Electronic Supplementary Material (ESI) for Chemical Science.

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_2Cl_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_3N 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).

^1H 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.

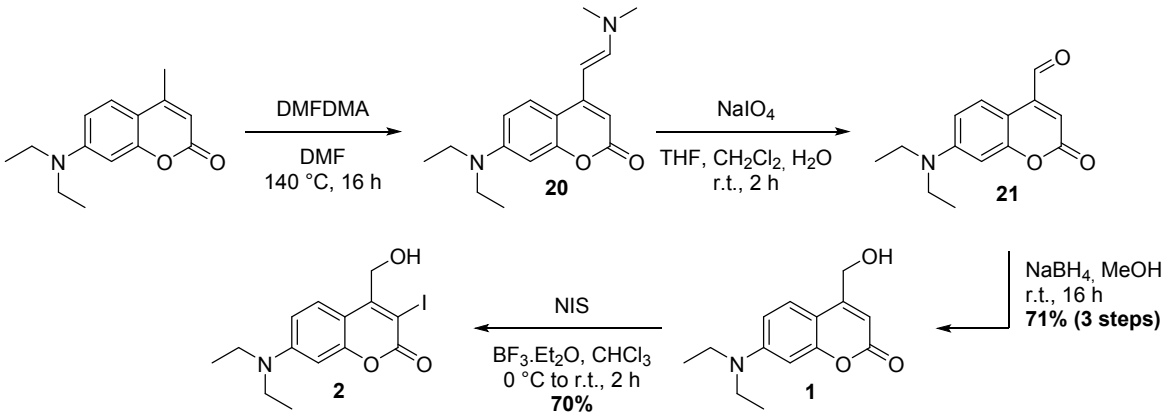
HRMS-ESI spectra were recorded by the CESAMO (Bordeaux, France) on a QStar Elite mass spectrometer (Applied Biosystems). The instrument is equipped with an ESI source and spectra were recorded in the negative/positive mode. The electrospray needle was maintained at 4500 V and operated at room temperature. 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

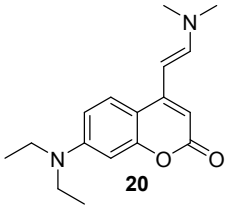
The formation of the alcohol **1** has been extensively described in literature using selenium dioxide as oxidizing agent. This method proved somewhat unreliable in our hands, affording poor yields and reproducibility, and remnant traces of toxic selenium salts in the product. Consequently, an

original alternative pathway was implemented (Scheme S1).[1] 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.



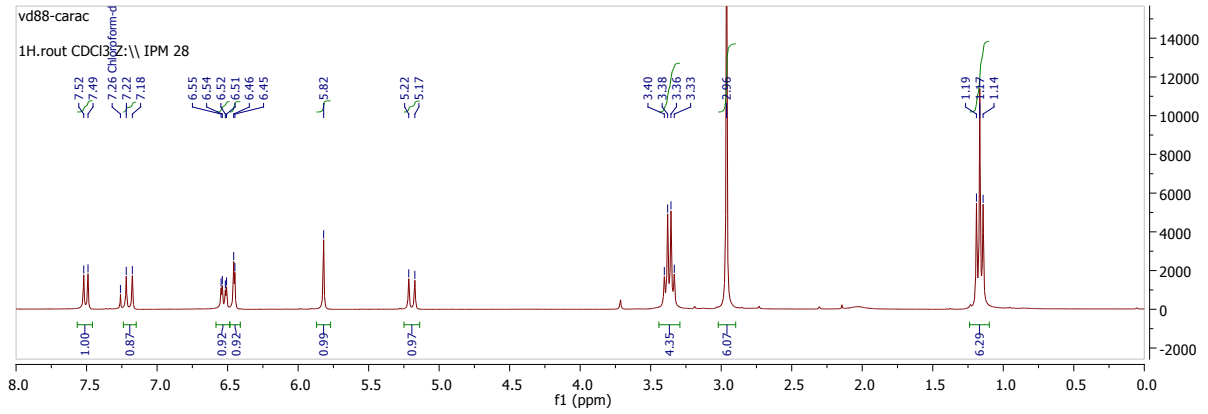
Scheme S1. Synthesis of the iodinated intermediate.

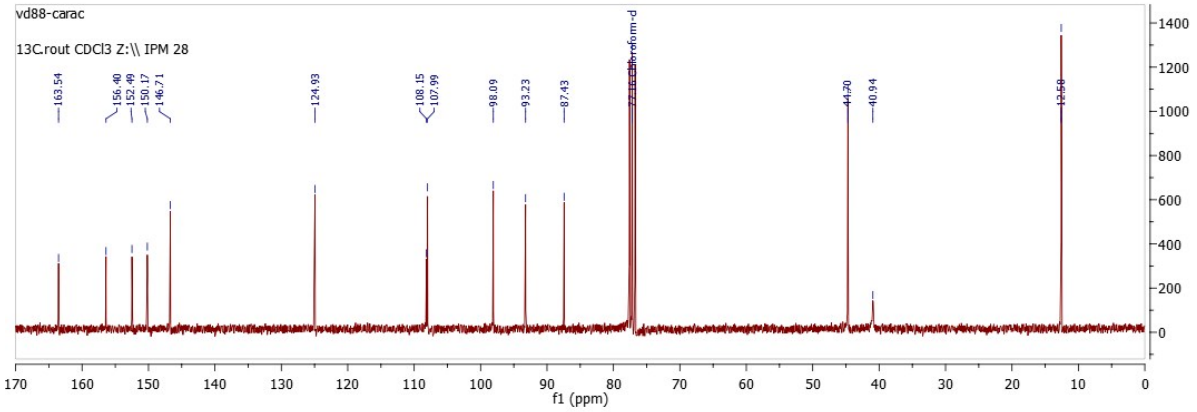
7-(diethylamino)-4-(2-dimethylamino)vinylcoumarin (20):



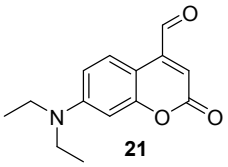
To a stirred solution of 7-(diethylamino)-4-methylcoumarin (20 g, 86.5 mmol, 1 eq.) in dry DMF (10 mL) under argon atmosphere was added DMFDMA (11.7 mL, 86.7 mmol, 1 eq.). The mixture was heated to 140 °C and heavily stirred for 8 h, then more DMFDMA (0.46 mL, 34.6 mmol, 0.4 eq.). Heating was maintained for 8 h, then the solvent was removed under vacuum. The residue was then suspended in cyclohexane (40 mL), filtered, washed with Et_2O (40 mL) and dried under vacuum to afford **20** as a bright yellow powder. (24.6 g, **quant.**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{17}H_{23}N_2O_2$ $(M+H)^+$: 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.



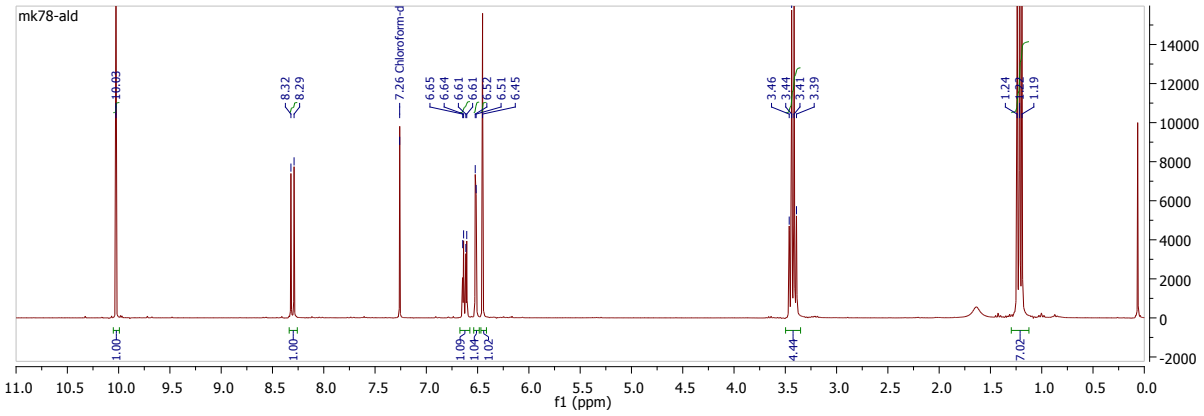


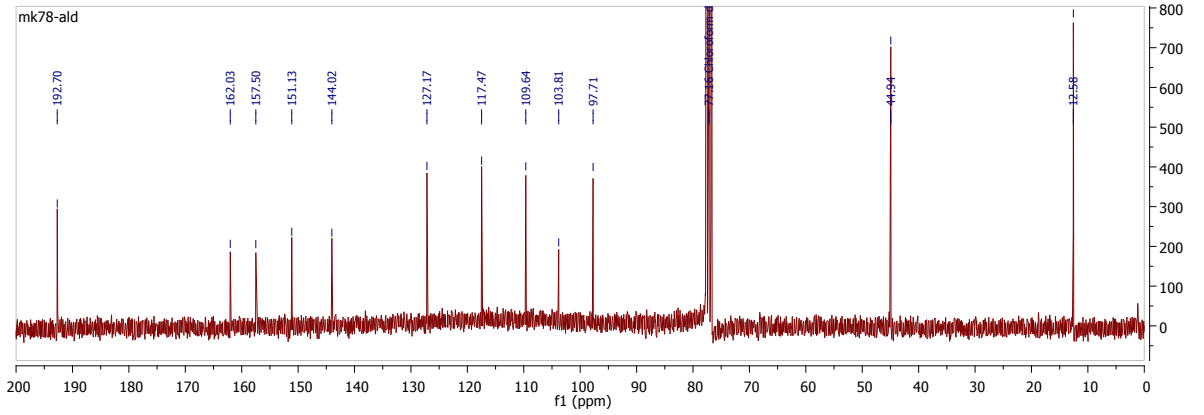
7-diethylamino-4-formylcoumarin (21):



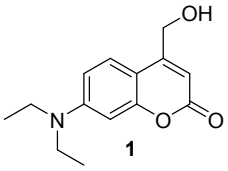
The enamine **20** (24.6 g, 85.9 mmol, 1 eq.) was dissolved in a mixture of THF and CH_2Cl_2 (300 mL, 5/1, v/v). A solution of $NaIO_4$ (55.1 g, 258 mmol, 3 eq.) in H_2O (250 mL) was then added, and the resulting mixture was stirred at room temperature for 2 h. Upon completion of the reaction, the mixture was filtered on Celite®, and the filtrate was partially concentrated under vacuum. The aqueous layer was then extracted with CH_2Cl_2 (5 x 100 mL), and the combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, 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.**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{15}H_{20}NO_4$ $(M+H+MeOH)^+$: 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 °C.





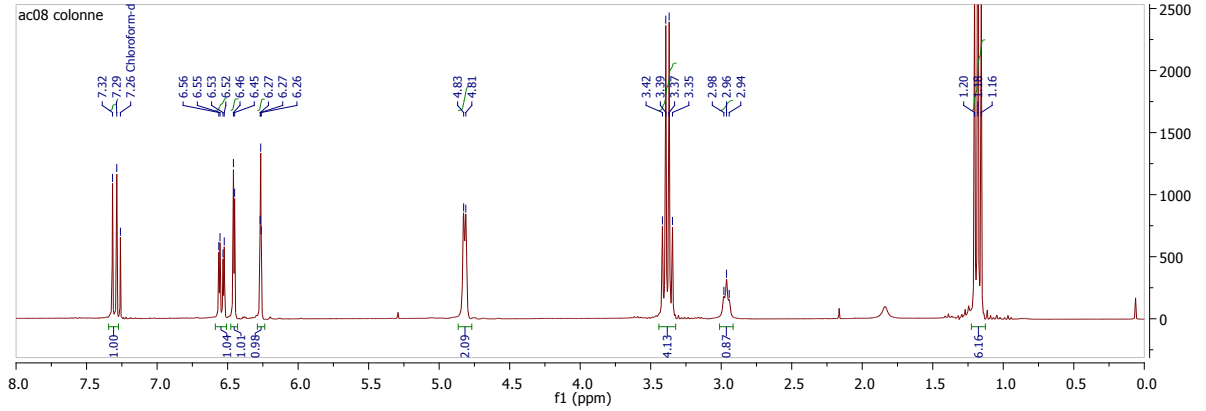
7-diethylamino-4-hydroxymethylcoumarin (1):

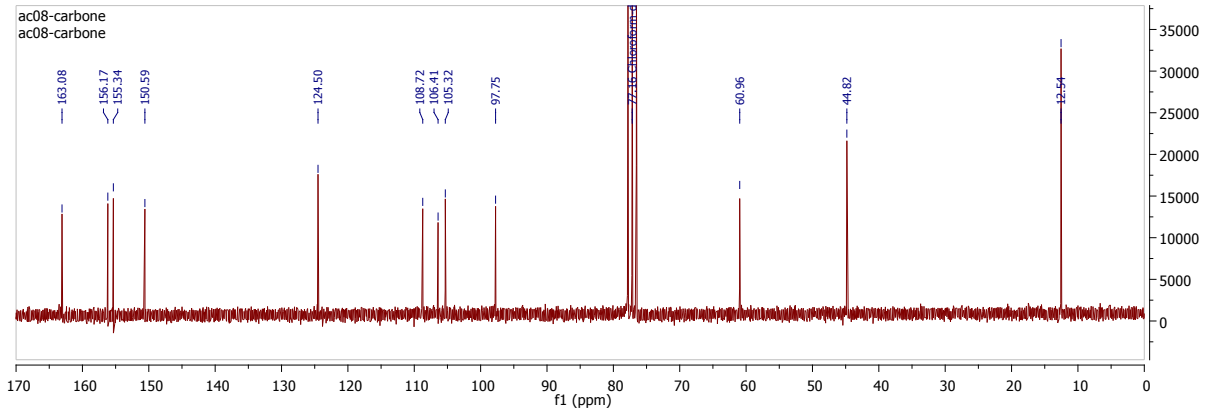


Sodium borohydride (3.90 g, 103 mmol, 1.2 eq.) was added portionwise to a stirred solution of the aldehyde **21** (21.0 g, 85.6 mmol, 1 eq.) in dry methanol (400 mL) at 0 °C and under argon atmosphere. The resulting mixture was allowed to warm up to room temperature and stirred for 16 h. The reaction 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 $NaHCO_3$ solution, then the aqueous layer was extracted with CH_2Cl_2 (4 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by filtration over a pad of silica gel (eluent: CH_2Cl_2, then CH_2Cl_2:acetone, 90:10), to give **1** as a pale greenish solid (15.0 g, **71%**).

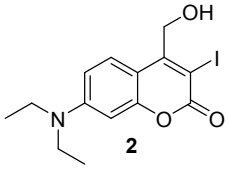
^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.30 (d, J = 9.0 Hz, 1H), 6.54 (dd, J = 9.0, 2.6 Hz, 1H), 6.45 (d, J = 2.6 Hz, 1H), 6.27 (t, J = 1.1 Hz, 1H), 4.82 (dd, J = 5.7, 1.1 Hz, 2H), 3.38 (q, J = 7.1 Hz, 4H), 2.96 (t, J = 5.7 Hz, 1H), 1.18 (t, J = 7.1 Hz, 6H). **^{13}C NMR ($CDCl_3$, 50 MHz), δ (ppm):** 163.1, 156.2, 155.3, 150.6, 124.5, 108.7, 106.4, 105.3, 97.8, 61.0, 44.8, 12.5. **HRMS (ESI):** m/z = 270.1109, calcd

for $C_{14}H_{17}NO_3Na$ $(M+Na)^+$: 270.1100. **FTIR ν (cm^{-1}):** 3435.3, 2970.3, 2929.0, 1678.8, 1599.6, 1523.8, 1324.1, 1086.4, 831.6. **Melting point:** 149 °C.





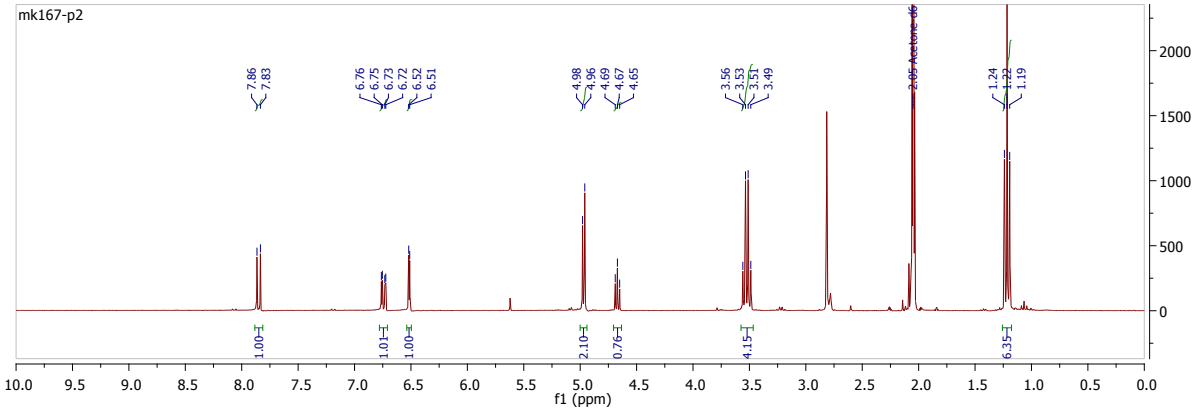
7-diethylamino-4-hydroxymethyl-3-iodocoumarin (2):

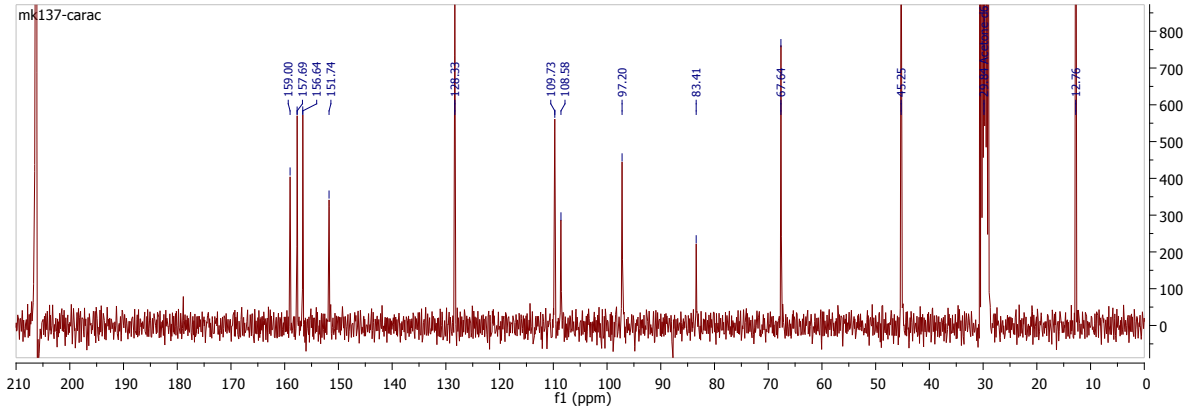


To a stirred solution of **1** (17.2 g, 69.6 mmol, 1 eq.) in $CHCl_3$ (350 mL) under argon atmosphere at 0 °C were added successively *N*-iodosuccinimide (17.2 g, 76.5 mmol, 1.1 eq.) and $BF_3.Et_2O$ (48%, 9.2 mL, 34.8 mmol, 0.5 eq.). The resulting mixture was allowed to warm up to room temperature, and stirred in the dark for 2 h. The pink mixture was quenched with an aqueous $Na_2S_2O_3$ saturated solution, then extracted with AcOEt (3 x 75 mL). The combined organic layers were washed with water, dried over Na_2SO_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%**).

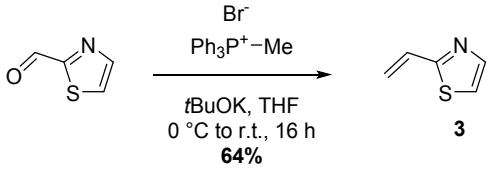
^{1}H NMR (acetone-d_6, 300 MHz), δ (ppm): 7.85 (d, J = 9.2 Hz, 1H), 6.74 (dd, J = 9.2, 2.6 Hz, 1H), 6.52 (d, J = 2.6 Hz, 1H), 4.97 (d, J = 5.9 Hz, 2H), 4.67 (t, J = 5.9 Hz, 1H), 3.52 (q, J = 7.1 Hz, 4H), 1.22 (t, J = 7.1 Hz, 6H). **^{13}C NMR (acetone-d_6, 75 MHz), δ (ppm):** 159.0, 157.7, 156.6, 151.7, 128.3, 109.7, 108.6, 97.2, 83.4, 67.6, 45.3, 12.8. **HRMS (ESI):** m/z = 396.0069, calcd for $C_{14}H_{16}INO_3Na$

$(M+Na)^+$: 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.



