{ref}} \times \left( \frac{n_{\text{ech}}}{n_{\text{ref}}} \right)^2 \times \frac{1 - 10^{-A_{\text{ech}}(\lambda_{\text{exc}})}}{1 - 10^{-A_{\text{ref}}(\lambda_{\text{exc}})}} \times \frac{\int_{0}^{\infty} I_{\text{ech}}(\lambda_{\text{exc}}) d\lambda_{\text{f}}}{\int_{0}^{\infty} I_{\text{ref}}(\lambda_{\text{exc}}) d\lambda_{\text{f}}}
\]

The reported fluorescence quantum yield values obtained via this method are within ± 0.005.

2PA cross sections (\( \sigma_2 \)) were derived from the two-photon excited fluorescence (TPEF) cross sections (\( \sigma_2 \Phi_i \)) and the fluorescence emission quantum yield (\( \Phi_i \)). TPEF cross sections were measured relative to fluorescein in 0.01M aqueous NaOH in the 680-1000 nm spectral range, and relative to Nile Red in DMSO in the 1000-1160 nm spectral range, using the well-established method described by Xu and Webb and the appropriate solvent-related refractive index corrections. The quadratic dependence of the fluorescence intensity on the excitation power was checked for each sample and all wavelengths. Measurements were conducted using an excitation source delivering fs pulses.

A Chameleon Ultra II (COHERENT) with Nd:YVO4-pumped Ti:Sapphire oscillator, generating 140 fs pulses at an 80 MHz repetition rate, was used to scan the 680-1080 nm range. To scan the 1000–1200 nm range, an optical parametric oscillator (OPO, PP-BBO) was added to the setup to collect and modulate the output signal of the Ti:Sapphire oscillator. The excitation was focused into the cuvette through a microscope objective (10X, NA 0.25). The fluorescence was detected in epifluorescence mode via a dichroic mirror (Chroma 675dcxru) and a barrier filter (Chroma e650sp-2p) by a compact CCD spectrometer module BWTek BTC112E. Total fluorescence intensities were obtained by integrating the corrected emission.
2. 1PA, emission and 2PA spectra

Dimethylsulfoxide was chosen as a common solvent for the comprehensive photophysical study of the series of compounds for optimal solubility reasons. However, compound 16 emits in the far-red NIR region in DMSO, thus preventing to access the higher energy band located around 700 nm by 2PEF measurements. Hence 2PA measurements were conducted in toluene (in which compound 16 emits in the visible) allowing 2PEF measurements in the full 700-1100 nm range.

| Compound | Normalized absorption & emission | 1PA vs 2PA spectra |
|----------|---------------------------------|--------------------|
| DEAC in DMSO | ![Normalized absorption & emission](image1) | ![1PA vs 2PA spectra](image2) |
| DEAC450 in DMSO | ![Normalized absorption & emission](image3) | ![1PA vs 2PA spectra](image4) |
| 13 in DMSO | ![Normalized absorption & emission](image5) | ![1PA vs 2PA spectra](image6) |
Normalized absorption & emission

1PA vs 2PA spectra

14 in DMSO

15 in DMSO

16 in DMSO
3. Solvatochromic behavior

The solvatochromic behaviour of the dipolar extended coumarin derivatives was investigated in solvent of various polarities. Derivatives 13-1, 17 and 19 showed only weak solvatochromic behaviour, as is the case of compound DEAC450. At opposite, compound 16 which bears a strong EW heterocyclic end-group, clearly showed distinct positive solvatochromic behaviour. A slight red shift of the absorption spectra and a marked red shift of the emission spectra were observed with increasing solvent polarity. This is indicative of an increased in electric dipole moment upon excitation, leading to a polar emissive excited state. This ICT phenomenon is further corroborated by the satisfactory Lippert-Mataga correlation observed for compound 16. In that case only, the Stokes shift values were found to depend linearly on the polarity-polarizability parameter $\Delta f'$ according to the following Equation: 13,14

$$\nu_{abs} - \nu_{em} = 2\Delta \mu \Delta f'/(hc\alpha) + \text{const}$$

where $\nu_{abs}$ ($\nu_{em}$) is the wavenumber of the absorption (fluorescence) maximum, $\Delta \mu$ is the change of dipole moment between the relaxed emissive excited state and corresponding Frank Condon ground state, $h$ is the Planck constant, $c$ is the light velocity, $a$ is the radius of the Onsager spherical cavity, and $\Delta f' = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$, where $\varepsilon$ is the dielectric constant and $n$ the refractive index of the solvent.

