Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Tandem triad systems based on FRET for two-photon induced release of glutamate

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A. General methods for the synthetic procedures:

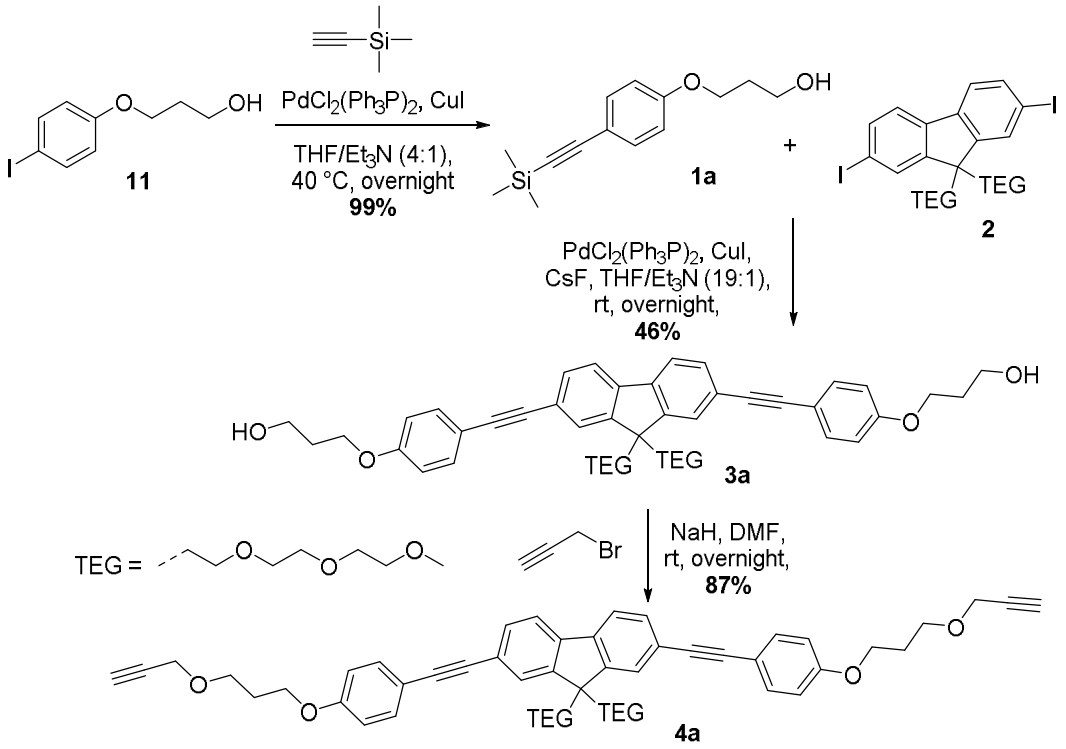
Melting points were measured on Stuart SMP 10. Infrared spectra were measured on a Perkin Elmer Spectrum 100 Optica. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance III 200 spectrometer at 200 MHz and 50 MHz respectively, on a Bruker Advance I 300 spectrometer at 300 MHz and 75 MHz respectively, on a Bruker Advance II 400 spectrometer at 400 MHz and 100 MHz respectively, 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. Column chromatography was performed on Fluka silica gel 60 (40-63 µm). Solvent were freshly distilled before to be used over CaH_2 (for toluene, DMF, CH_2Cl_2 and Et_3N) or benzophenone/Na (for THF).

Mass spectra were performed 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.

MALDI-MS spectra were performed by the CESAMO (Bordeaux, France) on a Voyager mass spectrometer (Applied Biosystems). The instrument is equipped with a pulsed N_2 laser (337 nm) and a time-delayed extracted ion source. Spectra were recorded in the positive-ion mode using the reflectron and with an accelerating voltage of 20 kV. Samples were dissolved in CH_2Cl_2 at 10 mg/ml. The DCTB matrix (T-2-(3-(4-t-butyl-phenyl)-2-methyl-2-propenylidene)malononitrile) solution was prepared by dissolving 10 mg in 1 ml of CH_2Cl_2. A MeOH solution of cationisation agent (NaI, 10 mg/ml) was also prepared. The solutions were combined in a 10:1:1 volume ratio of matrix to sample to cationisation agent. One to two microliters of the obtained solution was deposited onto the sample target and vacuum-dried.

B. Synthesis of chromophore 4a:

Synthetic route



3-((4-((trimethylsilyl)ethynyl)phenoxy)propan-1-ol (1a).