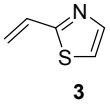


3. Synthesis of the vinyl moieties



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

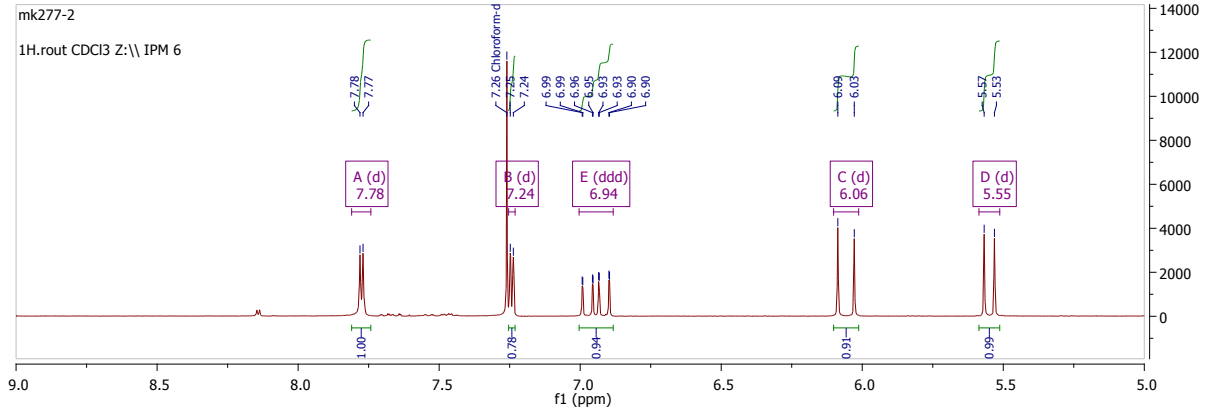
2-vinylthiazole (3):

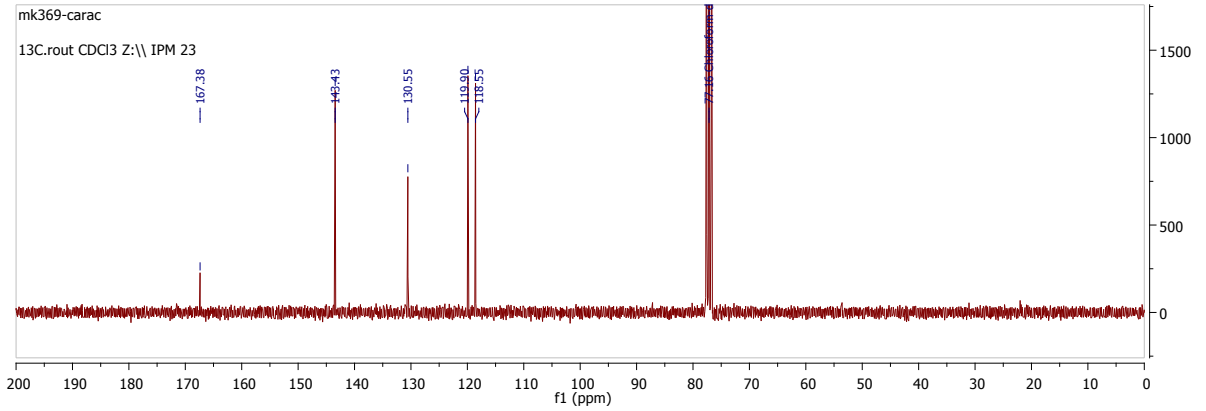


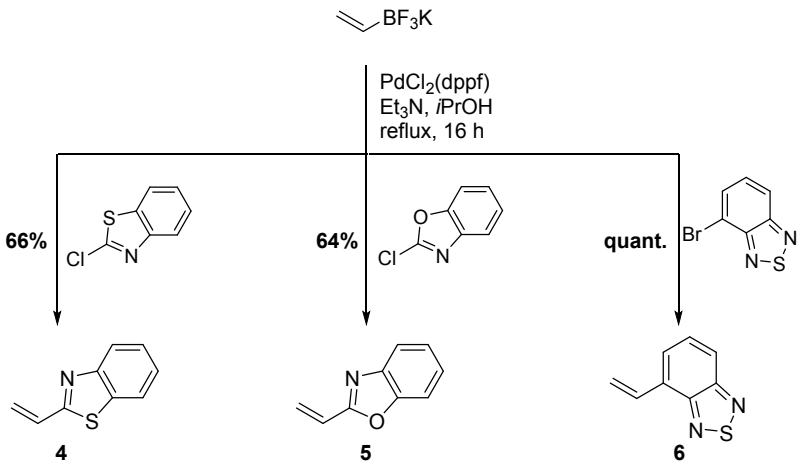
Argon was bubbled into a stirred suspension of methyltriphenylphosphonium bromide (3.47 g, 9.72 mmol, 1.1 eq.) in dry THF (20 mL) for 15 min, then potassium *tert*-butoxide (1.09 g, 9.72 mmol, 1.1 eq.) at 0 °C. The resulting yellow mixture was stirred at 0 °C for 1 h, then a solution of thiazole-2-carbaldehyde (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 Et_2O (3 x 20 mL). The combined organic layers were washed with water, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography of silicagel (gradient eluent: pentane:Et_2O, 95:5 to 80:20) to give **3** as a pale yellow oil (624 mg, **64%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm):** 167.4, 143.4, 130.6, 119.9, 118.6.

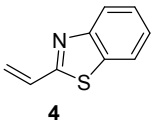






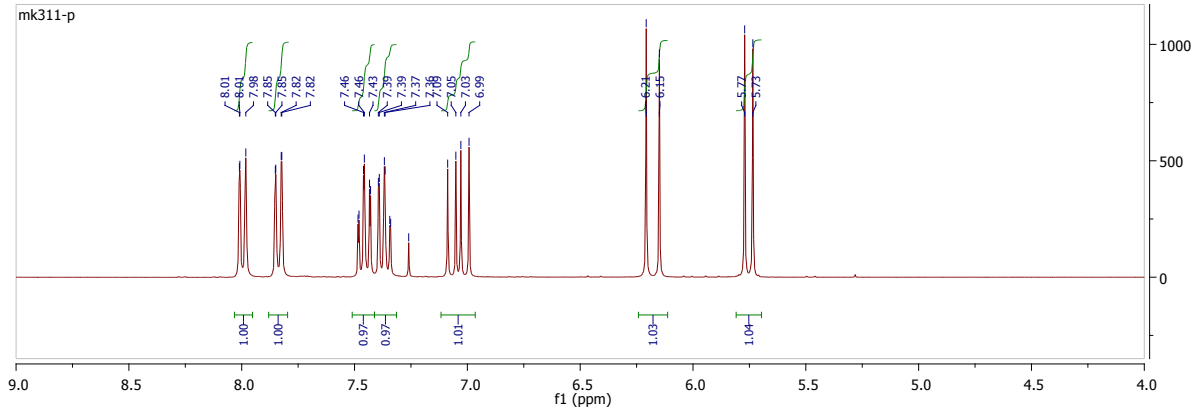
Scheme S3. Synthesis of electron-withdrawing intermediates by Suzuki coupling.

2-vinylbenzo[*d*]thiazole (4):

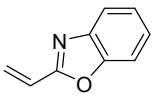


Compound **4** was synthesized according to a procedure described in literature.[2] Pale yellow oil which crystallized upon standing. (977 mg, **66%**). Analytical data were in accordance with literature.

^{1}H NMR ($CDCl_3$, 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).



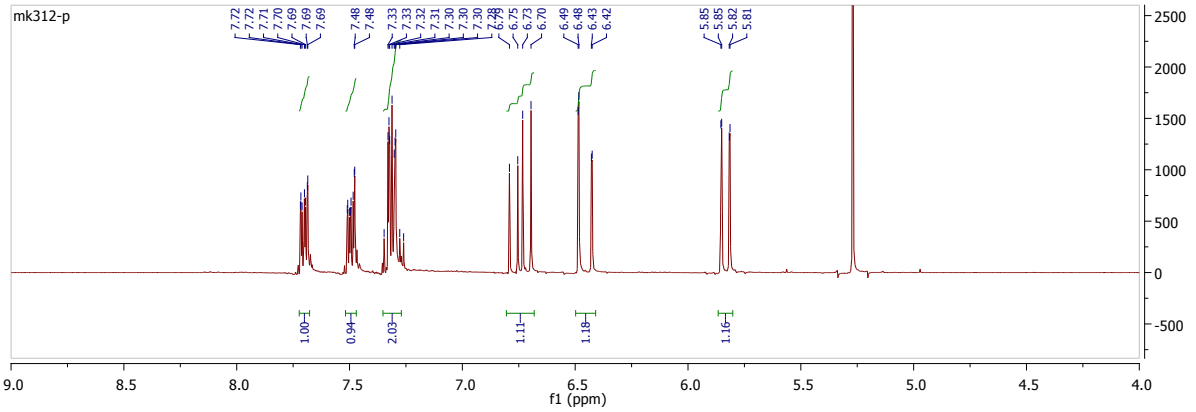
2-vinylbenzo[*d*]oxazole (5):



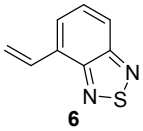
5

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.

^{1}H NMR ($CDCl_3$, 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):

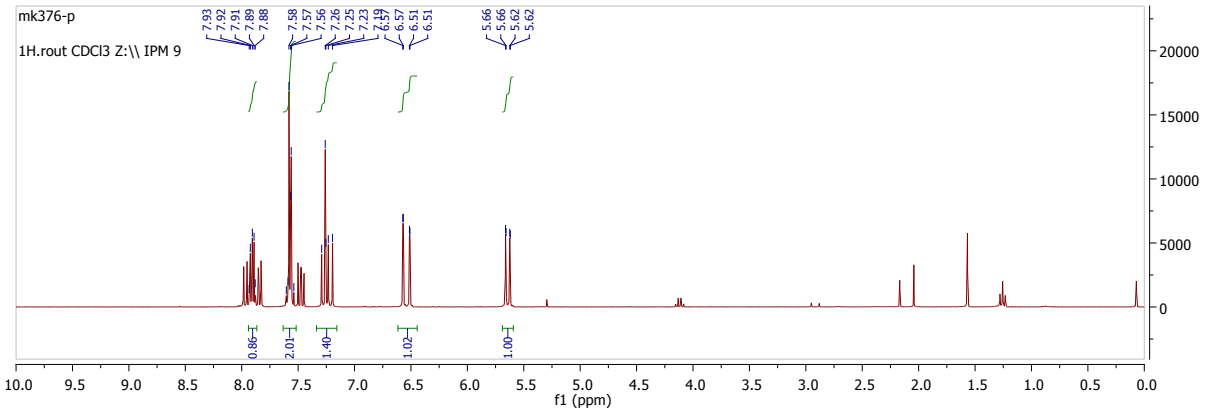


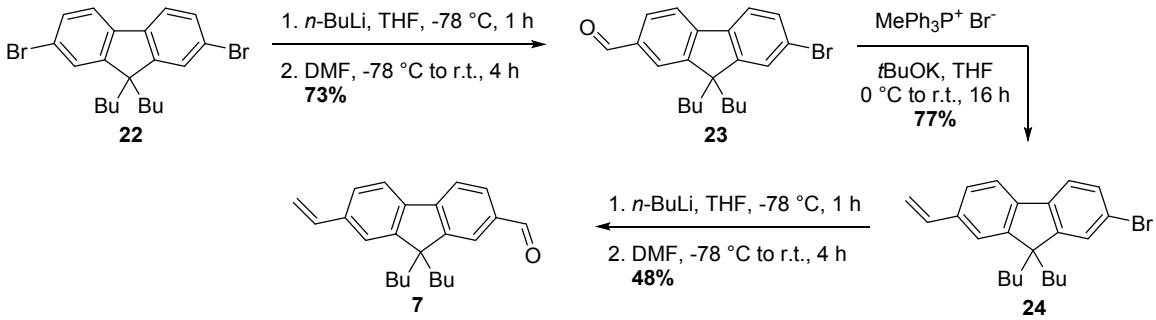
6

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 *i*PrOH (19 mL) and Et_3N (0.26 mL, 1.86 mmol, 1 eq.) was degassed with argon for 20 min, then $PdCl_2$(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.**)

^{1}H NMR ($CDCl_3$, 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).

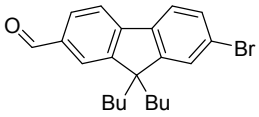
*NB: Compound **6** was not further characterized due to its tendency to polymerize, and was used shortly after synthesis.*





Scheme S4. Synthesis of the intermediate 9,9-dibutyl-7-vinyl-9*H*-fluorene-2-carbaldehyde **7**.

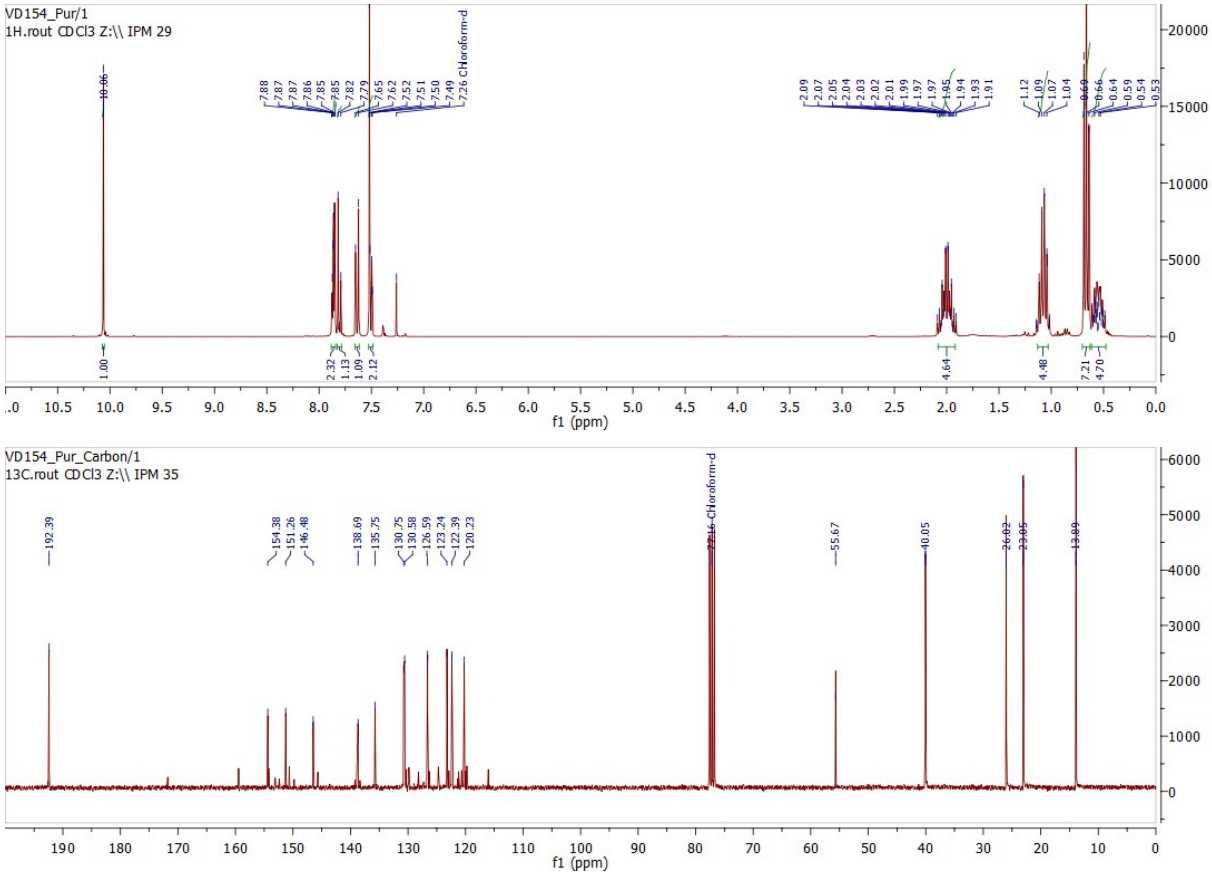
7-bromo-9,9-dibutyl-9*H*-fluorene-2-carbaldehyde (23):



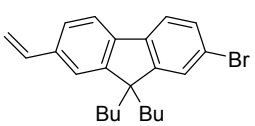
23

To a stirred solution of 2,7-dibromo-9,9-dibutyl-9*H*-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_4Cl solution, then extracted with CH_2Cl_2. The combined organic layers were washed with brine, then dried over Na_2SO_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), δ (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), δ (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-9*H*-fluorene (24):

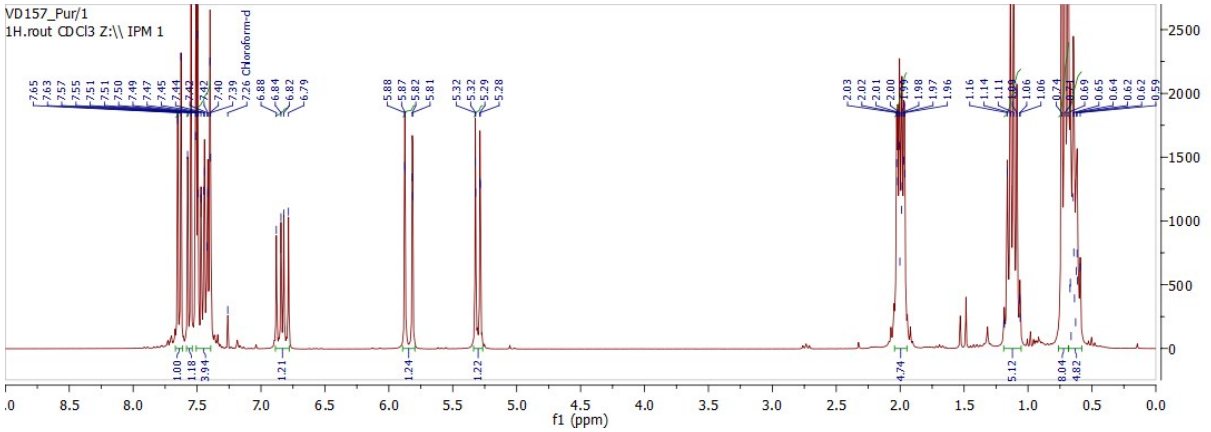


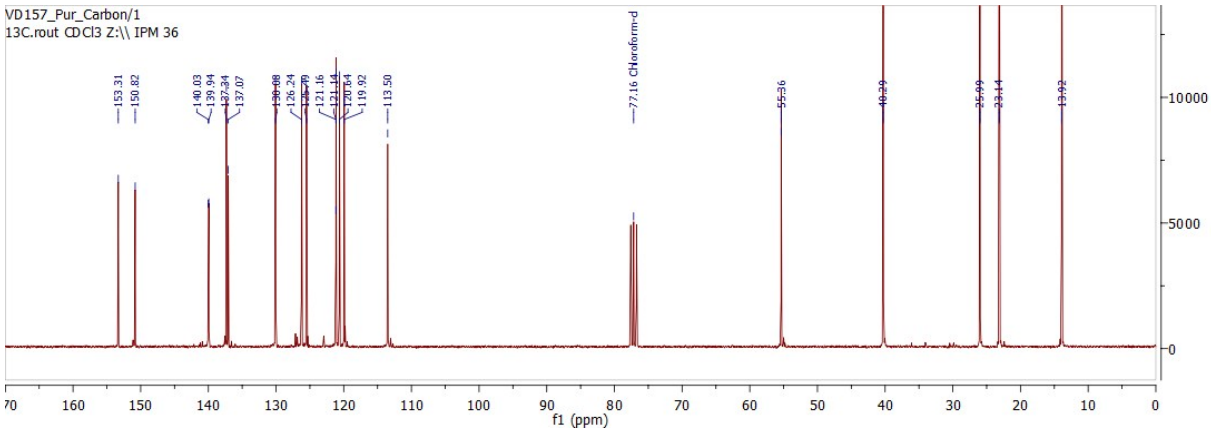
24

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

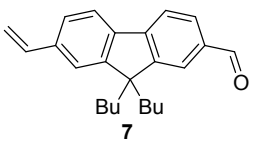
potassium *tert*-butoxide (960 mg, 8.56 mmol, 1.1 eq.) at 0 °C. The resulting yellow mixture was stirred at 0 °C for 1 h, then a solution of **23** (3.0 g, 7.79 mmol, 1 eq.) in dry degassed THF (25 mL) was added dropwise. The resulting mixture was allowed to warm up to r.t. overnight, then it was quenched with a saturated NH_4Cl solution and extracted with Et_2O (3 x 30 mL). The combined organic layers were washed with water, then dried over Na_2SO_4, 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%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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-9*H*-fluorene-2-carbaldehyde (7):