| Solvent        | $\lambda_{Abs}$ [nm] | $\lambda_{em}$ [nm] | Stoke Shift $[10^3 \text{ cm}^{-1}]$ | $\Delta f'$ |
|----------------|-----------------------|----------------------|--------------------------------------|------------|
| Toluene        | 459                   | 568                  | 4.2                                  | 0.01314    |
| CHCl$_3$       | 462                   | 601                  | 5.0                                  | 0.14816    |
| Ethyl Acetate  | 458                   | 600                  | 5.2                                  | 0.20020    |
| THF            | 462                   | 610                  | 5.3                                  | 0.20888    |
| CH$_2$Cl$_2$   | 464                   | 612                  | 5.2                                  | 0.21710    |
| Acetone        | 459                   | 649                  | 6.4                                  | 0.28427    |
| CH$_3$CN       | 461                   | 673                  | 6.8                                  | 0.30492    |
| DMSO           | 473                   | 694                  | 6.7                                  | 0.30394    |
Normalized absorption spectra of DEAC450

| Solvent  | $\lambda_{\text{Abs}}$ [nm] | $\lambda_{\text{em}}$ [nm] | Stoke Shift [10$^3$ cm$^{-1}$] | $\Delta f$ |
|----------|-----------------------------|-----------------------------|--------------------------------|-----------|
| Toluene  | 431                         | 500                         | 3.2                            | 0.01314   |
| CHCl$_3$ | 443                         | 505                         | 2.8                            | 0.14816   |
| Ethyl Acetate | 429                 | 502                         | 3.4                            | 0.20020   |
| THF      | 431                         | 505                         | 3.4                            | 0.20888   |
| CH$_2$Cl$_2$ | 441                 | 503                         | 2.8                            | 0.21710   |
| Acetone  | 433                         | 512                         | 3.6                            | 0.28427   |
| CH$_3$CN | 436                         | 519                         | 3.7                            | 0.30492   |
| DMSO     | 444                         | 525                         | 3.5                            | 0.30393   |

Normalized emission spectra of compound DEAC450

Normalized absorption spectra of compound 13

| Solvent  | $\lambda_{\text{Abs}}$ [nm] | $\lambda_{\text{em}}$ [nm] | Stoke Shift [10$^3$ cm$^{-1}$] | $\Delta f$ |
|----------|-----------------------------|-----------------------------|--------------------------------|-----------|
| Toluene  | 445                         | 527                         | 3.5                            | 0.01314   |
| CHCl$_3$ | 451                         | 532                         | 3.4                            | 0.14816   |
| Ethyl Acetate | 443                 | 518                         | 3.3                            | 0.20020   |
| THF      | 445                         | 524                         | 3.4                            | 0.20888   |
| CH$_2$Cl$_2$ | 450                 | 525                         | 3.2                            | 0.21710   |
| Acetone  | 447                         | 527                         | 3.4                            | 0.28427   |
| CH$_3$CN | 449                         | 534                         | 3.5                            | 0.30492   |
| DMSO     | 458                         | 542                         | 3.6                            | 0.30393   |
Normalized absorption spectra of compound 14