A solution of 3-(4-iodophenoxy)propan-1-ol[1] **11** (1.20 g, 4.3 mmol), in dry THF/Et_3N (4:1, 25 mL) was degased by bubbling argon. Then, cupper iodide (17 mg, 87 µmol), $PdCl_2(Ph_3P)_2$ (61 mg, 87 µmol) and ethynyltrimethylsilane (760 µL, 5.4 mmol). The reaction mixture was stirred overnight at 40 °C. The reaction mixture was filtrated through Celite®, and the filtrate was quenched with saturated aqueous NH_4Cl and then extracted with CH_2Cl_2. The combined organic was dried (Na_2SO_4) and concentrated under reduced pressure. The crude was purified by a short column of silica gel (Petroleum Ether/CH_2Cl_2, 1:4 as eluent) to give **1a** (1.00 g, 99%) as a brown viscous oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.39 (d, *J* = 8.9 Hz, 2H), 6.81 (d, *J* = 8.9 Hz, 2H), 4.10 (t, *J* = 6.0 Hz, 2H), 3.67-3.78 (m, 2H), 3.78-3.91 (m, 2H), 1.96-2.09 (m, 2H), 1.77 (s, 1H), 0.24 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 0.2, 32.1, 60.3, 65.7, 92.6, 105.3, 114.5, 115.6, 133.6, 159.1; ESIHMRS: $C_{14}H_{20}O_2Si$ calculated for $[M+Na]^+$: 271.1130, found 271.1133.

3,3'-(4,4'-((9,9-bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-9*H*-fluorene-2,7-diyl)bis(ethyne-2,1-diyl))bis(4,1-phenoxy))bis(propan-1-ol) (3a).

Cesium fluoride (430 mg, 2.81 mmol) was introduced into a Schlenk tube, and was dried under high vacuum and heating, and then Argon was introduced to break the vacuum. A solution of 2,7-diiodo-9,9-bis[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-9*H*-fluorene[2] **2** (499.5 mg, 703 µmol) and 3-((4-((trimethylsilyl)-ethynyl)phenoxy)propan-1-ol **1a** (420 mg, 1.69 mmol) in dry THF (8.3 mL) was added,

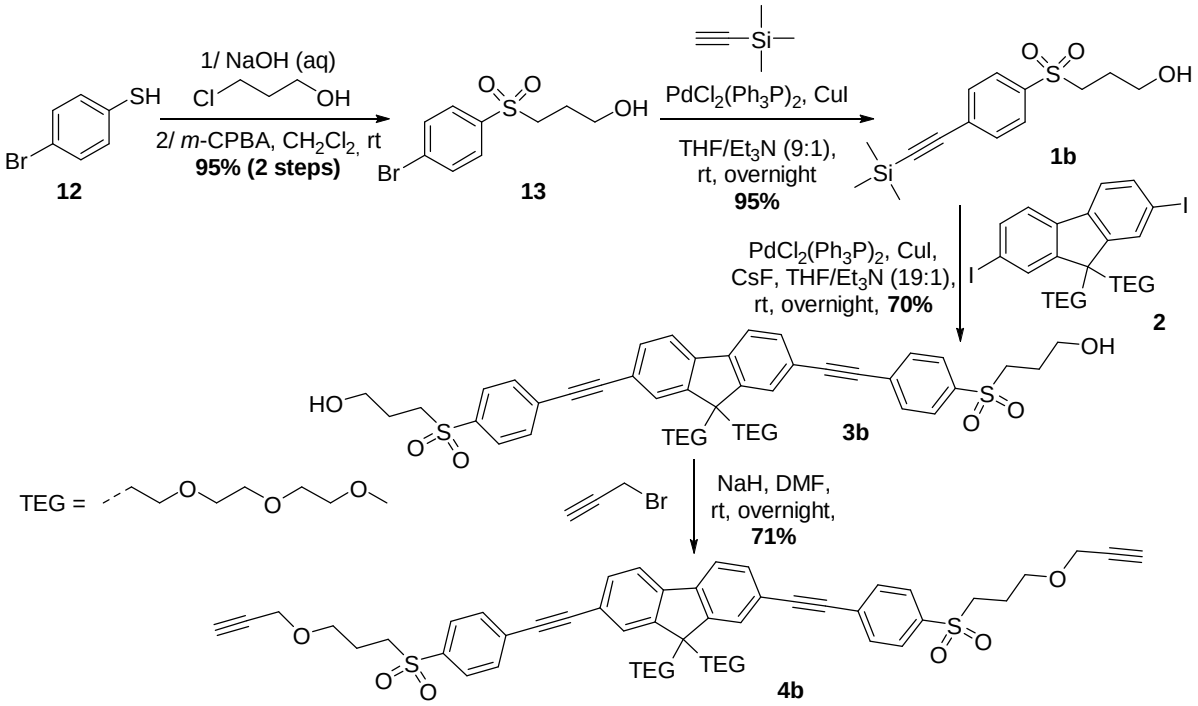
followed by the addition of $PdCl_2(Ph_3P)_2$ (9.8 mg, 14 µmol), CuI (5.3 mg, 28 µmol), and Et_3N (0.42 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated aqueous NH_4Cl and then extracted with EtOAc. The combined organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The crude was purified on silica gel (EtOAc as eluent) to give **3a** (261 mg, 46%) as a brown powder: 66 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.45 (m, 10H), 6.86-6.94 (m, 4H), 4.16 (t, *J* = 6.0 Hz, 4H), 3.89 (t, *J* = 6.0 Hz, 4H), 3.36-3.56 (m, 12H), 3.92 (s, 6H), 3.16-3.26 (m, 4H), 2.72-2.84 (m, 4H), 2.35-2.44 (m, 4H), 2.02-2.14 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 32.1, 39.8, 51.4, 59.1, 60.3, 65.7, 67.0, 70.1, 70.6, 72.0, 88.9, 90.3, 114.7, 115.6, 120.1, 122.7, 126.3, 131.1, 133.2, 139.8, 149.9, 159.0; ESIHMRS: $C_{49}H_{58}O_{10}$ calculated for $[M+Na]^+$: 829.3922, found 829.3934.