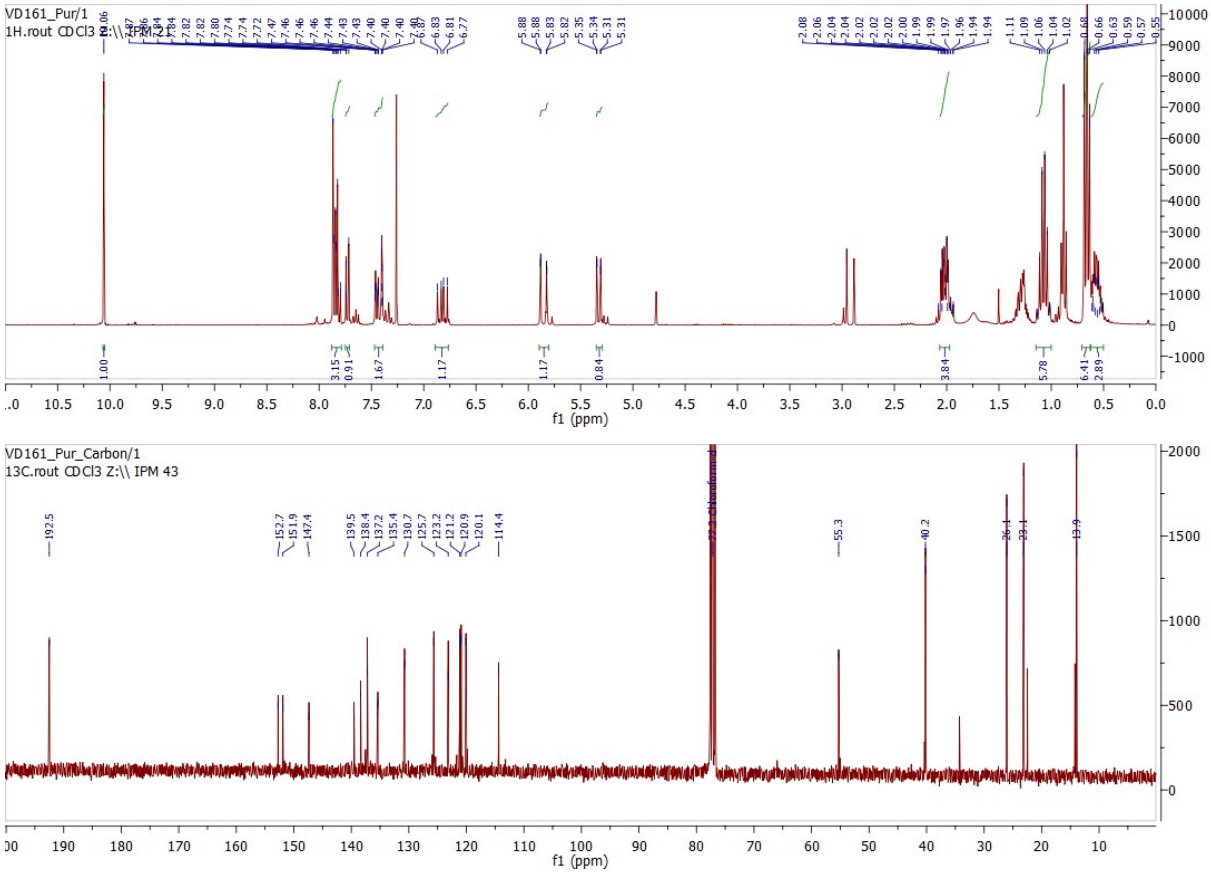


7

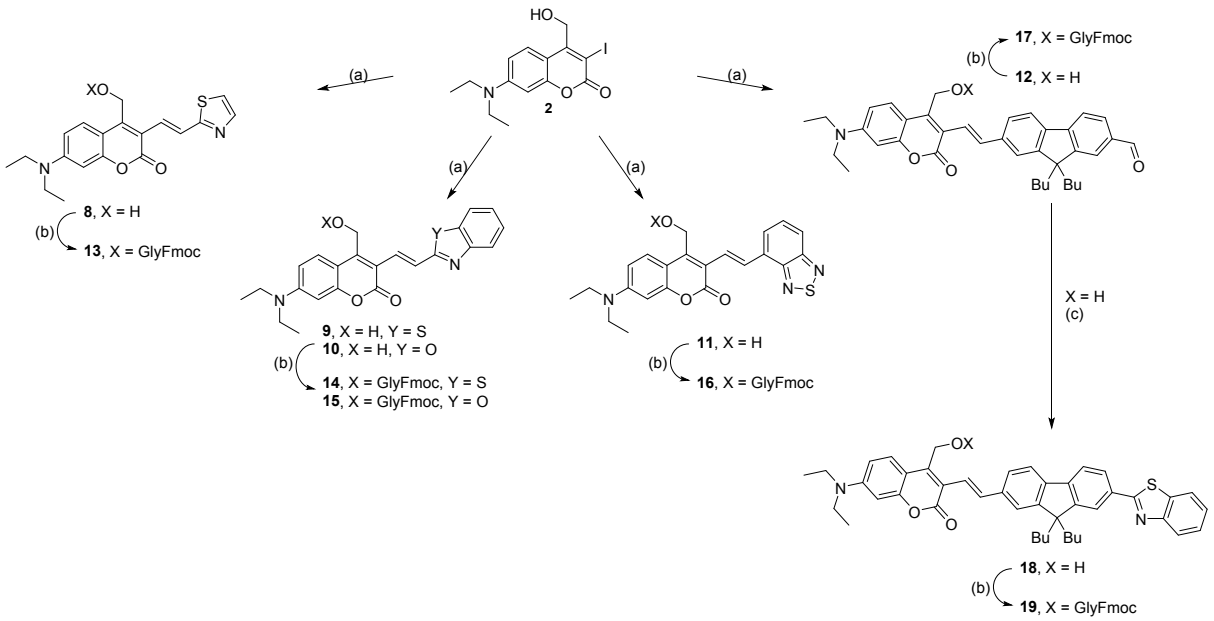
To a stirred solution of **24** (2.0 g, 5.22 mmol, 1 eq.) in dry THF (25 mL) at -78 °C under argon atmosphere was added dropwise *n*-BuLi (2.5 mL, 2.3 M in

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 NH_4Cl solution, then extracted with CH_2Cl_2. The combined organic layers were washed with brine, then dried over Na_2SO_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 **7** as a pale yellow solid (830 mg, **48%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{24}H_{29}O$ $(M+H)^+$: 333.2212.



4. Synthesis of the new PPGs



Scheme S5. Synthesis of the π-extended coumarin cages. Reagents and conditions: (a) **3-7**, $Pd(OAc)_2$, *n*-Bu_4NCl, LiCl, $NaHCO_3$, DMF, 130°C (**8**, 90%; **9**, 96%; **10**, 92%, **11**, 76%; **12**, 89%). (b) Fmoc-Gly-OH, EDC.HCl, DMAP (cat.), CH_2Cl_2, r.t. (**13**, 52%; **14**, 59%; **15**, 69%; **16**, 58%; **17**, 84%, **19**, 64%). (c) 2-Aminothiophenol hydrochloride, DMF, 120 °C. (42%).

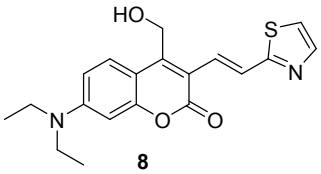
General procedure for the Heck coupling between 2 and the vinyl moieties:

In a Schlenk tube were introduced under argon atmosphere lithium chloride (1.7 eq.), tetra-*n*-butylammonium chloride (1.1 eq.), $NaHCO_3$ (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.

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

Finally, $Pd(OAc)_2$ (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.

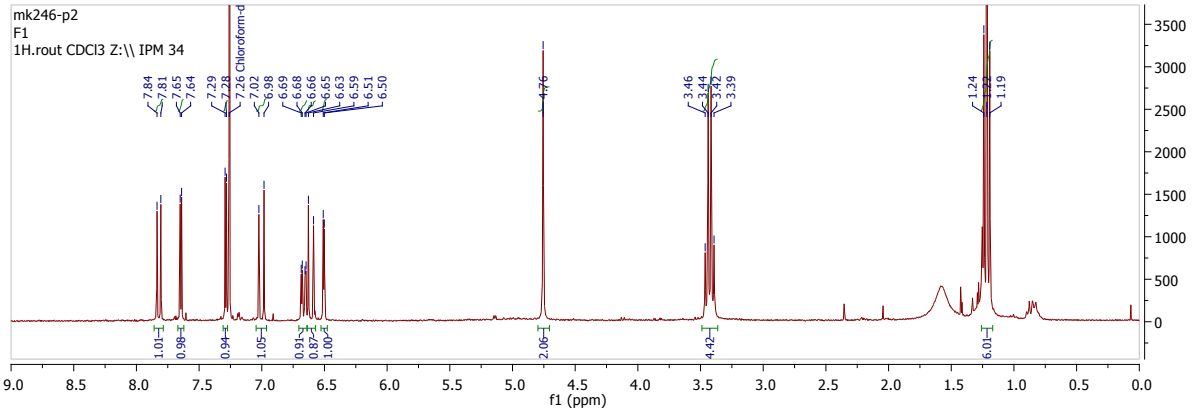
(*E*)-7-(diethylamino)-4-(hydroxymethyl)-3-(2-(thiazol-2-yl)vinyl)coumarin (8):

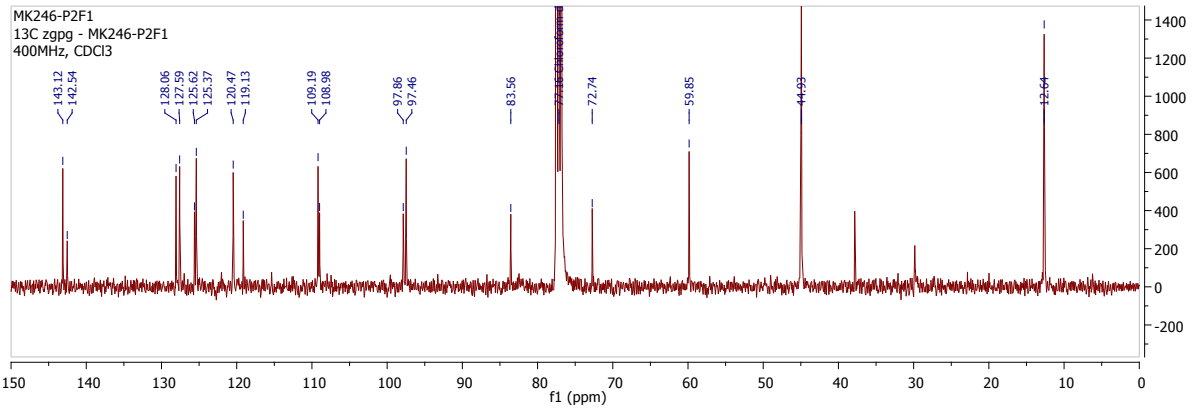


8

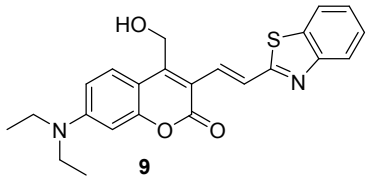
Following the general procedure for Heck coupling, with **2** (200 mg, 0.536 mmol, 1 eq.) and **3** (89.4 mg, 0.804 mmol, 1.5 eq.). Upon cooling down to room temperature, the reaction was quenched with water, and evaporated. The resulting precipitate was collected by filtration, washed with a large amount of water, then with Et_2O e (5 mL). The resulting powder was dried under vacuum to give **8** as an orange solid (171 mg, **90%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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.





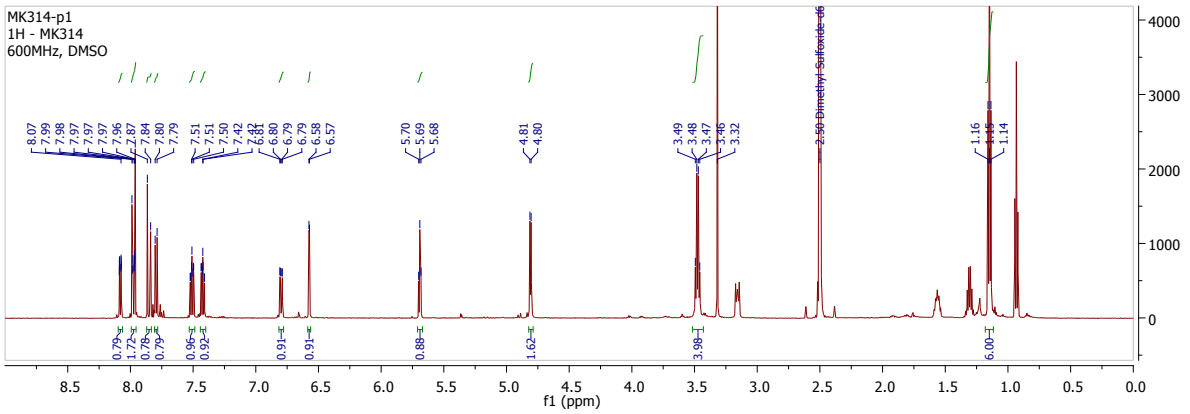
(*E*)-3-(2-(benzo[*d*]thiazol-2-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)coumarin (9):

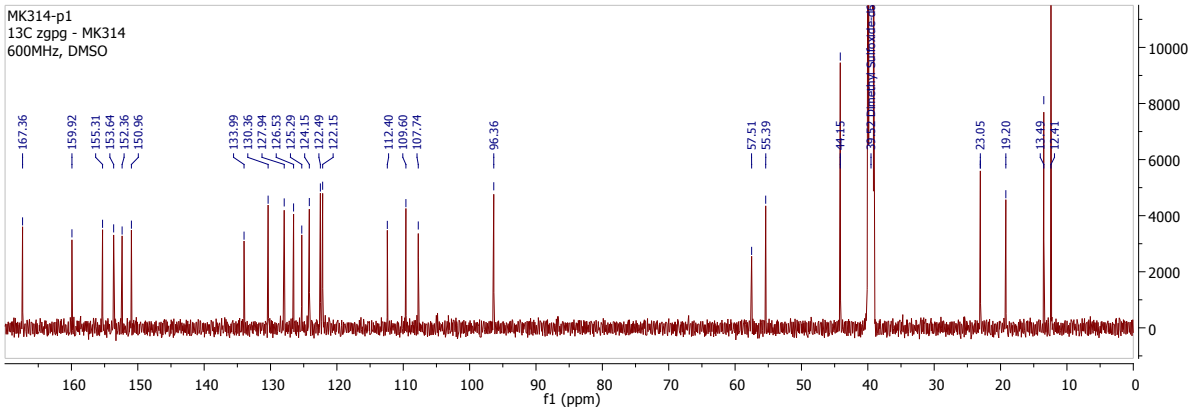


9

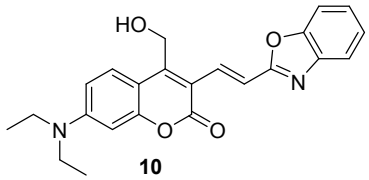
Following the general procedure for Heck coupling, with **2** (120 mg, 0.322 mmol, 1 eq.) and **4** (51.8 mg, 0.322 mmol, 1 eq.) The crude residue was precipitated in H_2O. The precipitate was collected by filtration, washed with a large amount of water, then with Et_2O (5 mL) and dried under vacuum. Dark purple solid (125 mg, **96%**).

^{1}H NMR (DMSO-d_6, 600 MHz), δ (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), δ (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, calcd for $C_{23}H_{22}N_2O_3NaS$ $(M+Na)^+$: 429.1243. **FTIR ν (cm^{-1}):** 3452.2, 2969.6, 1707.0, 1620.4, 1589.4, 1567.0, 1417.4, 1353.3, 1281.1, 1146.0, 752.0.



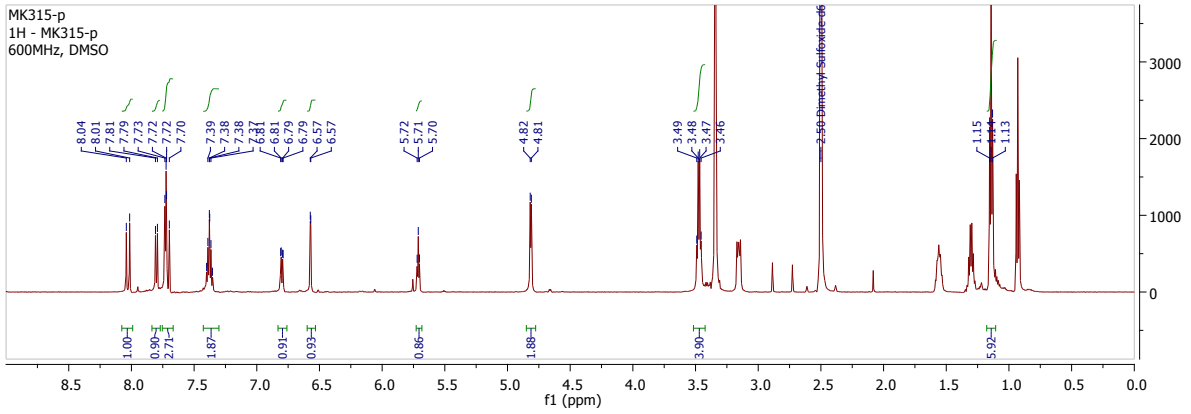


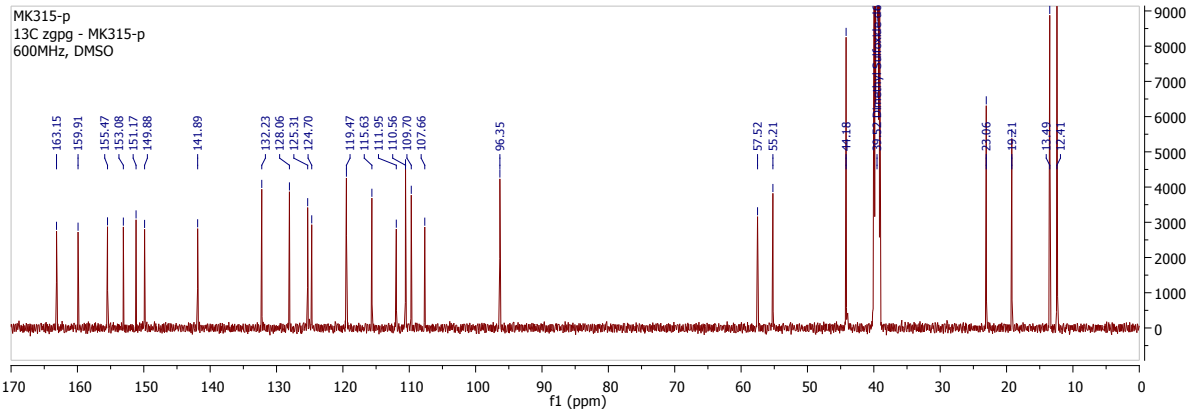
(*E*)-3-(2-(benzo[*d*]oxazol-2-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)coumarin (10):



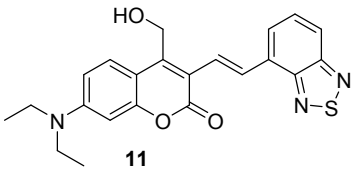
Following the general procedure for Heck coupling, with **2** (120 mg, 0.322 mmol, 1 eq.) and **5** (46.7 mg, 0.322 mmol, 1 eq.) The crude residue was precipitated in H_2O. The precipitate was collected by filtration, washed with a large amount of water, then with Et_2O (5 mL) and dried under vacuum. Orange solid (116 mg, **92%**).

^{1}H NMR (DMSO-d_6, 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, 4H), 1.14 (t, J = 7.0 Hz, 6H). **^{13}C NMR (DMSO-d_6, 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 $C_{23}H_{22}N_2O_4Na$ $(M+Na)^+$: 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.





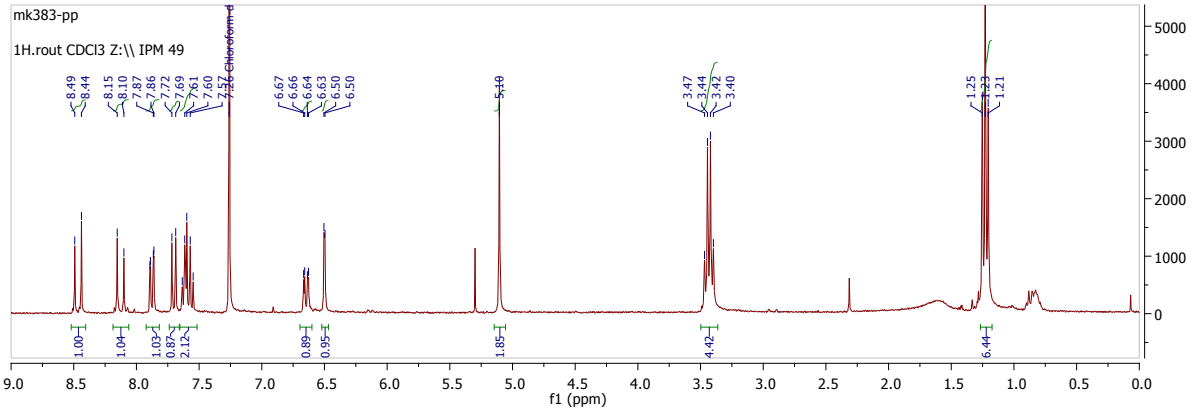
(*E*)-3-(2-(benzo[*c*][1,2,5]thiadiazol-4-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)coumarin (11):

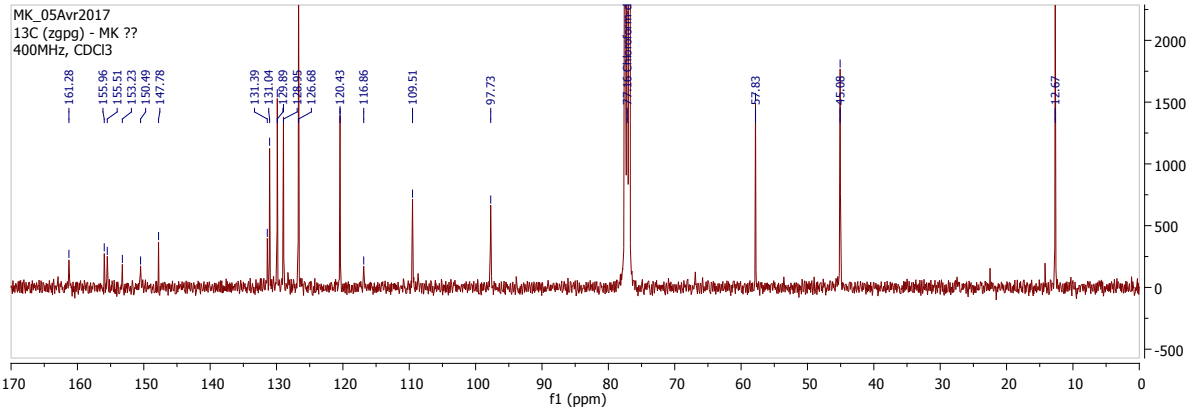


11

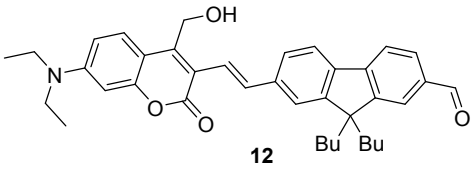
Following the general procedure for Heck coupling, with **2** (300 mg, 0.804 mmol, 1 eq.) and **6** (260.8 mg, 1.61 mmol, 2 eq.) The crude residue was purified by column chromatography of silicagel (eluent: CH_2Cl_2). Red solid (248 mg, **76%**).