| Solvent   | $\lambda_{\text{Abs}}$ [nm] | $\lambda_{\text{em}}$ [nm] | Stoke Shift [$10^4$ cm$^{-1}$] | $\Delta_f$ |
|-----------|----------------------------|-----------------------------|---------------------------------|------------|
| Toluene   | 458                        | 544                         | 3.5                             | 0.01314    |
| CHCl$_3$  | 463                        | 547                         | 3.3                             | 0.14816    |
| Ethyl Acetate | 455                      | 535                         | 3.3                             | 0.20020    |
| THF       | 459                        | 539                         | 3.2                             | 0.20888    |
| CH$_2$Cl$_2$ | 463                      | 532                         | 2.8                             | 0.21710    |
| Acetone   | 460                        | 544                         | 3.4                             | 0.28427    |
| CH$_3$CN  | 462                        | 548                         | 3.4                             | 0.30492    |
| DMSO      | 472                        | 558                         | 3.3                             | 0.30393    |

Normalized emission spectra of compound 14

Normalized absorption spectra of compound 15

| Solvent   | $\lambda_{\text{Abs}}$ [nm] | $\lambda_{\text{em}}$ [nm] | Stoke Shift [$10^4$ cm$^{-1}$] | $\Delta_f$ |
|-----------|----------------------------|-----------------------------|---------------------------------|------------|
| Toluene   | 455                        | 536                         | 3.3                             | 0.01314    |
| CHCl$_3$  | 461                        | 542                         | 3.2                             | 0.14815    |
| Ethyl Acetate | 454                      | 528                         | 3.1                             | 0.20019    |
| THF       | 458                        | 530                         | 3.0                             | 0.20888    |
| CH$_2$Cl$_2$ | 461                      | 529                         | 2.8                             | 0.21710    |
| Acetone   | 461                        | 537                         | 3.1                             | 0.28427    |
| CH$_3$CN  | 461                        | 540                         | 3.2                             | 0.30492    |
| DMSO      | 472                        | 563                         | 3.4                             | 0.30394    |

Normalized emission spectra of compound 15

$y = -364.18x + 3340.1$
$r^2 = 0.0313$

$\Delta f = \frac{\lambda_{\text{em}} - \lambda_{\text{Abs}}}{\lambda_{\text{em}}} = K \lambda_{\text{em}}^2 + x \lambda_{\text{em}} + y$
Normalized absorption spectra of compound 17

| Solvent    | $\lambda_{\text{Abs}}$ [nm] | $\lambda_{\text{em}}$ [nm] | Stoke Shift $10^3$ cm$^{-1}$ | $\Delta f$ |
|------------|----------------------------|----------------------------|-------------------------------|------------|
| Toluene    | 446                        | 529                        | 3.5                           | 0.01314    |
| CHCl$_3$   | 449                        | 540                        | 3.8                           | 0.14816    |
| Ethyl Acetate | 443                        | 531                        | 3.7                           | 0.20020    |
| THF        | 445                        | 528                        | 3.5                           | 0.20888    |
| CH$_2$Cl$_2$ | 450                        | 536                        | 3.6                           | 0.21710    |
| Acetone    | 445                        | 538                        | 3.9                           | 0.28427    |
| CH$_3$CN   | 447                        | 542                        | 3.9                           | 0.30492    |
| DMSO       | 457                        | 553                        | 3.8                           | 0.30394    |

Normalized emission spectra of compound 17

$\gamma = 1140.3x + 3474.8$

$R^2 = 0.4913$

Normalized absorption spectra of compound 19

| Solvent    | $\lambda_{\text{Abs}}$ [nm] | $\lambda_{\text{em}}$ [nm] | Stoke Shift $10^3$ cm$^{-1}$ | $\Delta f$ |
|------------|----------------------------|----------------------------|-------------------------------|------------|
| Toluene    | 449                        | 533                        | 3.5                           | 0.01314    |
| CHCl$_3$   | 451                        | 541                        | 3.7                           | 0.14816    |
| Ethyl Acetate | 443                        | 529                        | 3.7                           | 0.20020    |
| THF        | 448                        | 529                        | 3.4                           | 0.20888    |
| CH$_2$Cl$_2$ | 450                        | 521                        | 3.0                           | 0.21710    |
| Acetone    | 446                        | 536                        | 3.8                           | 0.28427    |
| CH$_3$CN   | 448                        | 540                        | 3.8                           | 0.30492    |
| DMSO       | 458                        | 547                        | 3.6                           | 0.30393    |