9,9-bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,7-bis((4-(3-(prop-2-yn-1-yloxy)propoxy)phenyl)-ethynyl)-9*H*-fluorene (4a).

To a solution of **3a** (94 mg, 120 µmol), in dry DMF (1.5 mL), at 0 °C, was added NaH (60 % on mineral oil, 19 mg, 470 µmol). The reaction mixture was stirred 10 min at 0 °C and then propargyl bromide (80% in toluene, 60 µL, 470 µmol) was added. The reaction mixture was stirred overnight at room temperature, quenched with saturated aqueous NH_4Cl and then extracted with CH_2Cl_2. The combined organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated under reduced pressure. The crude was purified on silica gel (CH_2Cl_2/EtOAc, 3:2 as eluent) to give **4a** (90 mg, 87%) as a brown powder: mp 66 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.63 (d, *J* = 7.9 Hz, 2H), 7.56 (s, 2H), 7.46-7.52 (m, 6H), 6.89 (d, *J* = 8.9 Hz, 4H), 4.16 (d, *J* = 2.4 Hz, 4H), 4.10 (t, *J* = 6.3 Hz, 4H), 3.72 (t, *J* = 6.0 Hz, 4H), 3.43-3.54 (m, 8H), 3.37-3.41 (m, 4H), 3.32 (s, 6H), 3.18-3.24 (m, 4H), 2.74-2.81 (m, 4H), 2.35-2.43 (m, 6H), 2.03-2.12 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 29.6, 39.8, 51.4, 58.4, 59.1, 64.9, 66.7, 67.0, 70.2, 70.6, 72.0, 74.5, 79.9, 88.9, 90.4, 114.8, 115.4, 120.1, 122.8, 126.3, 131.1, 133.2, 139.8, 149.5, 159.2; ESIHMRS: $C_{55}H_{62}O_{10}$ calculated for $[M]^+$: 882.4343, found 882.4348.

C. Synthesis of chromophore 4b:

Synthetic route



3-((4-bromophenyl)sulfonyl)propan-1-ol (13).

Sodium hydroxide (2.12 g, 52.8 mmol) was dissolved in 12.5 mL of distillated water, and 4-bromothiophenol **12** (10 g, 52.8 mmol) was added. The reaction mixture was heated at 40°C for 1 h until suspension turned into a clear solution. At this temperature, 3-chloropropan-1-ol (4.6 mL, 54.9 mmol) was added dropwise. The reaction mixture was cooled at room temperature and stirred overnight. The mixture was diluted with brine, and then extracted with EtOAc. The combined organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was controlled by ^{1}H NMR, and was used for the next step without further purification. The crude was diluted in dry CH_2Cl_2 (200 mL), cooled at 0° C and *m*-CPBA (77%, 39.0 g, 158.4 mmol) was added by portion. The resulting mixture was stirred overnight at room temperature, filtrated trough Celite®, and the filtrate was washed with an aqueous KOH solution (1 M). Then, the organic layer was dried, and concentrated under reduced pressure. The crude was purified by a short column of silica gel (CH_2Cl_2/Et_2O 7:3 as eluent) to give **13** (13.9 g, 95%) as a white powder: mp 75 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.70 (d, *J* = 8.9 Hz, 2H), 7.64 (d, *J* = 8.9 Hz, 2H), 3.54-3.63 (m, 2H), 3.12-3.21 (m, 2H), 2.90 (s, 1H), 1.78-1.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 25.6, 53.1, 60.0, 129.0, 129.5, 132.6, 137.7; ESIHMRS: $C_9H_{11}BrO_3S$ calculated for $[M+Na]^+$: 300.9504, found 300.9523.