^{1}H NMR ($CDCl_3$, 300 MHz), δ (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), δ (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, calcd for $C_{22}H_{21}N_3O_3S$ $(M)^{+\cdot}$: 407.13036. **FTIR ν (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.





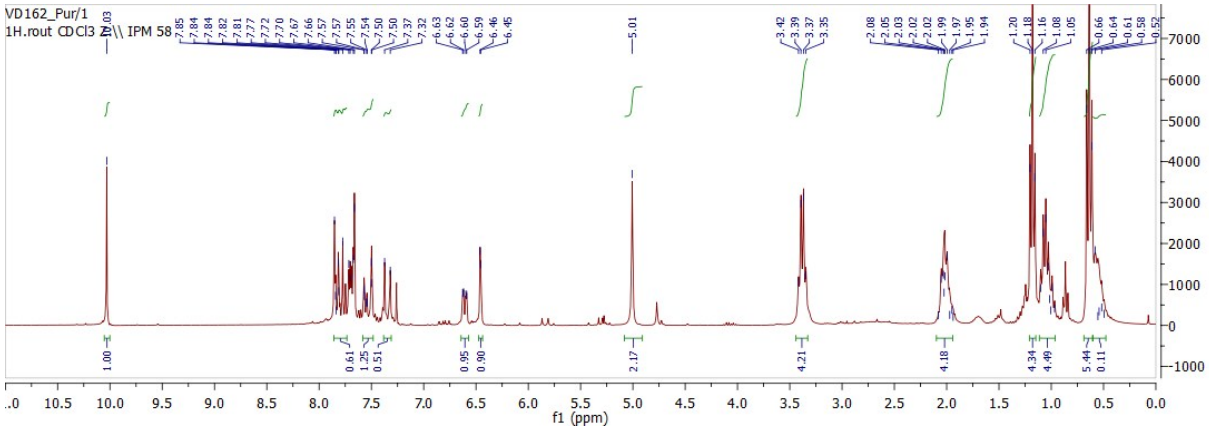
(*E*)-9,9-dibutyl-7-(2-(7-(diethylamino)-4-(hydroxymethyl)-coumarin-3-yl)vinyl)-9*H*-fluorene-2-carbaldehyde (12):

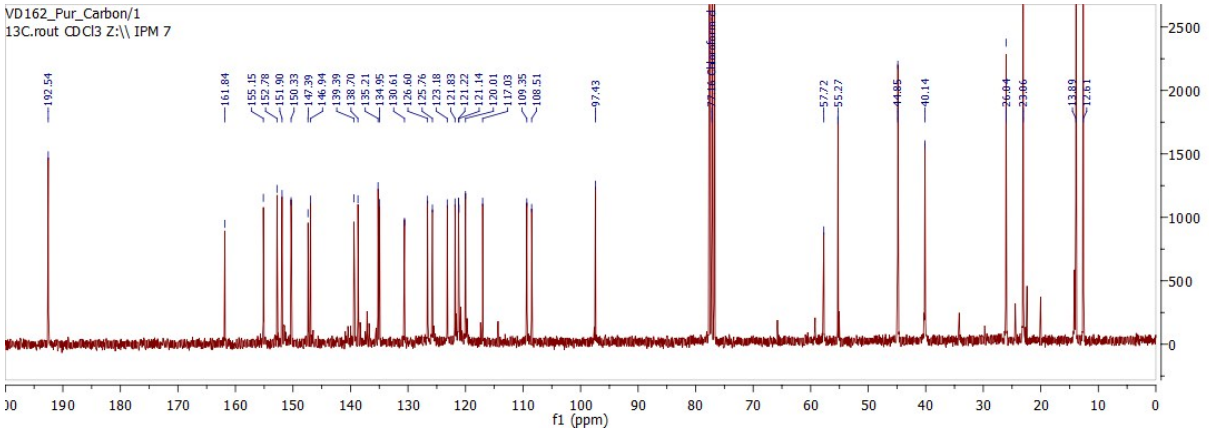


12

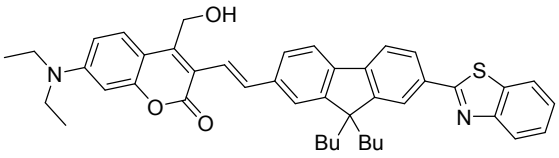
Following the general procedure for Heck coupling, with **2** (745 mg, 2.0 mmol, 1 eq.) and **7** (830 mg, 2.5 mmol, 1.25 eq.) The crude residue was precipitated in H_2O. The precipitate was collected by filtration, washed with a large amount of water, then with Et_2O (5 mL) and dried under vacuum. Brown solid (1.0 g, **89%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{38}H_{43}NO_4Na$ $(M+Na)^+$: 600.3084.





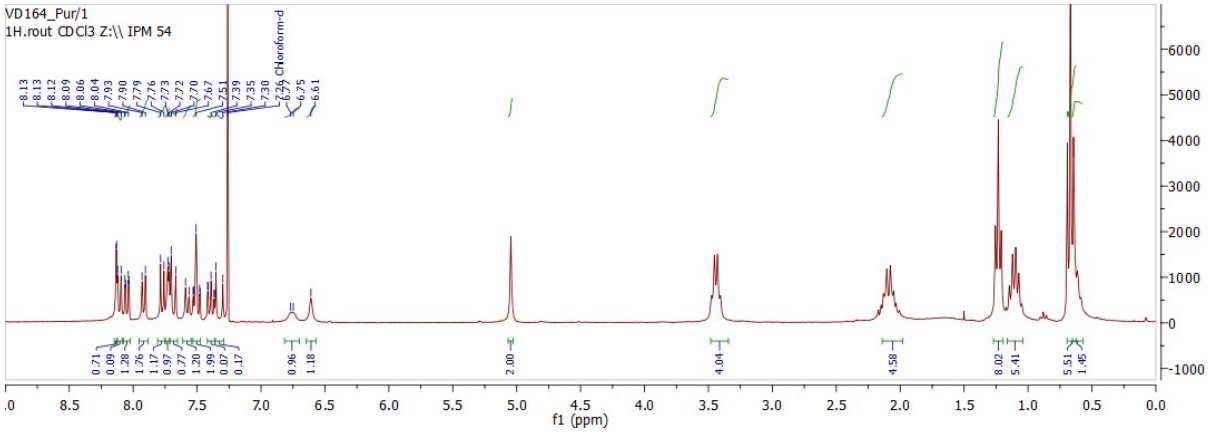
(*E*)-3-(2-(7-(benzo[*d*]thiazol-2-yl)-9,9-dibutyl-9*H*-fluoren-2-yl)vinyl)-7-(diethylamino)-4-(hydroxymethyl)-coumarin (18):

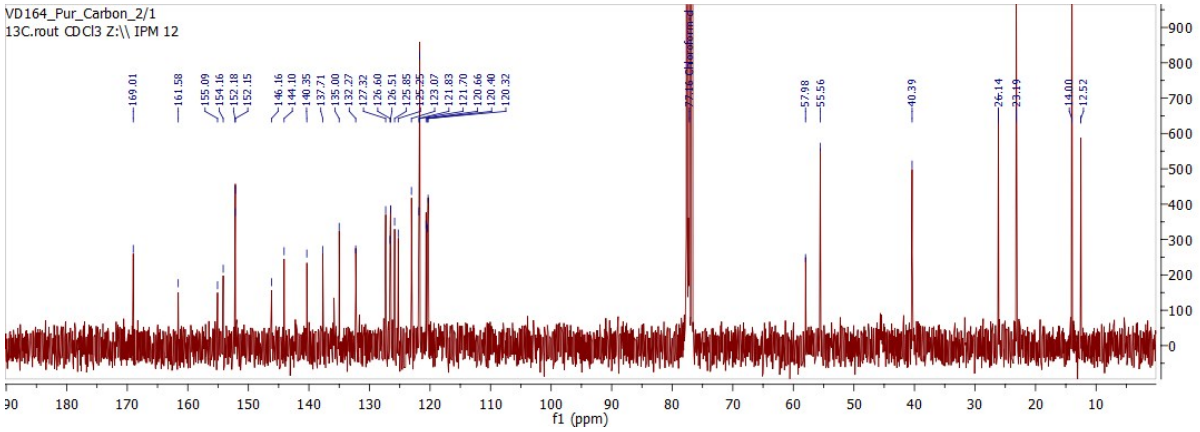


18

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 Na_2SO_4, 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%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{44}H_{46}N_2O_3NaS$ $(M+Na)^+$: 705.3121.

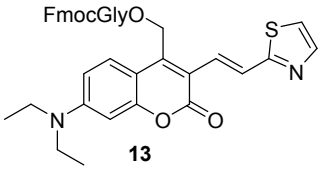




General procedure for the preparation of caged Fmoc-protected glycin (esterification):

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 CH_2Cl_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.

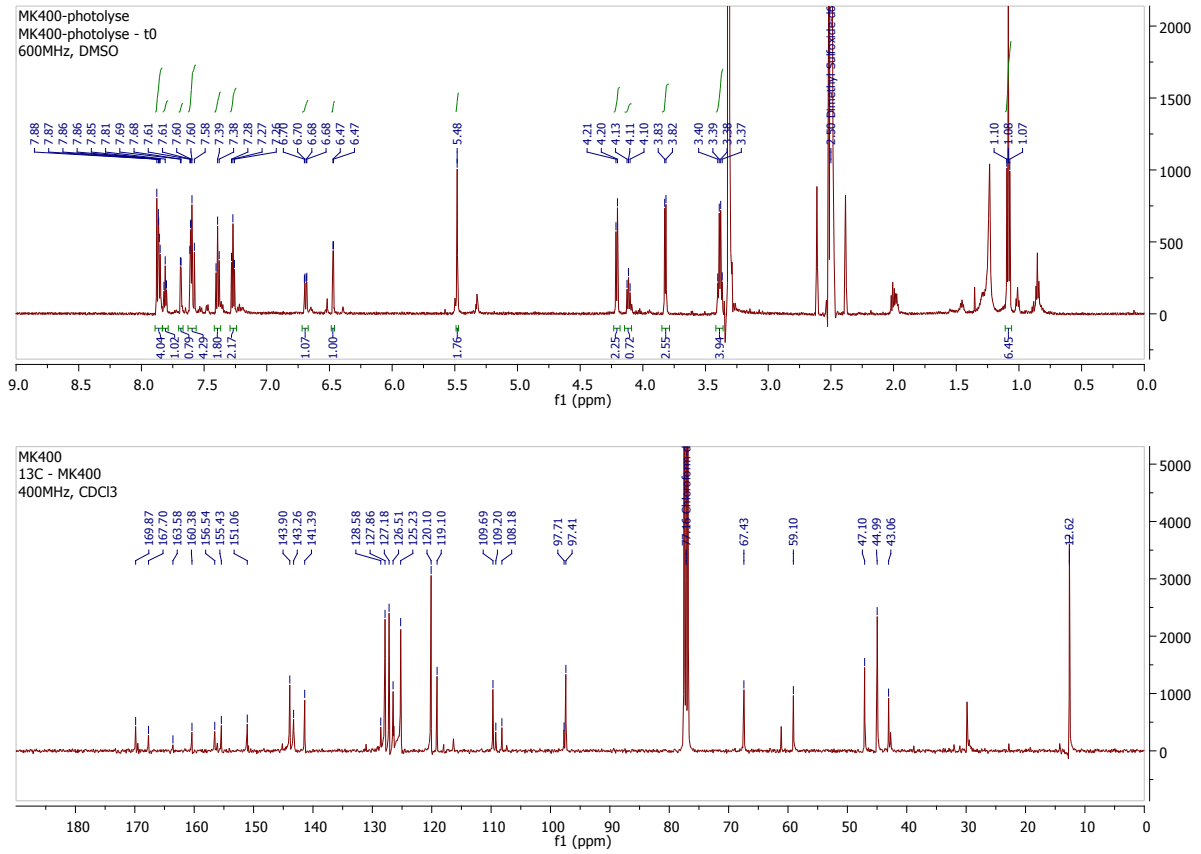
(*E*)-(7-(diethylamino)-2-oxo-3-(2-(thiazol-2-yl)vinyl)-coumarin-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (13):



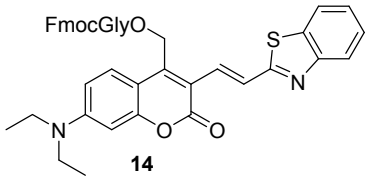
13

Following the general procedure for esterification, with **8** (35 mg, 98.2 µmol, 1 eq.) and Fmoc-Gly-OH (32.1 mg, 108 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH_2Cl_2:acetone, 1:0 to 7:3) to give **13** as an orange powder (33 mg, **52%**).

^{1}H NMR (DMSO-d_6, 600 MHz), δ (ppm): 7.89 – 7.83 (m, 4H), 7.81 (t, *J* = 6.0 Hz, 1H), 7.69 (d, *J* = 3.2 Hz, 1H), 7.63 – 7.56 (m, 4H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 2H), 6.69 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.47 (d, *J* = 2.5 Hz, 1H), 5.48 (s, 2H), 4.21 (d, *J* = 7.3 Hz, 2H), 4.11 (t, *J* = 7.3 Hz, 1H), 3.82 (d, *J* = 6.0 Hz, 2H), 3.38 (q, *J* = 7.1 Hz, 4H), 1.08 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR ($CDCl_3$, 101 MHz), δ (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. **FTIR ν (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. **Melting point:** 170 °C.



(*E*)-(3-(2-(benzo[*d*]thiazol-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (14):

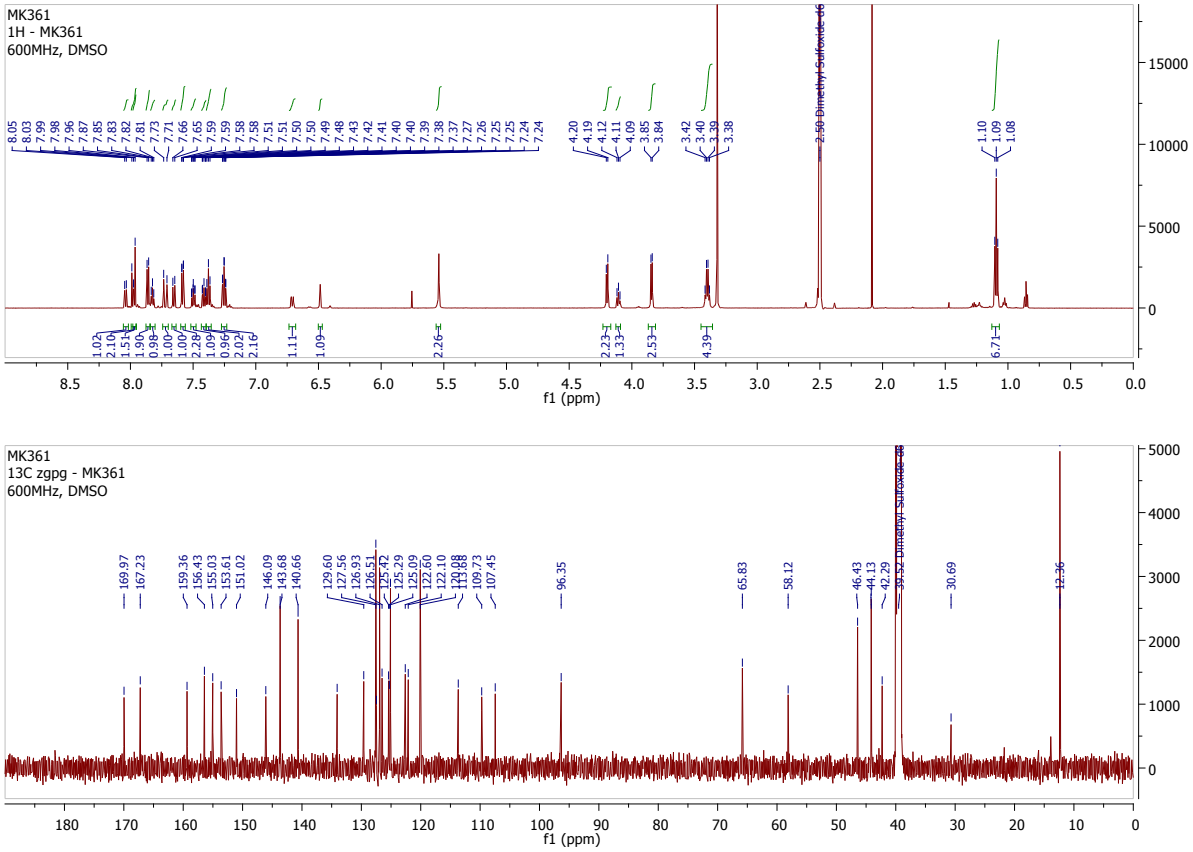


14

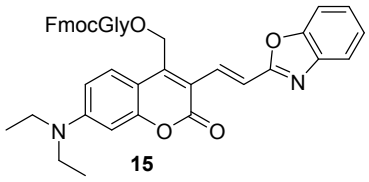
Following the general procedure for esterification, with **9** (60 mg, 147.6 µmol, 1 eq.) and Fmoc-Gly-OH (48.3 mg, 162 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH_2Cl_2:acetone, 100:0 to 94:6). to give **14** as an orange powder (60 mg, **59%**).

^{1}H NMR (DMSO-d_6, 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). **^{13}C NMR (DMSO-d_6, 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 $C_{40}H_{35}N_3O_6NaS$ $(M+Na)^+$: 708.2138. **FTIR ν (cm^{-1}):** 3420.7, 3060.4,

2967.0, 2923.1, 2868.1, 1721.7, 1712.2, 1620.1, 1599.5, 1513.4, 1419.8, 1354.5, 1267.7, 1147.5, 1050.8, 758.5. **Melting point:** 142 °C.



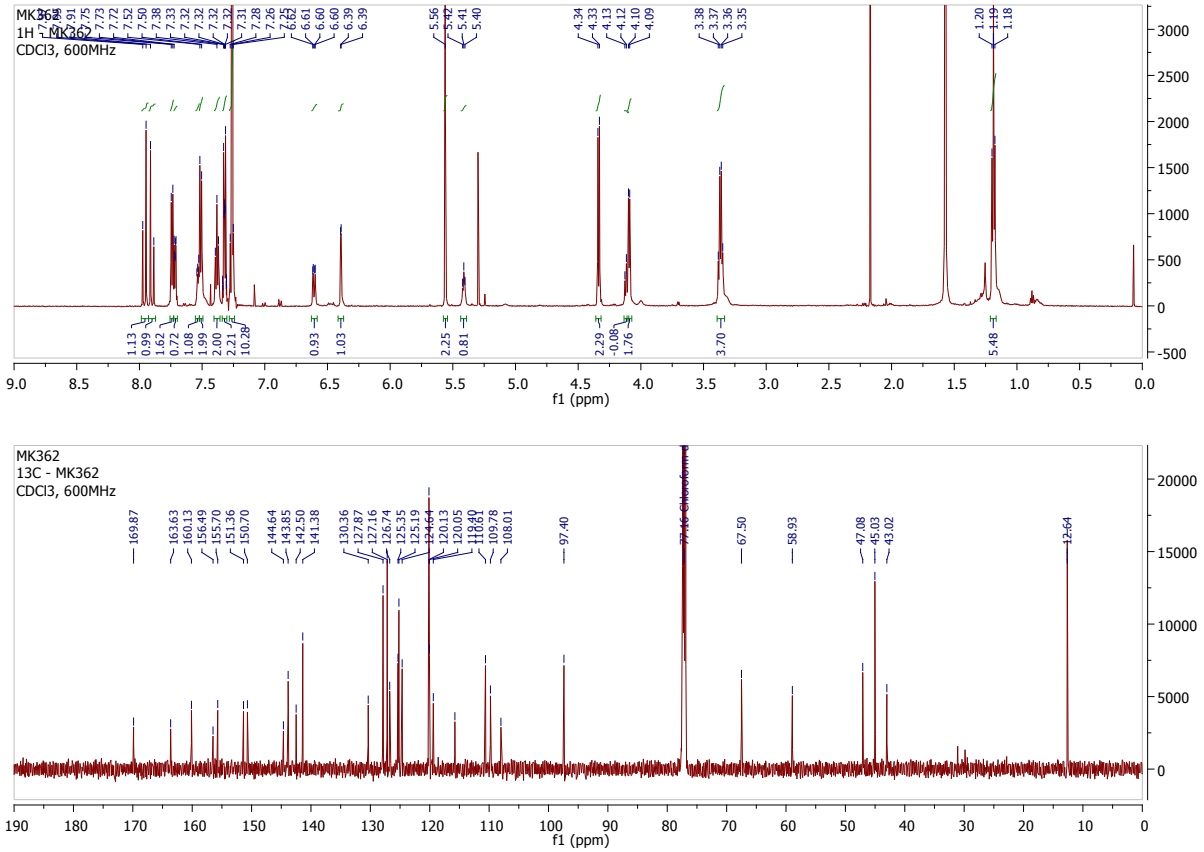
(*E*)-(3-(2-(benzo[*d*]oxazol-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (15):



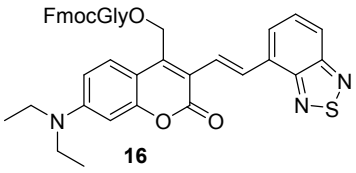
Following the general procedure for esterification, with **10** (60 mg, 153.6 µmol, 1 eq.) and Fmoc-Gly-OH (50.3 mg, 169 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH_2Cl_2:acetone, 1:0 to 9:1). to give **15** as an orange powder (71 mg, **69%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{40}H_{35}N_3O_7Na$ $(M+Na)^+$: 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 (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (16):