Normalized emission spectra of compound 19

$\gamma = 526.15x + 3443.8$

$R^2 = 0.0421$
III. Comparative photolysis experiments

Uncaging quantum yield $\Phi_u$ of a PPG is usually defined as $\Phi_u = (I \cdot 1000 \cdot \varepsilon \cdot t_{90})^{-1}$, where $I$ is the irradiation intensity (in einstein.cm$^{-2}$s$^{-1}$), $\varepsilon$ the molar extinction coefficient (in M$^{-1}$cm$^{-1}$), and $t_{90}$ the irradiation time necessary to reach 90% conversion (in s).

The one-photon uncaging sensitivity of our new PPGs was determined in a relative way, by comparing their conversion kinetics with respect to an appropriate reference (DEAC450-[Gly] ($\Phi_u = 39\%$ in phosphate buffer) for photolysis at 455 nm). According to the previous equation, the ratio of the rate constants is indeed directly proportional to the 1P uncaging sensitivities of the new compound ($\varepsilon_{ec}^Q/\Phi_u^Q$) and the reference compound ($\varepsilon_{ref}^Q/\Phi_{ref}^Q$):

$$\frac{k_{ec}^Q}{k_{ref}^Q} = \frac{\varepsilon_{ec}^Q \Phi_u^Q}{\varepsilon_{ref}^Q \Phi_{ref}^Q}$$

Determination of the $\varepsilon$ values at the excitation wavelength, measured in the solvent of the photolysis, then allowed the determination of the $Q_u/\Phi_u^Q$ ratio.

The conversion rate ($\xi$) was monitored over time by $^1$H NMR. Solutions of the unknown and reference samples of same concentration (either 0.7 or 0.25 mM, depending on the solubility) were prepared in a mixture of CD$_3$CN/D$_2$O (9/1, v/v) and introduced in a quartz NMR tube. The resulting samples (0.6 mL) were irradiated at maximum power in an optical cage cube equipped with a mounted high-power LED (Thorlabs M455L3, 900 mW at 455 nm). The beam was collimated onto the sample with a Ø2” lense, and light was collected at the end of the optical path with an aluminum mirror. During the course of the irradiation, the intensity of the singlet related to caged Fmoc-Gly-OR (3.91 ppm) decreased, while a second singlet (3.76 ppm) appeared for the released acid Fmoc-Gly-OH. In parallel, the doublet related to the CH$_2$ of the Fmoc group in caged glycine (4.14 ppm) was shifted to 4.32 ppm in free glycine. A first order kinetics was observed for all derivatives.

IV. Dark stability

The stability of our compounds towards dark hydrolysis was assessed by monitoring their absorption spectra in the solvent of photolysis (CH$_3$CN/H$_2$O, 9/1, v/v) over one week at room temperature (21 °C). Figure S1,a) shows the decrease of the recorded absorbance at $\lambda_{abs}$max over time, starting from the normalized absorption spectra at t = 0. No significant change in the shape or the intensity of the absorption band were observed in such conditions, evidencing that the rate of absorption decrease was not competitive with the rate of the uncaging reactions upon photolysis.
Fluorenyl derivative 17 showed slightly sharper evolution of the absorption maximum, but still retained very good stability.

The dark stability of compound 19 was verified from a structural point of view by monitoring the evolution of the $^1$H NMR spectrum in the same conditions as the photolysis, but without irradiation (Fig. S1,b). Comparison of hydrolysis under irradiation and in the dark shows the relatively good dark stability of compound 19. 25% of hydrolysis of the glycine ester was observed after 8 days at room temperature.