3-((4-((trimethylsilyl)ethynyl)phenyl)sulfonyl)propan-1-ol (1b).

To a solution of **13** (3.98 g, 14.2 mmol), cupper iodide (54 mg, 0.028 mmol), and $PdCl_2(Ph_3P)_2$ (196.5 mg, 0.28 mmol) in a THF/Et_3N mixture (50 mL, 9:1) was added ethynyltrimethylsilane (2.4 mL,

1.71 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was filtrated through Celite®, and the filtrate was quenched with saturated aqueous NH_4Cl and then extracted with EtOAc. The combined organic was dried (Na_2SO_4) and concentrated under reduced pressure. The crude was purified by a short column of silica gel (Petroleum Ether/EtOAc 3:2 as eluent) to give **1b** (4.01 g, 95%) as an orange powder: mp 92 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.84 (d, *J* = 8.5 Hz, 2H), 7.67 (d, *J* = 8.5 Hz, 2H), 3.67-3.78 (m, 2H), 3.17-3.26 (m, 2H), 1.86-2.05 (m, 2H), 1.69 (t, *J* = 5.3 Hz, 1H), 0.26 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): -0.1, 25.8, 52.5, 60.6, 99.5, 103.0, 128.1, 129.2, 132.8, 138.5; ESIHMRS: $C_{14}H_{20}O_3SiS$ calculated for $[M+Na]^+$: 319.0794, found 319.0810.

3,3'-(4,4'-((9,9-bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-9*H*-fluorene-2,7-diyl)bis(ethyne-2,1-diyl))bis(4,1-phenylenesulfonyl))bis(propan-1-ol) (3b).

Cesium fluoride (102.6 mg, 675.6 µmol) was introduced into a Schlenk tube, and was dried under strong vacuum and heating, and then Argon was introduced to break the vacuum. A solution of 2,7-diiodo-9,9-bis[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-9*H*-fluorene **2** (120 mg, 168.9 µmol) and 3-((4-((trimethylsilyl)-ethynyl)phenyl)sulfonyl)propan-1-ol **1b** (120.2 mg, 405.4 µmol) in dry THF (1.9 mL) was added, followed by the addition of $PdCl_2(Ph_3P)_2$ (2.4 mg, 3.4 µmol), CuI (1.3 mg, 6.8 µmol), and Et_3N (0.1 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated aqueous NH_4Cl and then extracted with EtOAc. The combined organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The crude was purified on silica gel (EtOAc as eluent) to give **3b** (106 mg, 70%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.92 (d, *J* = 8.5 Hz, 4H), 7.74 (d, *J* = 8.5 Hz, 4H), 7.70 (d, *J* = 7.9 Hz, 2H), 7.63 (s, 2H), 7.57 (dd, *J* = 7.9 Hz, *J* = 1.2 Hz, 2H), 3.72-3.79 (m, 4H), 3.49-3.53 (m, 4H), 3.44-3.48 (m, 4H), 3.31 (s, 6H), 3.25-3.30 (m, 4H), 3.19-3.23 (m, 4H), 2.76-2.84 (m, 4H), 2.80 (t, *J* = 7.3 Hz, 4H), 2.43 (t, *J* = 7.3 Hz, 4H), 1.77 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 25.9, 39.7, 51.7, 53.5, 59.1, 60.7, 67.0, 70.2, 70.6, 70.7, 72.0, 88.7, 94.2, 120.5, 121.7, 126.9, 128.3, 129.3, 131.6, 132.4, 138.3, 140.7, 149.8. ESIHMRS: $C_{49}H_{58}O_{12}S_2$ calculated for $[M+Na]^+$: 925.3261, found 925.3277.