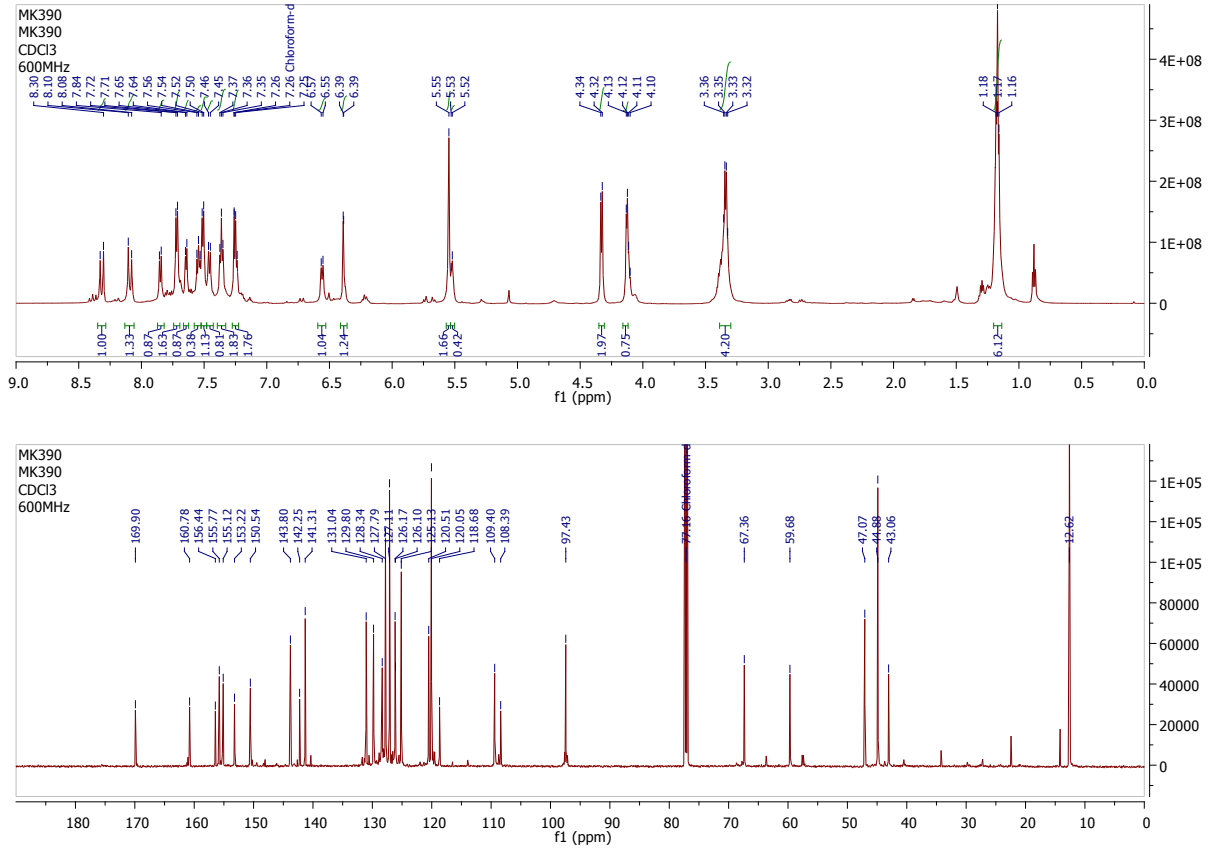


16

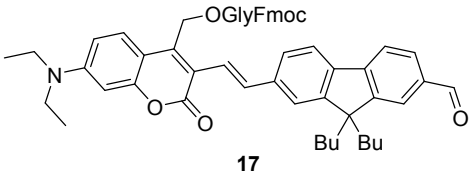
Following the general procedure for esterification, with **11** (76 mg, 186.5 µmol, 1 eq.) and Fmoc-Gly-OH (61 mg, 205 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: CH_2Cl_2:acetone, 100:0 to 98:2) to give **16** as a red powder (74 mg, **58%**).

^{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), δ (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 $C_{39}H_{34}N_4O_6S$ $(M)^{+\cdot}$: 686.21990. **FTIR ν (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.



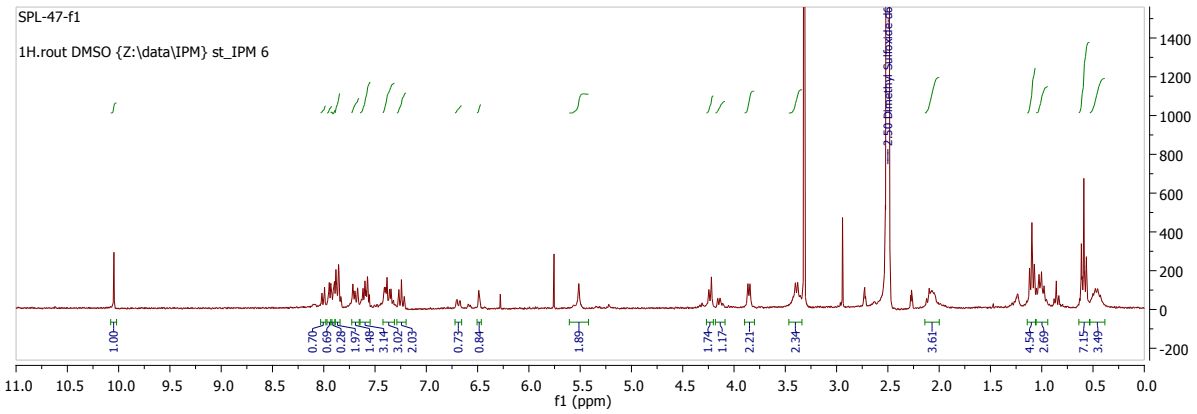
(*E*)-(3-(2-(9,9-dibutyl-7-formyl-9*H*-fluoren-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (17):

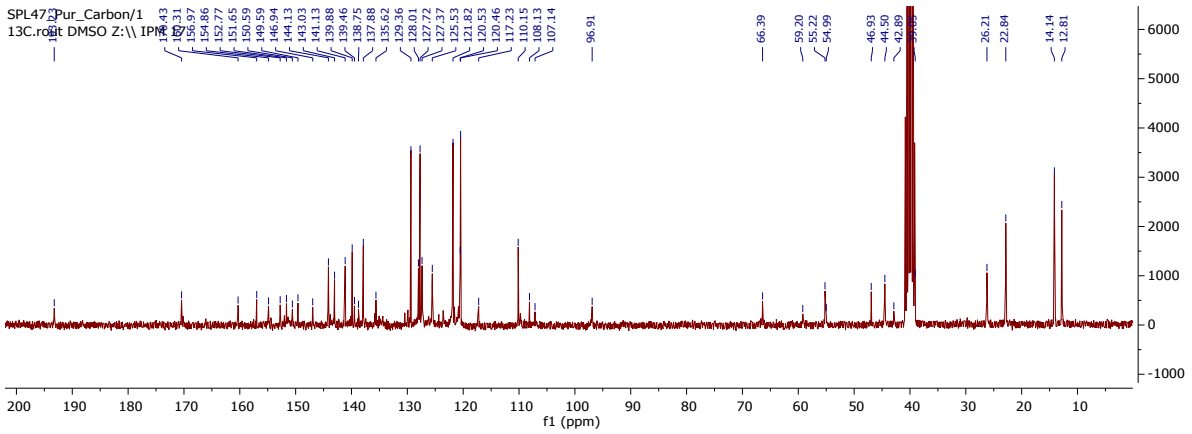


17

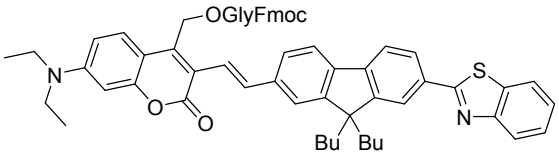
Following the general procedure for esterification, with **12** (200 mg, 348 µmol, 1 eq.) and Fmoc-Gly-OH (113.5 mg, 381 µmol, 1.1 eq.), the product was purified by a column chromatography of neutral alumina (gradient eluent: CH_2Cl_2:AcOEt, 100:0 to 95:5) to give **17** as an orange powder (250 mg, **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, 150.6, 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 $C_{55}H_{56}N_2O_7Na$ $(M+Na)^+$: 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.





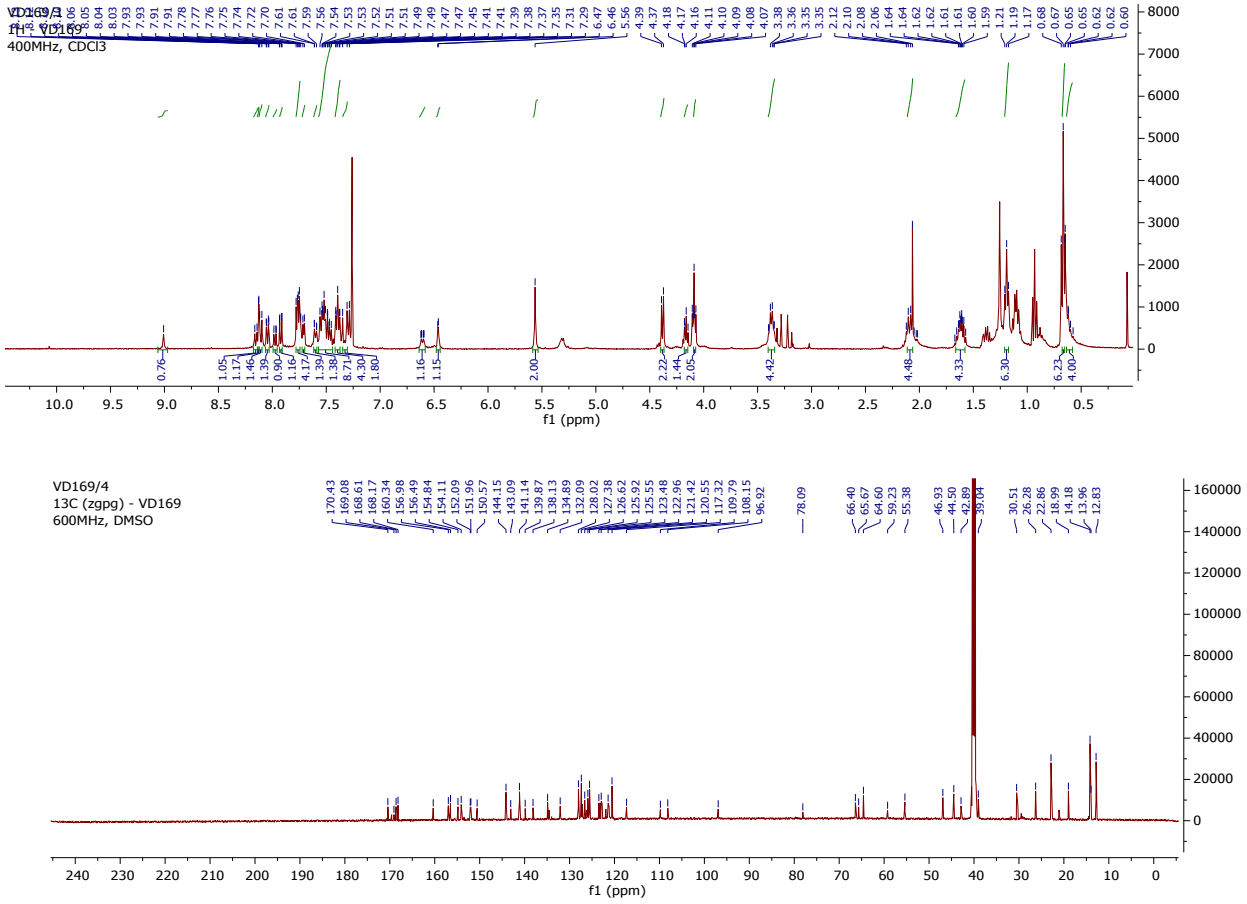
(*E*)-(3-(2-(9,9-dibutyl-7-formyl-9*H*-fluoren-2-yl)vinyl)-7-(diethylamino)-coumarin-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (19):



19

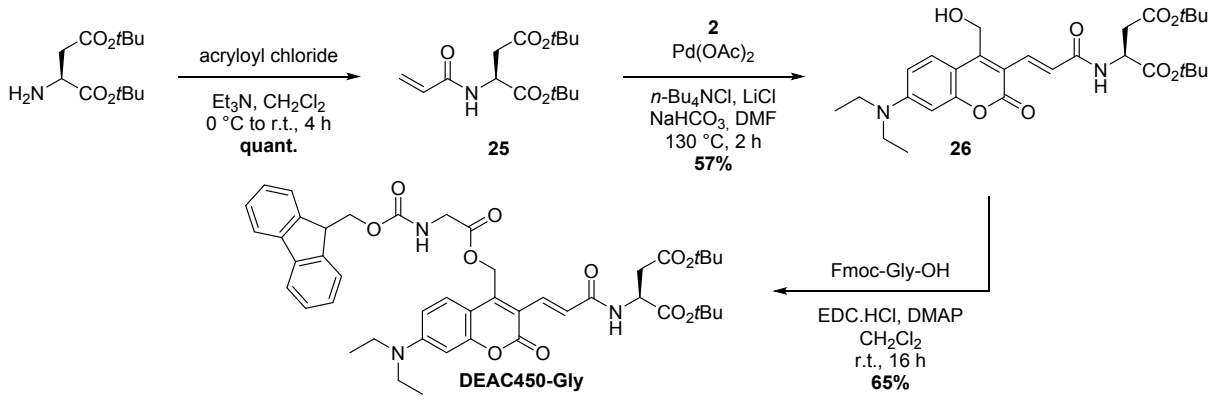
Following the general procedure for esterification, with **18** (32 mg, 46.9 µmol, 1 eq.) and Fmoc-Gly-OH (15.5 mg, 51.6 µmol, 1.1 eq.), the product was purified by a column chromatography of silicagel (gradient eluent: toluene:AcOEt, 9:1 to 8:2) to give **19** as an orange powder (28 mg, **64%**).

^{1}H NMR ($CDCl_3$, 400 MHz), δ (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). **^{13}C NMR (DMSO-d_6, 151 MHz), δ (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. **HRMS (FD):** m/z = 961.41500, calcd for $C_{61}H_{59}N_3O_6S$ $(M)^{+\cdot}$: 961.41246.



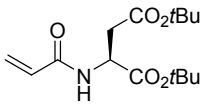
5. Synthesis of the reference compounds DEAC-Gly and DEAC450-Gly

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.[4] 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.

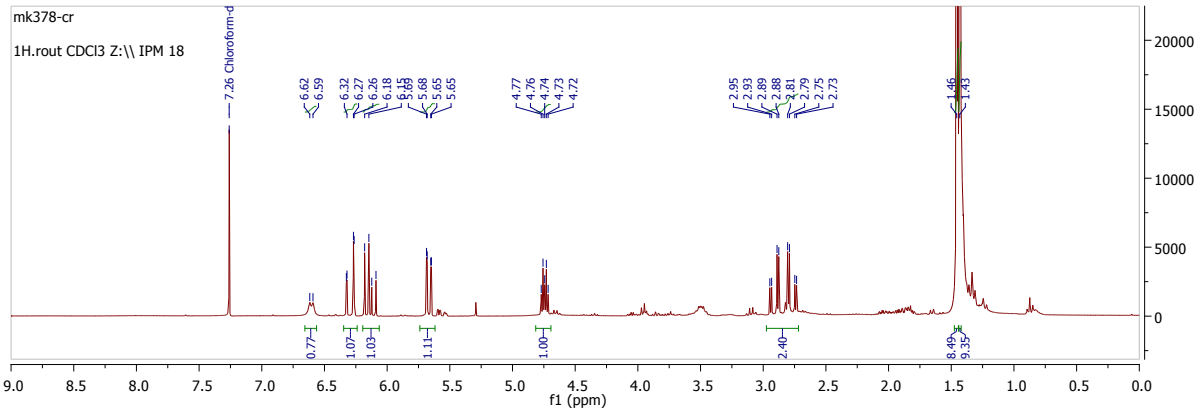
di-*tert*-butyl acryloyl-*L*-aspartate (25):

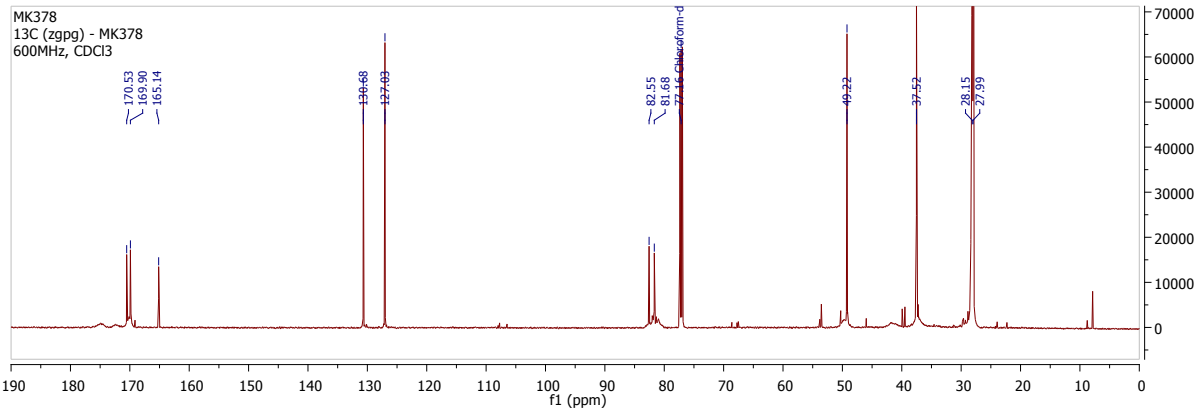


25

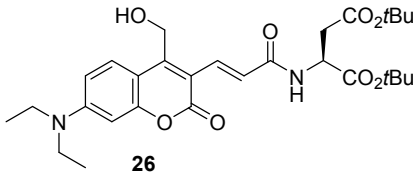
A stirred solution of di-*tert*-butyl *L*-aspartate (1.0 g, 3.55 mmol, 1 eq.) in dry CH_2Cl_2 (36 mL) was placed under argon at 0 °C. Triethylamine (1.05 mL, 7.45 mmol, 2.1 eq.) was added and the mixture was stirred for 10 min, then acryloyl chloride (0.29 mL, 3.55 mmol, 1 eq.) was added dropwise over 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, **quant.**).

^{1}H NMR ($CDCl_3$, 300 MHz), δ (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), δ (ppm):** 170.5, 169.9, 165.1, 130.7, 127.0, 82.6, 81.7, 49.2, 37.5, 28.2, 28.0. **FTIR ν (cm^{-1}):** 3426.9, 2982.8, 1732.3, 1665.9, 1525.2, 1369.9, 1155.2, 845.8.





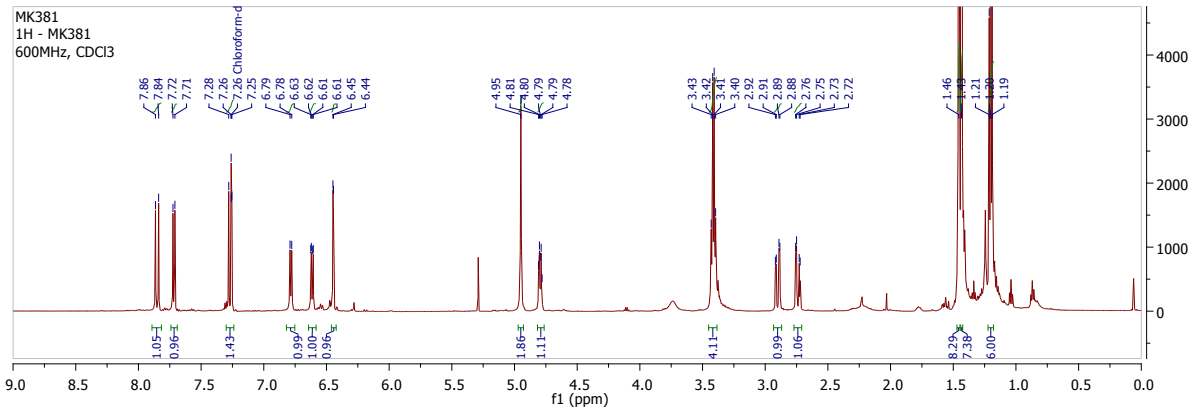
di-*tert*-butyl (*E*)-(3-(7-(diethylamino)-4-(hydroxymethyl)-coumarin-3-yl)acryloyl)-*L*-aspartate (26):

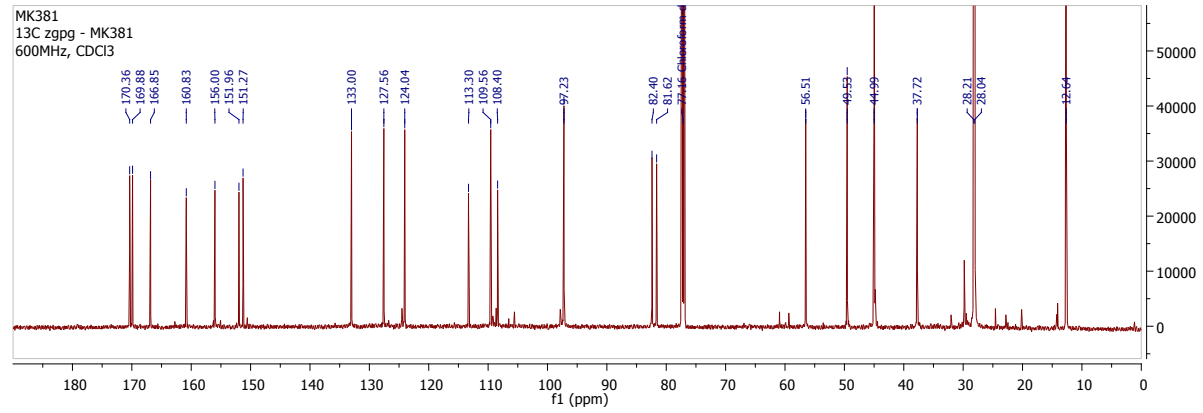


26

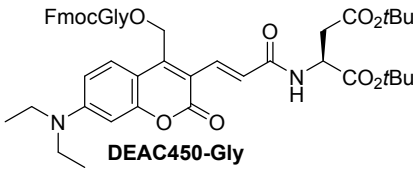
Following the general procedure for Heck coupling, with **2** (500 mg, 1.34 mmol, 1 eq.) and **25** (441 mg, 1.47 mmol, 1.1 eq.). The reaction was quenched with H_2O, and solvents were removed under vacuum. The residue was extracted with AcOEt, dried and evaporated. The crude was then purified by column chromatography of silicagel (gradient eluent: CH_2Cl_2:acetone, 98:2 to 70:30). Brown solid (257 mg, **57%**).