![Figure S1. a) Evolution of the absorbance maximum of compounds 13-17 over time starting from the normalized absorption spectrum at t = 0; b) Conversion of the PPG 19 to free Fmoc-Gly-OH over time upon irradiation or dark hydrolysis, monitored by $^1$H NMR.](image)

V. Two-photon photolysis

Two-photon photolysis experiments were performed with the TPEF setup (vide supra). Solutions of the PPGs prepared in CD$_3$CN/D$_2$O (9/1, v/v) at identical concentrations (0.25 mM) were introduced in a 10*4 mm quartz cuvette and irradiated with a Chameleon Ultra II (COHERENT) generating 140 fs pulses at a 80 MHz rate. Photolysis of the samples (1.2 mL, stirred at 500 rpm) were carried out either at 730 or 940 nm, with a power of 1.0 W. The two-photon induced photorelease of Fmoc-Gly-OH was monitored by $^1$H NMR after 7 h of irradiation.
VI. DFT calculations

Computational methods

The geometry of the ground (S\textsubscript{0}) and first singlet excited (S\textsubscript{1}) states were optimized in the gas phase in the framework of the Density Functional Theory (DFT) using the M06-2X exchange-correlation functional\textsuperscript{15} and the 6-311G(d) basis set. All structures were characterized as real minima of the potential energy surface on the basis on their positive vibrational force constants. Ground state optimized geometries were used to compute the vertical transition energies and excited state properties by employing the Time-Dependent Density Functional Theory (TD-DFT) at the M06-2X/6-311G(d) level. Solvent effects (acetonitrile) were taken into account in these calculations by using the Integral Equation Formalism of the Polarizable Continuum Model (IEF-PCM).\textsuperscript{16} The photo-induced charge transfer was analyzed on the basis of vertical electronic transitions, by considering the difference \(\Delta \mu_{\text{vert}}\) in the electric dipoles of the S\textsubscript{0} and S\textsubscript{1} states. Using the approach reported in refs \textsuperscript{17} and \textsuperscript{18}, \(\Delta \mu_{\text{vert}}\) was further decomposed as \(\Delta \mu_{\text{vert}} = q_{\text{CT}} \times d_{\text{CT}}\), where \(q_{\text{CT}}\) is the photo-induced charge transfer, \(i.e\). the global amount of charge transferred upon light excitation, and \(d_{\text{CT}}\) is the distance over which this charge is transferred.

Then, the effects of structural relaxation on the electronic structure of the S\textsubscript{1} state were addressed. The adiabatic energies, defined as the energy difference of the S\textsubscript{1} and S\textsubscript{0} states in their respective minimum (\(E_{\text{adia}} = E_{\text{opt}S1} - E_{\text{opt}S0}\)), were calculated on the basis of the gas phase geometries. The 0-0 energies were then evaluated as the sum of the adiabatic contribution and the difference of zero-point vibrational energy (ZPVE) between S\textsubscript{1} and S\textsubscript{0} (\(E_{0-0} = E_{\text{adia}} + \Delta E_{\text{ZPVE}}\)). Finally, IEF-PCM:TD-DFT single-point calculations in acetonitrile were performed on the (gas phase) relaxed geometries of S\textsubscript{1}, to evaluate the excited-state dipole moment and photo-induced dipole moment variation \(\Delta \mu_{\text{opt}+s}\) accounting for geometrical relaxation effects. All calculations were performed using the Gaussian 09 package.\textsuperscript{19}

1. Structures used for the calculations
Scheme S8: Simplified chemical structures of compounds 13-17 and 19 used in the DFT calculations.

2. Vertical absorption spectra
**Figure S2.** Vertical absorption spectra of the investigated compounds, calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile. Electronic transitions with oscillator strength $f \geq 0.1$ are reported as grey dashed vertical bars.

**Figure S3.** Vertical absorption spectra of the investigated compounds, calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile.
Figure S4. Electron density difference between the lowest-energy excited state and the ground state, $\Delta \rho = \rho(S_1) - \rho(S_0)$, as calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile. Dark (light) blue lobes are associated with negative (positive) $\Delta \rho$ values.