^{1}H NMR ($CDCl_3$, 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). **^{13}C NMR ($CDCl_3$, 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, 45.0, 37.7, 28.2, 28.0, 12.6. **HRMS (FD):** m/z = 544.27724, calcd for $C_{29}H_{40}N_2O_8$ $(M)^{+\cdot}$: 544.27846. **FTIR ν (cm^{-1}):** 3428.1, 2976.2, 2922.6, 1726.6, 1607.5, 1510.7, 1354.3, 1148.7, 845.1.



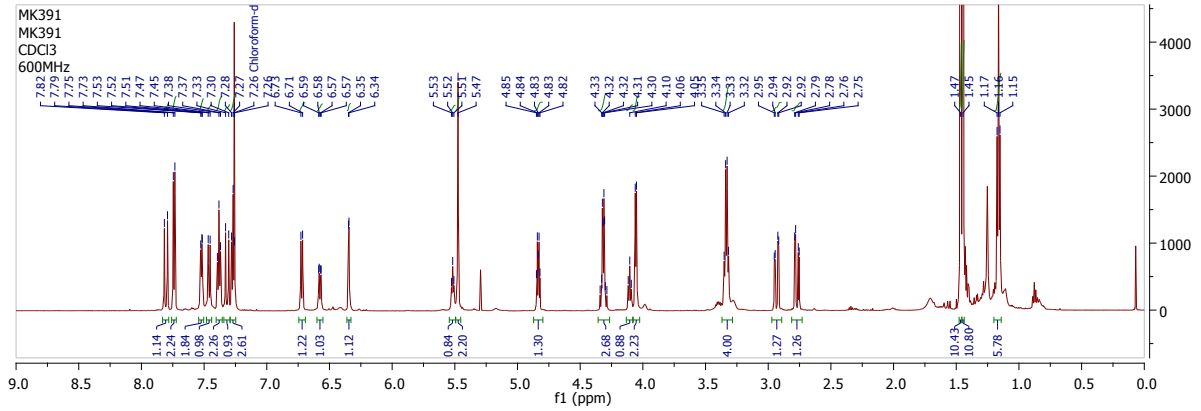


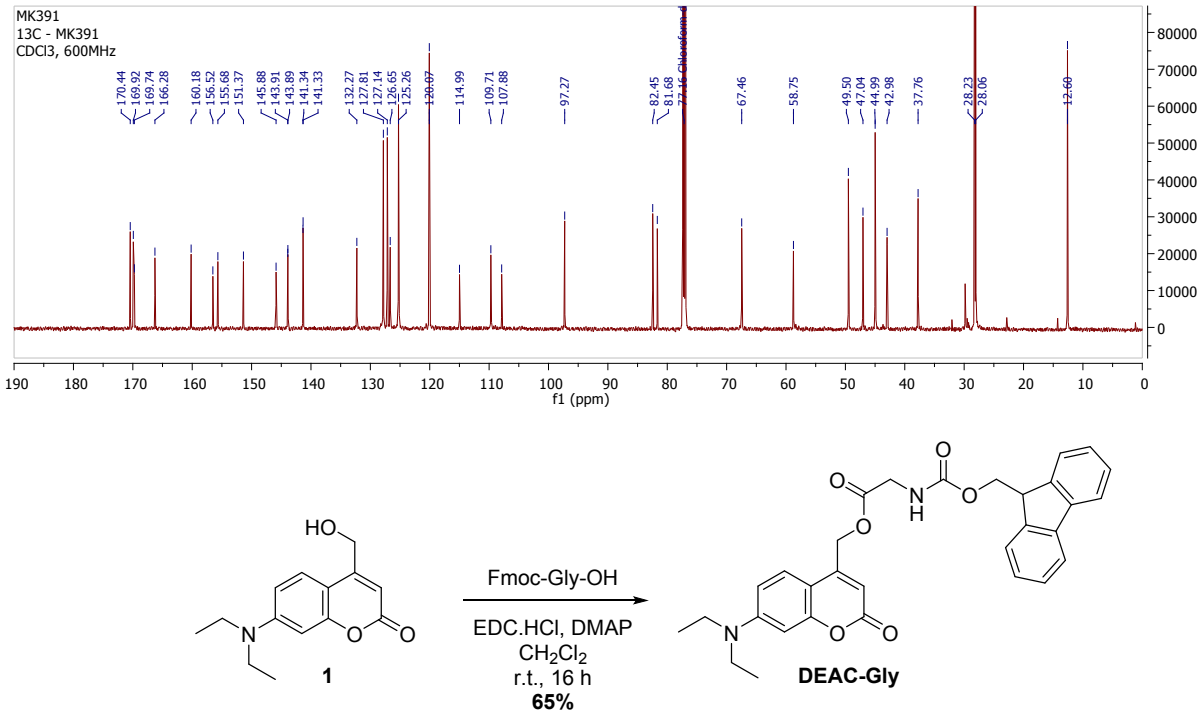
di-*tert*-butyl (*E*)-(3-(4-((((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)oxy)methyl)-7-(diethylamino)-coumarin-3-yl))acryloyl)-*L*-aspartate (DEAC450-Gly):



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: CH_2Cl_2:acetone, 1:0 to 8:2) to give **DEAC450-Gly** as a dark yellow powder (25 mg, **65%**).

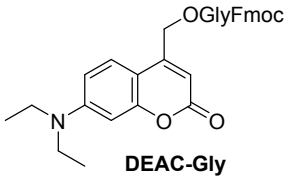
^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): 7.80 (d, *J* = 15.1 Hz, 1H), 7.74 (d, *J* = 7.3 Hz, 2H), 7.52 (dd, *J* = 7.3, 1.5 Hz, 2H), 7.46 (d, *J* = 9.2 Hz, 1H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.32 (d, *J* = 15.1 Hz, 1H), 7.27 (t, *J* = 7.3 Hz, 2H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.58 (dd, *J* = 9.2, 2.4 Hz, 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). **^{13}C NMR ($CDCl_3$, 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 $C_{46}H_{53}N_3O_{11}Na$ $(M+Na)^+$: 846.3572. **Melting point:** 132 °C.





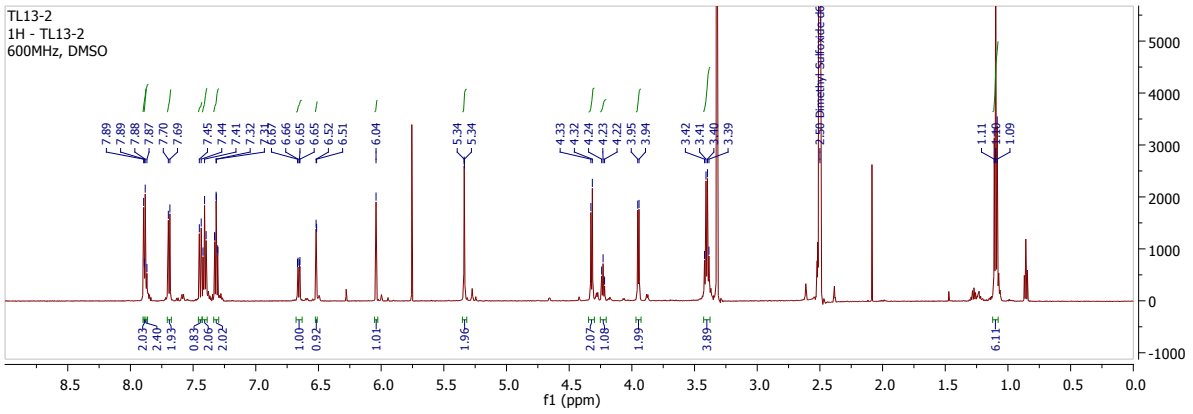
Scheme S7. Synthesis of the reference cage DEAC.

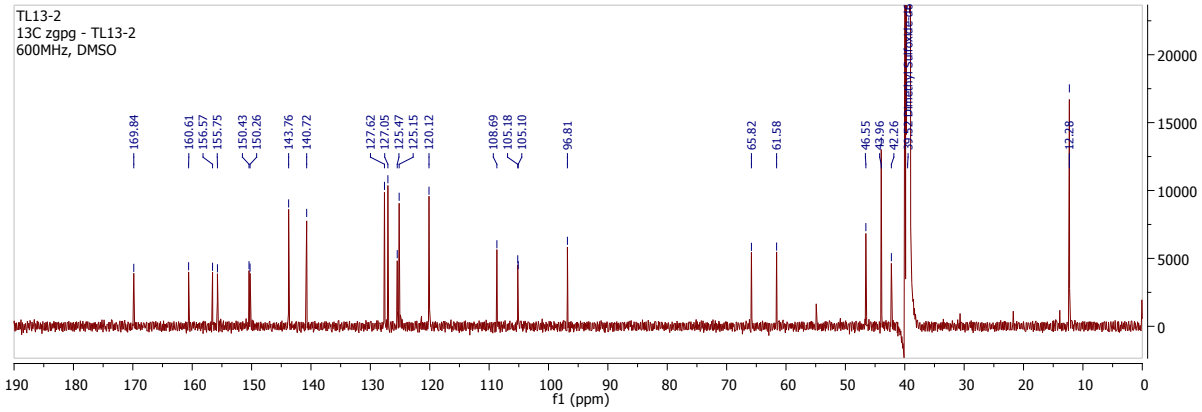
(7-(diethylamino)-coumarin-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)glycinate (DEAC-Gly):



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_2Cl_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), δ (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), δ (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 $C_{31}H_{30}N_2O_6Na$ $(M+Na)^+$: 549.1996. **FTIR ν (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 ~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 ≤ 0.1) contained in standard 1 cm quartz cuvettes using a Fluorolog spectro fluorometer. 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 $I_f(\lambda_{exc},\lambda_f)$:

$$\Phi_f^{ech} = \Phi_f^{ref} \times \left(\frac{n^{ech}}{n^{ref}}\right)^2 \times \frac{1 - 10^{-A^{ref}(\lambda_{exc})}}{1 - 10^{-A^{ech}(\lambda_{exc})}} \times \frac{\int_0^\infty I_f^{ech}(\lambda_{exc}, \lambda_f)\, d\lambda_f}{\int_0^\infty I_f^{ref}(\lambda_{exc}, \lambda_f)\, d\lambda_f}$$

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

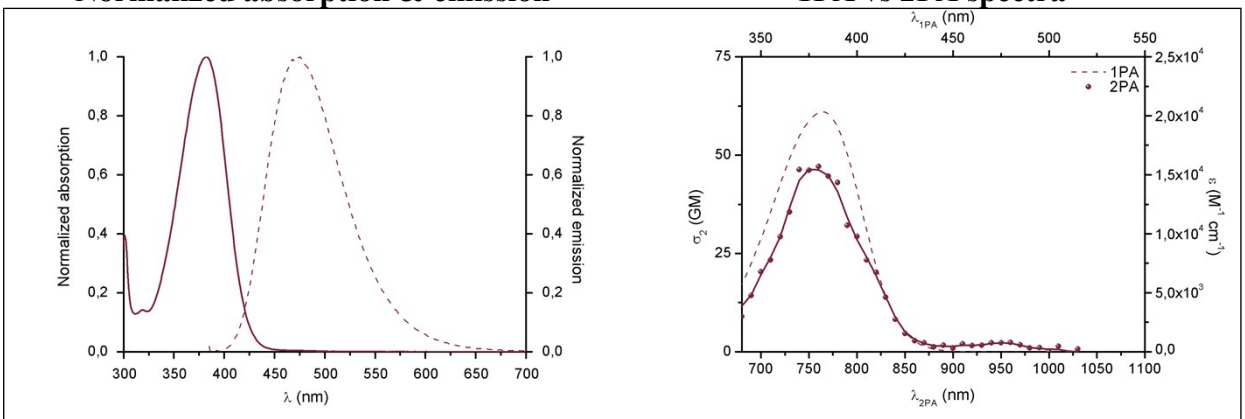
2PA cross sections (σ_2) were derived from the two-photon excited fluorescence (TPEF) cross sections ($\sigma_2\Phi_f$) and the fluorescence emission quantum yield (Φ_f). TPEF cross sections were measured relative to fluorescein in 0.01M aqueous NaOH in the 680-1000 nm spectral range,[8] and relative to Nile Red in DMSO in the 1000-1160 nm spectral range,[9,10] using the well-established method described by Xu and Webb[11] and the appropriate solvent-related refractive index corrections.[12] 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.

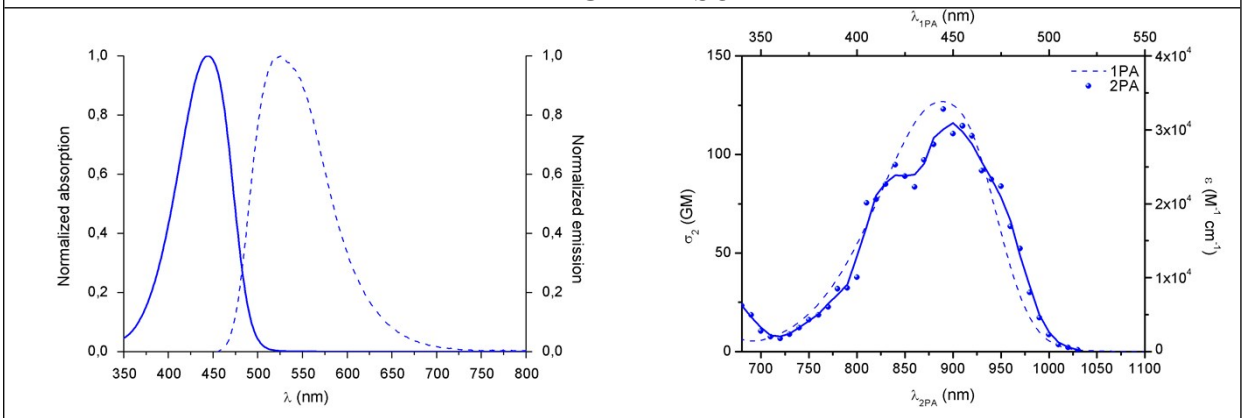
2. 1PA, emission and 2PA spectra

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.

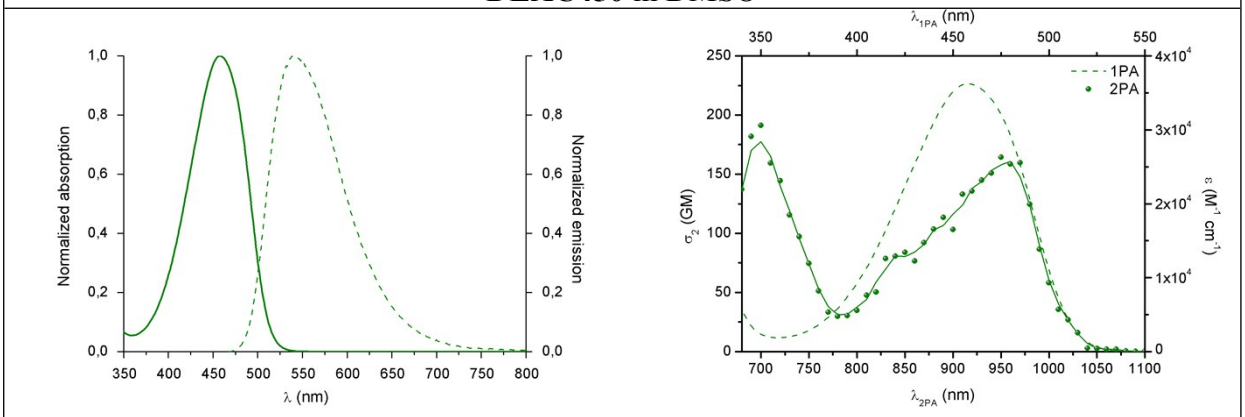
Normalized absorption & emission **1PA vs 2PA spectra**



DEAC in DMSO

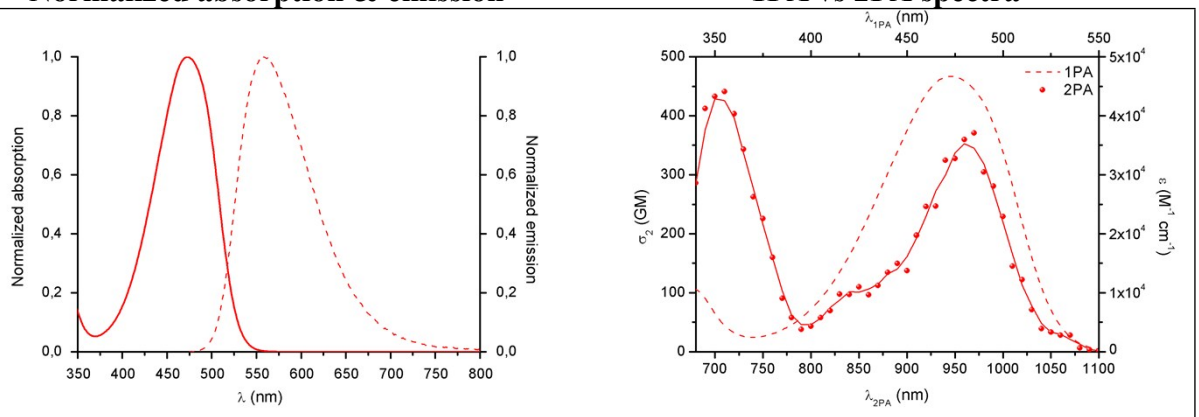


DEAC450 in DMSO

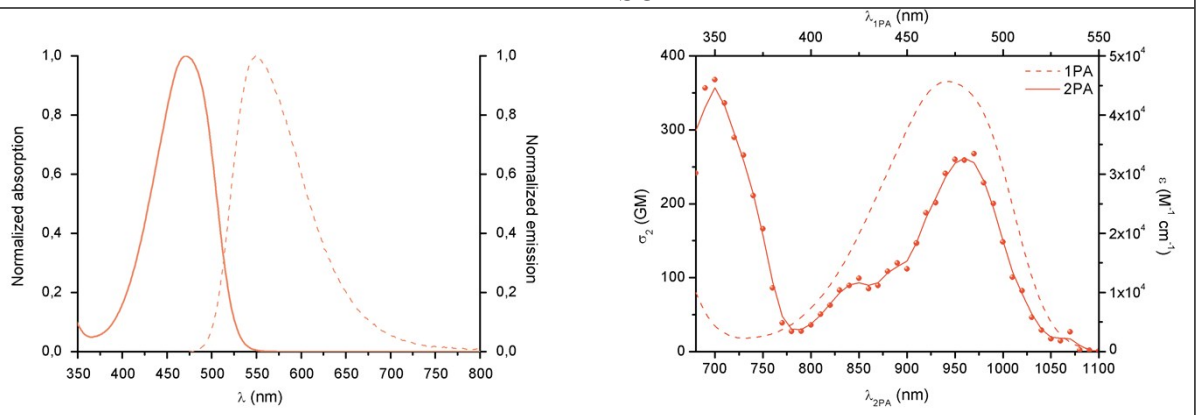


13 in DMSO

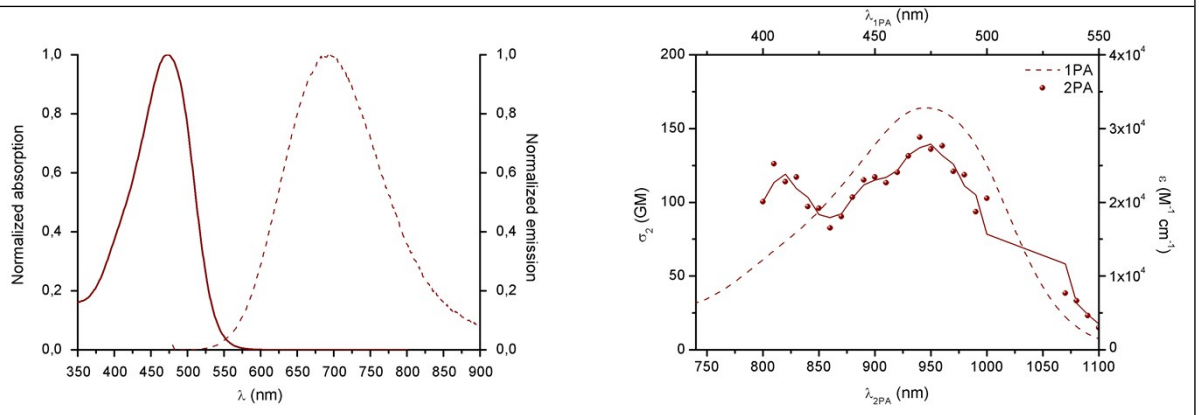
Normalized absorption & emission **1PA vs 2PA spectra**



14 in DMSO

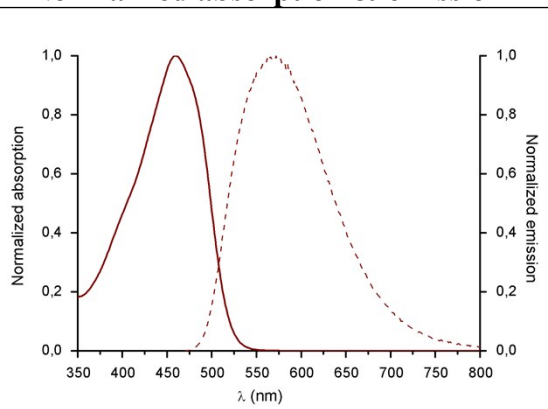


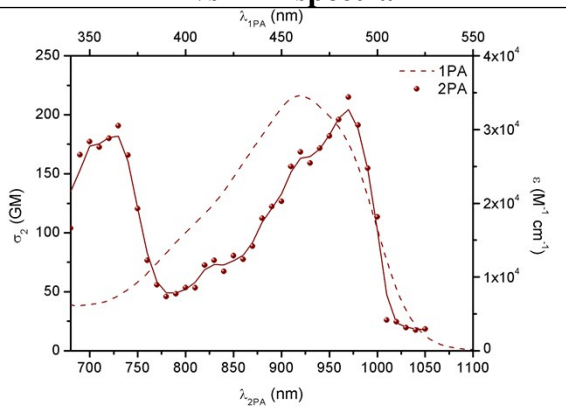
15 in DMSO



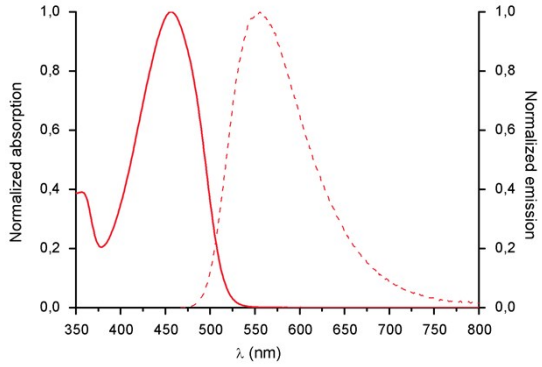
16 in DMSO

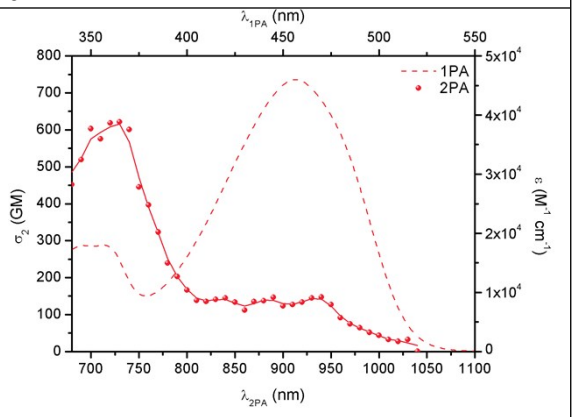
Normalized absorption & emission **1PA vs 2PA spectra**



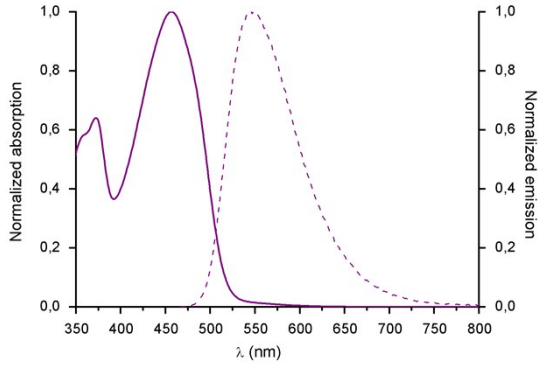


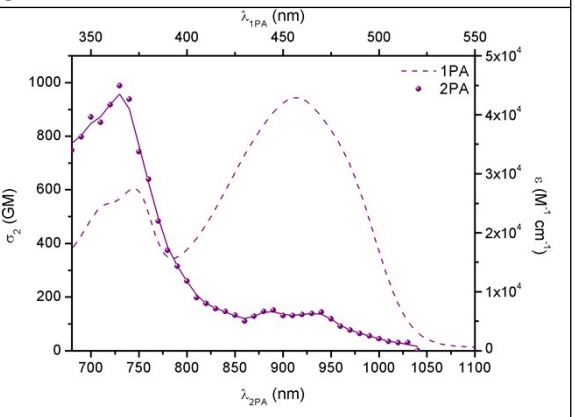
16 in toluene





17 in DMSO





19 in DMSO

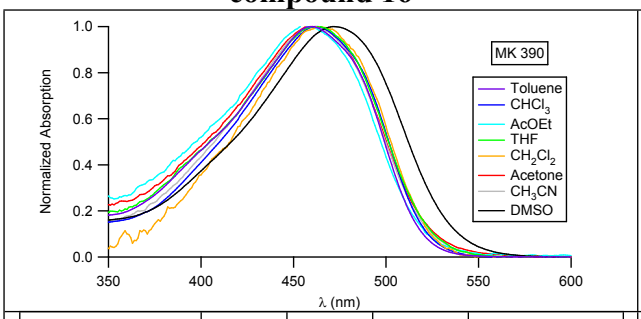
3. Solvatochromic behavior

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 Δf according to the following Equation: [13,14]

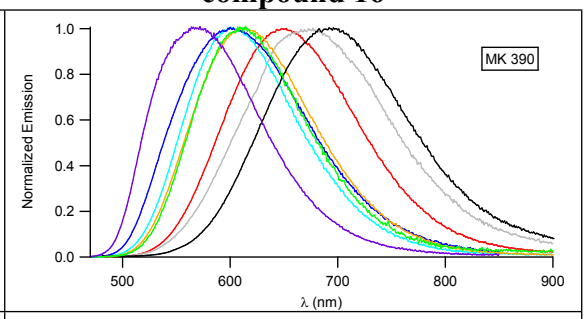
$$\nu_{abs} - \nu_{em} = 2\Delta\mu^2\Delta f/(hca^3) + \text{const}$$

where ν_{abs} (ν_{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 ε is the dielectric constant and n the refractive index of the solvent.

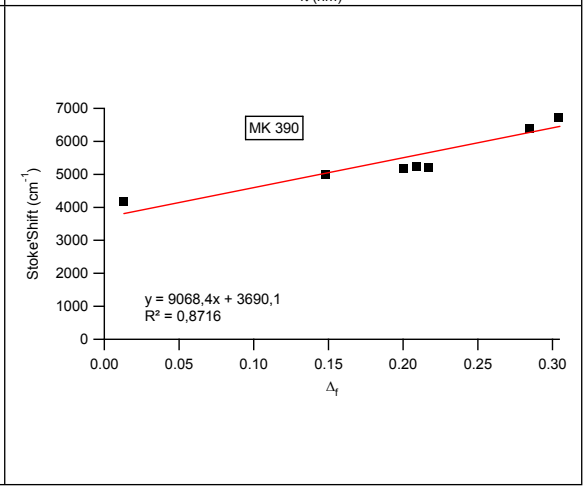
Normalized absorption spectra of compound 16



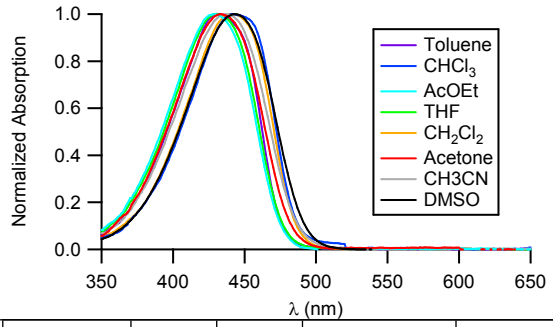
Normalized emission spectra of compound 16



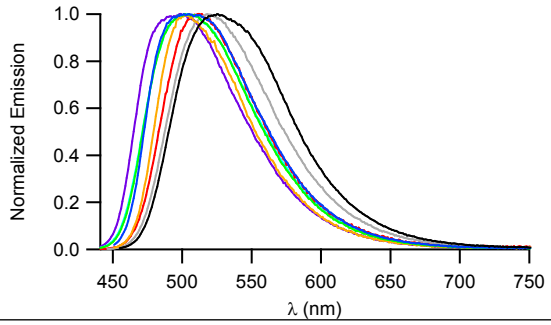
Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_f
Toluene	459	568	4.2	0.01314
$CHCl_3$	462	601	5.0	0.14816
Ethyl Acetate	458	600	5.2	0.20020
THF	462	610	5.3	0.20888
CH_2Cl_2	464	612	5.2	0.21710
Acetone	459	649	6.4	0.28427
CH_3CN	461	673	6.8	0.30492
DMSO	473	694	6.7	0.30394



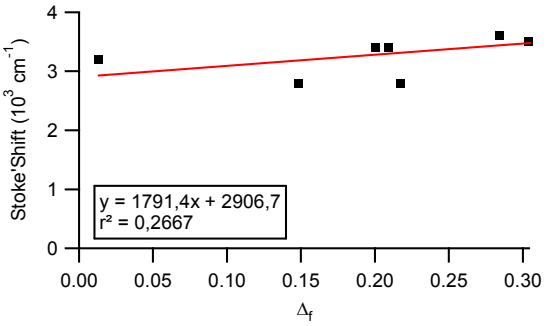
Normalized absorption spectra of DEAC450



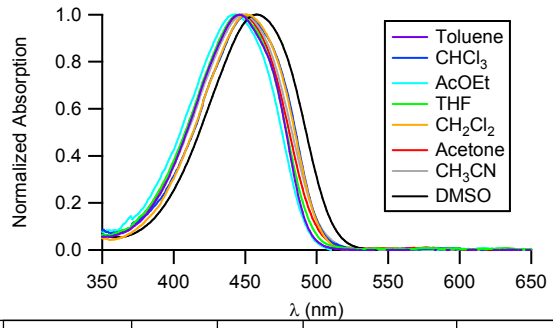
Normalized emission spectra of compound DEAC450



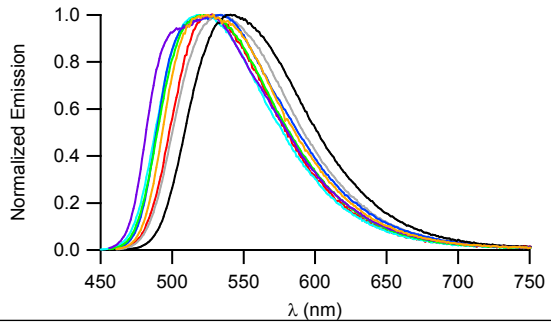
Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_f
Toluene	431	500	3.2	0.01314
$CHCl_3$	443	505	2.8	0.14816
Ethyl Acetate	429	502	3.4	0.20020
THF	431	505	3.4	0.20888
CH_2Cl_2	441	503	2.8	0.21710
Acetone	433	512	3.6	0.28427
CH_3CN	436	519	3.7	0.30492
DMSO	444	525	3.5	0.30393



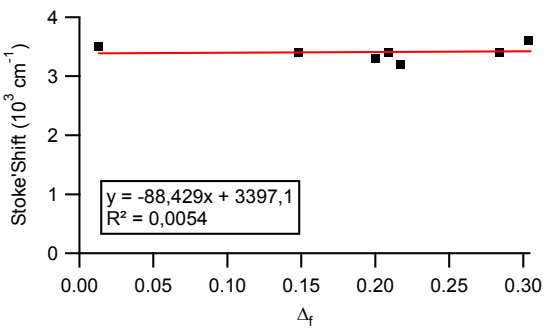
Normalized absorption spectra of compound 13



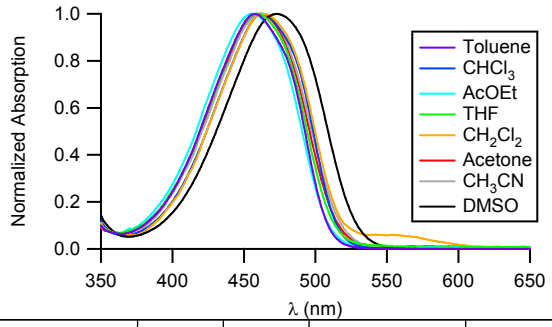
Normalized emission spectra of compound 13



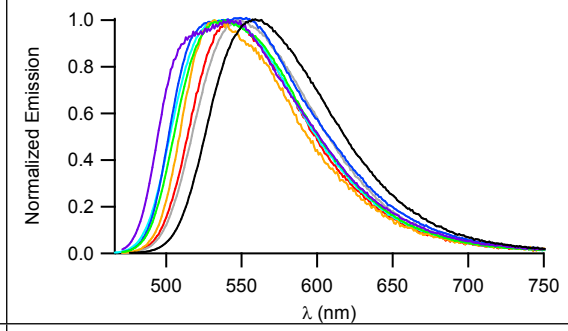
Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_f
Toluene	445	527	3.5	0.01314
$CHCl_3$	451	532	3.4	0.14816
Ethyl Acetate	443	518	3.3	0.20020
THF	445	524	3.4	0.20888
CH_2Cl_2	450	525	3.2	0.21710
Acetone	447	527	3.4	0.28427
CH_3CN	449	534	3.5	0.30492
DMSO	458	542	3.6	0.30393



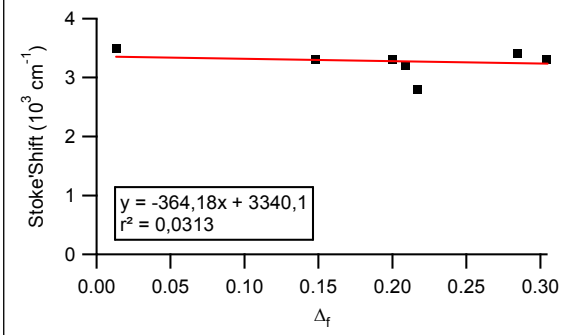
Normalized absorption spectra of compound 14



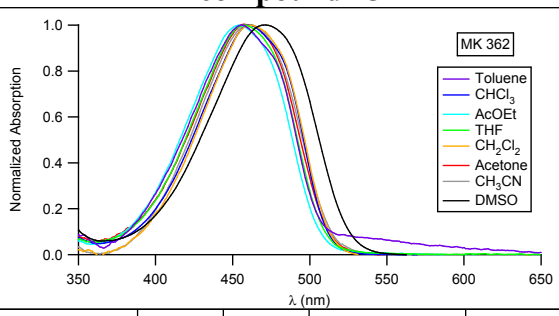
Normalized emission spectra of compound 14



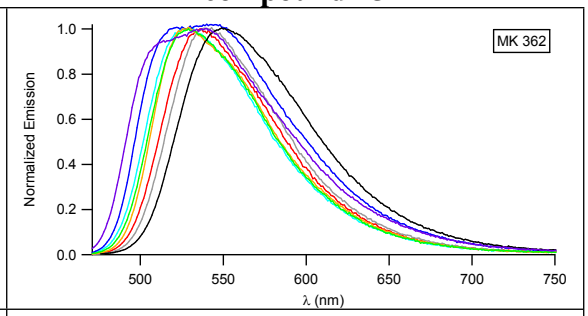
Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_f
Toluene	458	544	3.5	0.01314
$CHCl_3$	463	547	3.3	0.14816
Ethyl Acetate	455	535	3.3	0.20020
THF	459	539	3.2	0.20888
CH_2Cl_2	463	532	2.8	0.21710
Acetone	460	544	3.4	0.28427
CH_3CN	462	548	3.4	0.30492
DMSO	472	558	3.3	0.30393



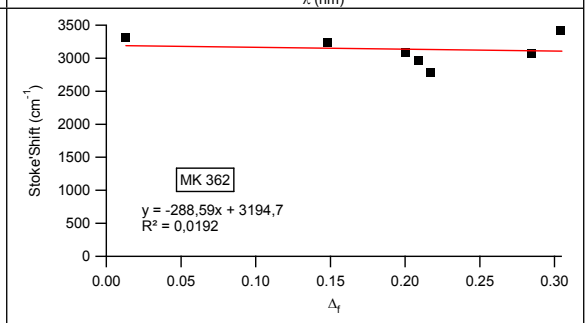
Normalized absorption spectra of compound 15



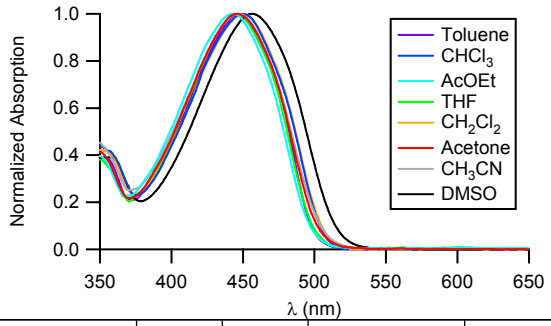
Normalized emission spectra of compound 15



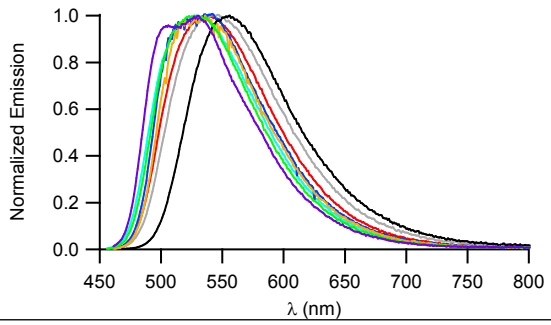
Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_f
Toluene	455	536	3.3	0.01314
$CHCl_3$	461	542	3.2	0.14815
Ethyl Acetate	454	528	3.1	0.20019
THF	458	530	3.0	0.20888
CH_2Cl_2	461	529	2.8	0.21710
Acetone	461	537	3.1	0.28427
CH_3CN	461	540	3.2	0.30492
DMSO	472	563	3.4	0.30394



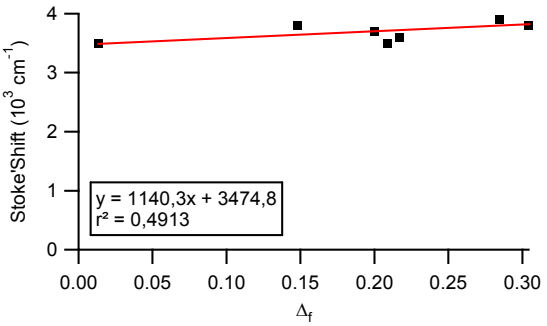
Normalized absorption spectra of compound 17



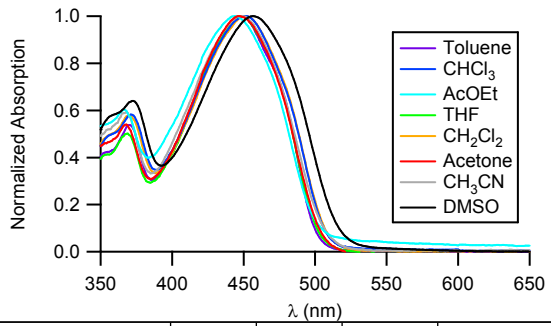
Normalized emission spectra of compound 17



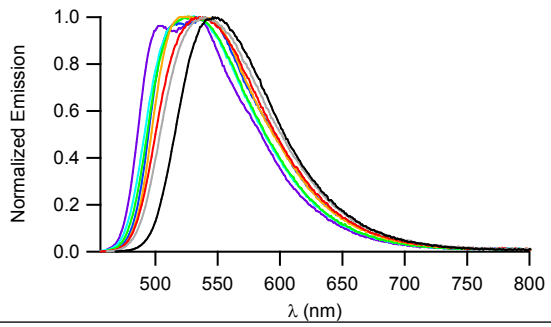
Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_f
Toluene	446	529	3.5	0.01314
$CHCl_3$	449	540	3.8	0.14816
Ethyl Acetate	443	531	3.7	0.20020
THF	445	528	3.5	0.20888
CH_2Cl_2	450	536	3.6	0.21710
Acetone	445	538	3.9	0.28427
CH_3CN	447	542	3.9	0.30492
DMSO	457	553	3.8	0.30394



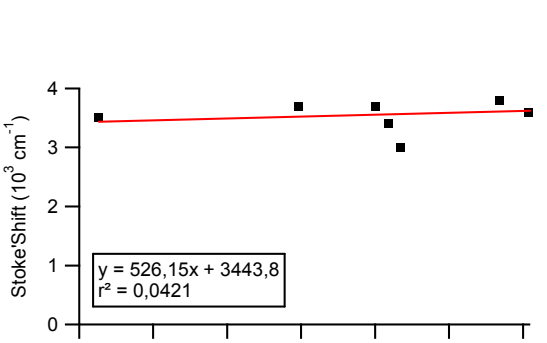
Normalized absorption spectra of compound 19



Normalized emission spectra of compound 19



Solvent	λ_{Abs} [nm]	λ_{em} [nm]	Stoke Shift [10^3 cm^{-1}]	Δ_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_2Cl_2	450	521	3.0	0.21710
Acetone	446	536	3.8	0.28427
CH_3CN	448	540	3.8	0.30492
DMSO	458	547	3.6	0.30393



III. Comparative photolysis experiments

Uncaging quantum yield Φ_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}), ε 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]** (Φ_u = 39% in phosphate buffer)[4] 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 ($\varepsilon^{ech} Q_u^{ech}$) and the reference compound ($\varepsilon^{ref} \Phi_u^{ref}$):

$$\frac{k^{ech}}{k^{ref}} = \frac{\varepsilon^{ech}\, Q_u^{ech}}{\varepsilon^{ref}\, \Phi_u^{ref}}$$

Determination of the ε values at the excitation wavelength, measured in the solvent of the photolysis, then allowed the determination of the Q_u^{ech}/Φ_u^{ref} ratio.