3. Geometrical relaxation in the $S_1$ state

Table S1. Torsional angles between the bridge and the EWG moiety, as calculated at the M06-2X/6-311G(d) level in the $S_0$ and $S_1$ states.

| torsion | $S_0$  | $S_1$  |
|---------|--------|--------|
| DEAC450 | 179.740 | 179.896 |
| 13      | 178.750 | 179.382 |
| 14      | 179.257 | 179.826 |
| 15      | 179.118 | 179.630 |
| 16      | 156.982 | 174.184 |
| 17      | 162.476 | 175.250 |
| 19      | 162.538 | 175.310 |
Figure S5. Rigid energy scans first performed at the M06-2X/6-311G(d) level in the $S_1$ state (left), and focus on the long distance energies (right).

Table S2. Bond dissociation energies (BDE, kcal/mol) evaluated from gas phase rigid energy scans performed at the M06-2X/6-311G(d) level in the $S_1$ state.

| Bond | BDE   |
|------|-------|
| DEAC450  | 125,3  |
| 13     | 112,8  |
| 14     | 116,6  |
| 15     | 118,7  |
| 16     | 112,3  |
| 17     | 106,7  |
| 19     | 105,7  |
Table S3. Relative carbocation stabilization energies evaluated as $E_{\text{stab}} = E_{\text{PPG-Gly}} - E_{\text{PPG}} + - E_{\text{Gly-}}$ where $E_{\text{PPG-Gly}}$, $E_{\text{PPG}}^+$, and $E_{\text{Gly-}}$ are respectively the ground-state electronic energies of the parent PPG compound and of the separated carbocation and counter anion, calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile (using gas phase geometries).

| DEAC450 | 95.2 |
| 13      | 82.6 |
| 14      | 86.9 |
| 15      | 89.7 |
| 16      | 81.4 |
| 17      | 77.5 |
| 19      | 76.2 |

Table S4. Vertical transition energy ($\Delta E_{\text{ge}}$, eV) as well as oscillator strength ($f_{\text{ge}}$), transition dipole moment ($\mu_{\text{ge}}$, D), MO contributions (%), dipole moment variation ($\Delta \mu_{01}$, D), charge transferred upon excitation ($q_{\text{CT}}$, |e|), and charge transfer distance ($d_{\text{CT}}$, Å), calculated at the TDDFT/M06-2X/6-311G(d) level in acetonitrile.

| state   | $\Delta E_{\text{ge}}$ | $f_{\text{ge}}$ | $\mu_{\text{ge}}$ | MO contributions % | $\Delta \mu_{01}$ | $q_{\text{CT}}$ | $d_{\text{CT}}$ |
|---------|-------------------------|-----------------|-------------------|--------------------|------------------|-----------------|-----------------|
| DEAC450 | 1                       | 3.125           | 1.385             | HOMO-1↑LUMO        | 96               | 8.25            | 0.584           | 2.940           |
| 13      | 1                       | 2.935           | 1.701             | HOMO-1↑LUMO        | 96               | 6.70            | 0.540           | 2.581           |
| 14      | 1                       | 2.874           | 1.869             | HOMO-1↑LUMO        | 95               | 8.09            | 0.568           | 2.967           |
|         | 2                       | 4.088           | 0.129             | HOMO-1↑LUMO        | 62              | 0.46            | 0.484           | 0.199           |
| 15      | 1                       | 2.902           | 1.844             | HOMO-1↑LUMO        | 95               | 7.59            | 0.566           | 2.789           |
| 16      | 1                       | 2.941           | 1.548             | HOMO-1↑LUMO        | 95               | 7.80            | 0.581           | 3.775           |
| 17      | 3                       | 3.820           | 0.289             | HOMO-1↑LUMO        | 2                | 7.18            | 0.551           | 2.714           |
| 19      | 1                       | 3.004           | 2.569             | HOMO-1↑LUMO        | 4                | 6.35            | 0.544           | 2.433           |
|         | 2                       | 3.653           | 0.549             | HOMO-1↑LUMO        | 2                | 6.89            | 0.504           | 2.842           |
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