The conversion rate (ξ) 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_3CN/D_2O (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_3CN/H_2O, 9/1, v/v) over one week at room temperature (21 °C). Figure S1,a) shows the decrease of the recorded absorbance at λ_{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.

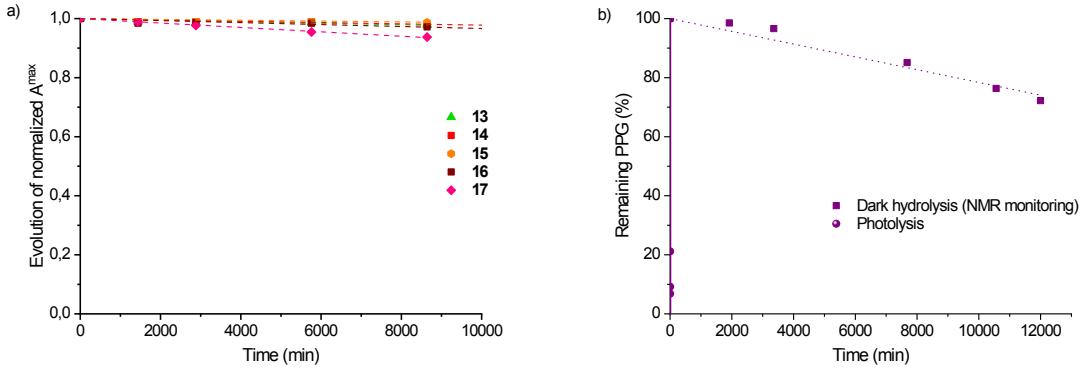


Figure S1. a) Evolution of the absorbance maximum of compounds **13-17** over time starting from the normalized absorption spectrum at t = 0; b) Conversion of the PPG **19** to free Fmoc-Gly-OH over time upon irradiation or dark hydrolysis, monitored by ^{1}H NMR.

V. Two-photon photolysis

Two-photon photolysis experiments were performed with the TPEF setup (*vide supra*). Solutions of the PPGs prepared in CD_3CN/D_2O (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.

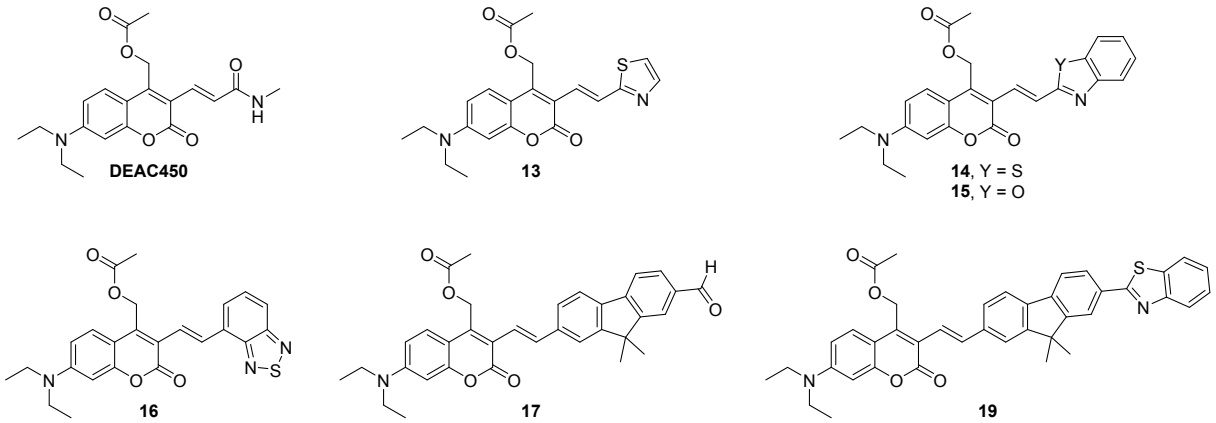
VI. DFT calculations

Computational methods

The geometry of the ground (S_0) and first singlet excited (S_1) states were optimized in the gas phase in the framework of the Density Functional Theory (DFT) using the M06-2X exchange-correlation functional[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).[16] The photo-induced charge transfer was analyzed on the basis of vertical electronic transitions, by considering the difference $\Delta\mu_{01}^{vert}$ in the electric dipoles of the S_0 and S_1 states. Using the approach reported in refs [17] and [18], $\Delta\mu_{01}^{vert}$ was further decomposed as $\Delta\mu_{01}^{vert} = q^{CT} \times d^{CT}$, where q^{CT} is the photo-induced charge transfer, *i.e*. the global amount of charge transferred upon light excitation, and d^{CT} is the distance over which this charge is transferred.

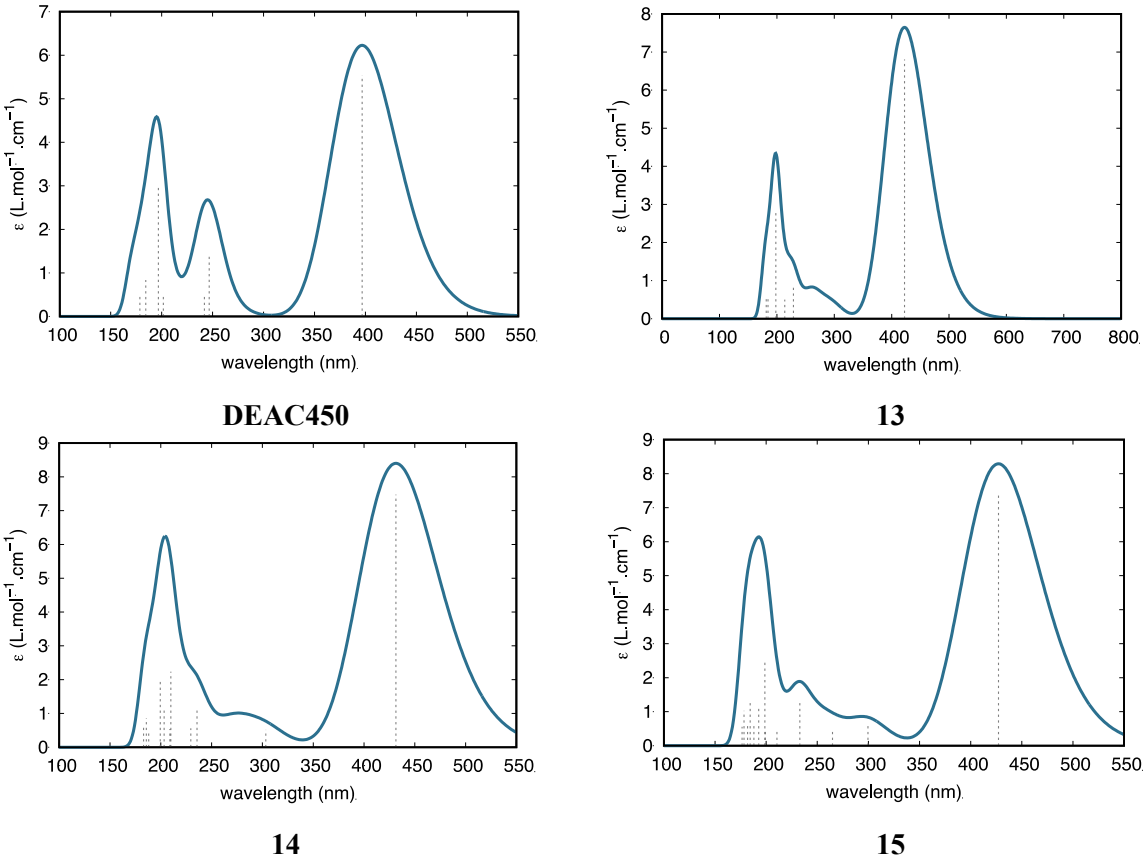
Then, the effects of structural relaxation on the electronic structure of the S_1 state were addressed. The adiabatic energies, defined as the energy difference of the S_1 and S_0 states in their respective minimum ($E_{adia} = E_{S1}^{opt} - E_{S0}^{opt}$), were calculated on the basis of the gas phase 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_1 and S_0 ($E_{0-0} = E_{adia} + \Delta E_{ZPVE}$). Finally, IEF-PCM:TD-DFT single-point calculations in acetonitrile were performed on the (gas phase) relaxed geometries of S_1, to evaluate the excited-state dipole moment and photo-induced dipole moment variation $\Delta\mu_{01}^{opt+s}$ accounting for geometrical relaxation effects. All calculations were performed using the Gaussian 09 package.[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



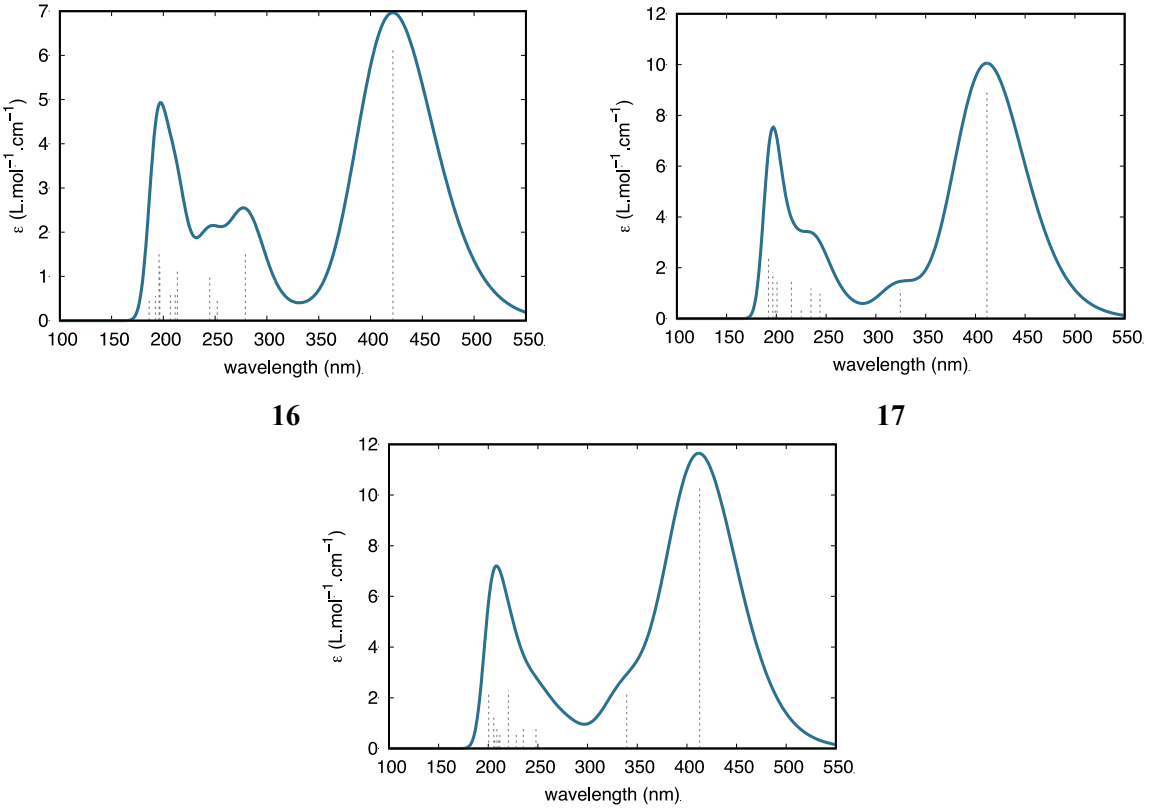


Figure S2. Vertical absorption spectra of the investigated compounds, calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile. Electronic transitions with oscillator strength $f \geq 0.1$ are reported as grey dashed vertical bars.

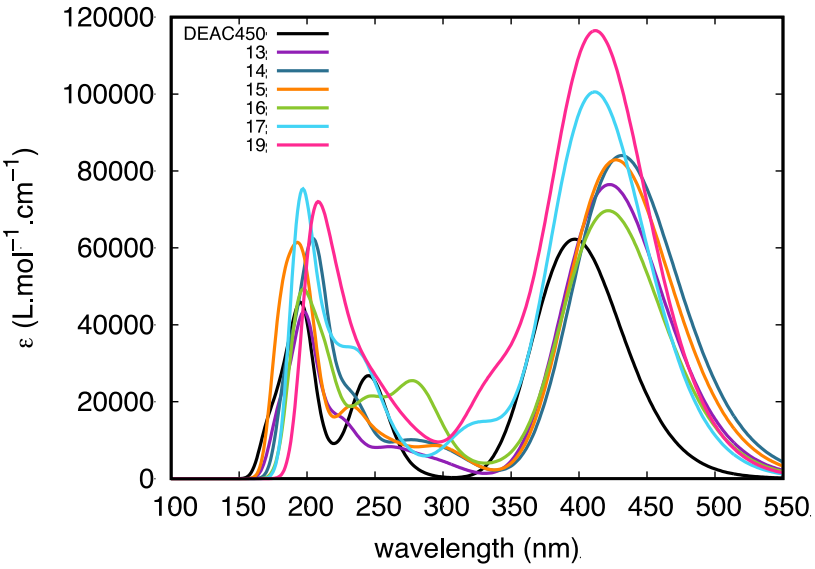


Figure S3. Vertical absorption spectra of the investigated compounds, calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile.

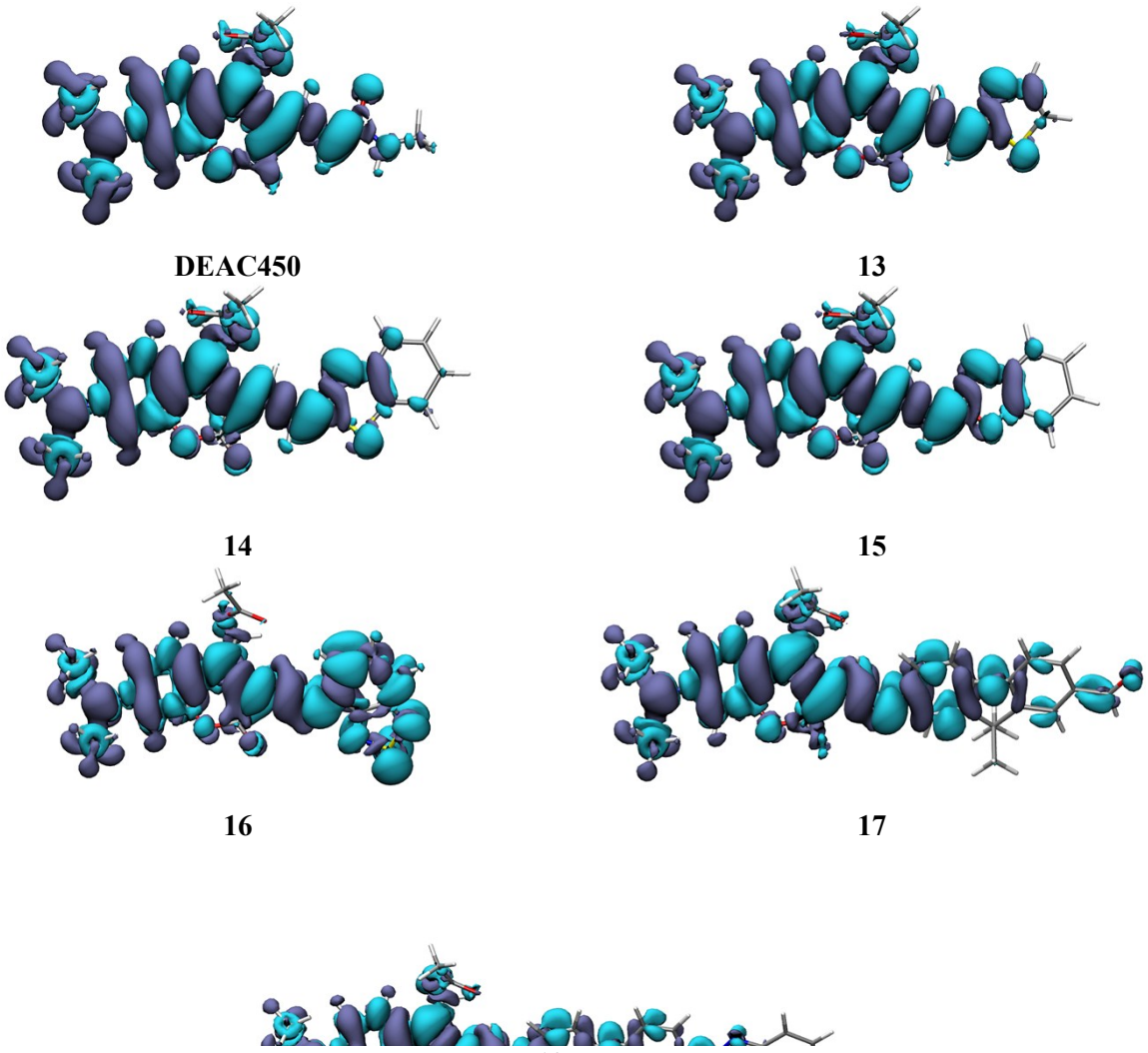
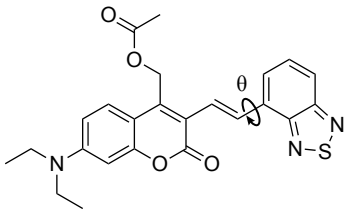


Figure S4. Electron density difference between the lowest-energy excited state and the ground state, $\Delta\rho = \rho(S_1) - \rho(S_0)$, as calculated at the IEFPCM:M06-2X/6-311G(d) level in acetonitrile. Dark (light) blue lobes are associated with negative (positive) $\Delta\rho$ values.

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



16

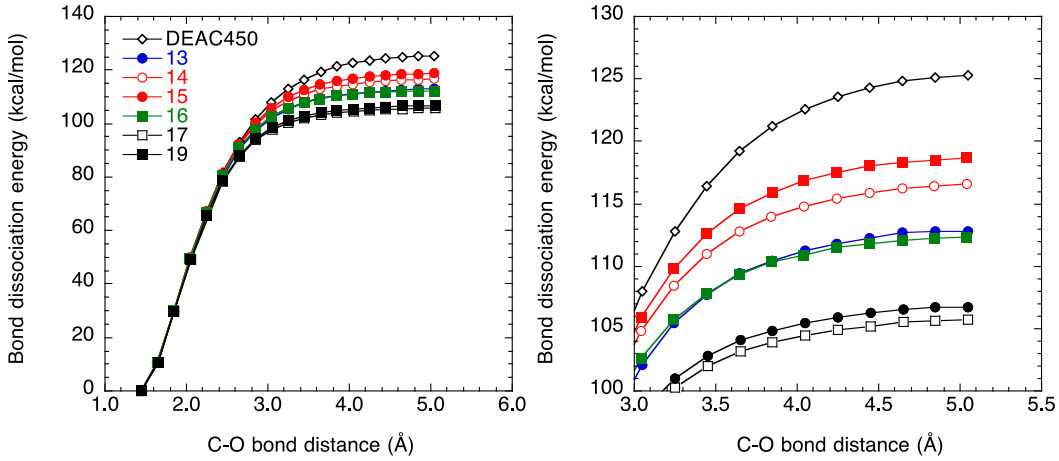


Figure S5. Rigid energy scans first performed at the M06-2X/6-311G(d) level in the S_1 state (left), and focus on the long distance energies (right).

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.

	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 $E_{stab} = E_{PPG-Gly} - E_{PPG^+} - E_{Gly^-}$ where $E_{PPG-Gly}$, E_{PPG^+}, and E_{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).

	E_{stab}
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 (ΔE_{01}, eV) as well as oscillator strength (f_{01}), transition dipole moment (μ_{01}, D), MO contributions (%), dipole moment variation ($\Delta\mu^{vert}_{01}$, D), charge transferred upon excitation (q^{CT}, |e|), and charge transfer distance (d^{CT}, Å), calculated at the TDDFT/M06-2X/6-311G(d) level in acetonitrile.

	state	ΔE_{ge}	f_{ge}	μ_{ge}	MO contributions	%	$\Delta\mu^{vert}_{ge}$	q^{CT}	d^{CT}
DEAC450	1	3.125	1.385	10.81	HOMO➌LUMO	96	8.25	0.584	2.940
13	1	2.935	1.701	12.36	HOMO➌LUMO	96	6.70	0.540	2.581
14	1	2.874	1.869	13.09	HOMO➌LUMO	95	8.09	0.568	2.967
	2	4.088	0.129	2.88	HOMO-1➌LUMO HOMO➌LUMO+1	62 30	0.46	0.484	0.199
15	1	2.902	1.844	12.94	HOMO➌LUMO	95	7.59	0.566	2.789
16	1	2.941	1.548	11.78	HOMO-1➌LUMO HOMO➌LUMO HOMO➌LUMO+1	6 78 12	10.53	0.581	3.775
17	1	3.012	2.235	13.99	HOMO-1➌LUMO+1 HOMO➌LUMO HOMO➌LUMO+1	2 84 9	7.18	0.551	2.714
	3	3.820	0.289	4.46	HOMO-6➌LUMO+1 HOMO-1➌LUMO HOMO-1➌LUMO+1 HOMO➌LUMO+1 HOMO➌LUMO+3	3 45 2 37 2	8.58	0.525	3.403
19	1	3.004	2.569	15.02	HOMO-1➌LUMO+1 HOMO➌LUMO HOMO➌LUMO+1	4 82 11	6.35	0.544	2.433
	2	3.653	0.549	6.30	HOMO-2➌LUMO+1 HOMO-1➌LUMO HOMO-1➌LUMO+1 HOMO➌LUMO+1 HOMO➌LUMO+2	2 49 5 35 3	6.89	0.504	2.842

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