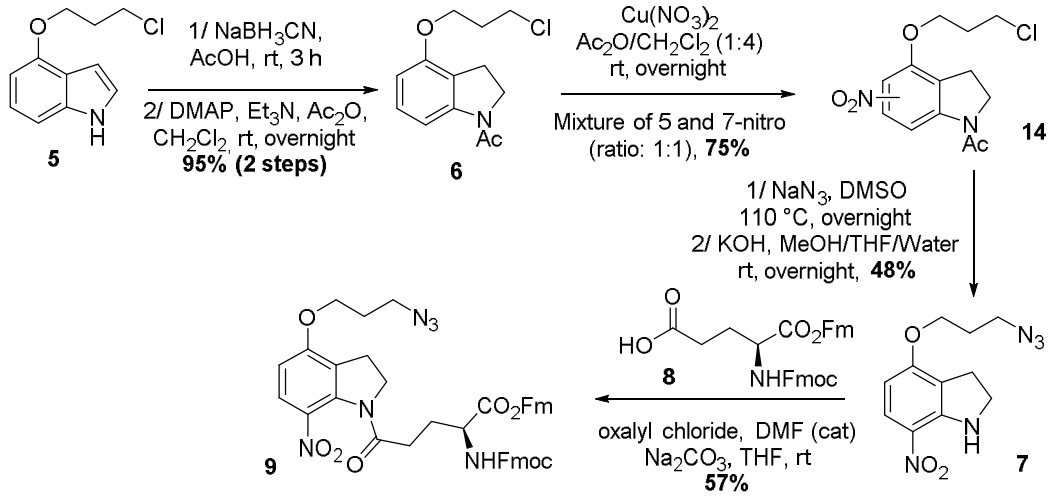
9,9-bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)-2,7-bis((4-((3-(prop-2-yn-1-yloxy)propyl)-sulfonyl)phenyl)ethynyl)-9*H*-fluorene (4b)

To a solution of **3b** (100 mg, 110 µmol), in dry DMF (0.5 mL), at 0 °C, was added NaH (60 % on mineral oil, 17.6 mg, 440 µmol). The reaction mixture was stirred for 10 min at 0 °C and then propargyl bromide (80% in toluene, 50 µL, 440 µmol) was added. The reaction mixture was stirred overnight at room temperature, quenched with saturated aqueous NH_4Cl and then extracted with CH_2Cl_2. The combined organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated under reduced pressure. The crude was purified on silica gel (CH_2Cl_2/EtOAc, 1:1 as eluent) to give **4b** (76 mg, 71%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.90 (d, *J* = 8.3 Hz, 4H), 7.72 (d, *J* = 8.3 Hz, 4H), 7.69 (d, *J* = 7.9 Hz, 2H), 7.62 (s, 2H), 7.56 (dd, *J* = 7.9 Hz, *J* = 1.1 Hz, 2H), 4.08 (d, *J* = 2.4 Hz, 4H) 3.58 (t, *J* = 5.9 Hz, 4H), 3.48-3.52 (m, 4H), 3.44-3.47 (m, 4H), 3.36-3.38 (m, 4H), 3.31 (s, 6H), 3.22-3.26 (m, 4H), 3.19-3.21 (m, 4H), 2.79 (t, *J* = 7.4 Hz, 4H), 2.39-2.44 (m, 6H), 2.01-2.06 (m,

4H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 23.3, 39.7, 51.6, 53.6, 58.3, 59.1, 66.9, 67.5, 70.2, 70.5, 70.6, 71.9, 74.8, 88.7, 94.1, 120.5, 121.6, 126.8, 128.2, 129.2, 131.6, 132.3, 138.3, 140.6, 149.7. ESIHMRS: $C_{55}H_{62}O_{12}S_2$ calculated for $[M+Na]^+$: 1001.3574, found 1001.3577.

D. Synthesis of NI-Glu 9:

Synthetic route



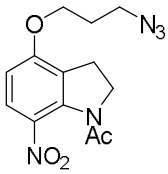
1-acetyl-(4-(3-chloropropoxy)indoline (6)

To a mixture of 4-(3-chloropropoxy)indole[3] **5** (6.3 g, 30 mmol), in AcOH (100 mL), was added $NaBH_3CN$ (5.65 g, 90 mmol) by portion. The reaction mixture was stirred 3 h at room temperature and then neutralized with saturated aqueous Na_2CO_3 and then extracted with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The crude was dissolved in dry CH_2Cl_2 (100 mL) and then DMAP (36.7 mg, 0.3 mmol), Et_3N (8.4 mL, 60 mmol), and Ac_2O (4.3 mL, 45 mmol) were added. The reaction mixture was stirred overnight at room temperature, quenched with a saturated aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The combined organic layer was dried and concentrated under reduced pressure. The crude was purified on silica gel (Petroleum Ether/EtOAC, 3:2 as eluent) to give **6** (7.23 g, 95%, purity ~98%) as a white powder: mp 102 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.84 (d, *J* = 7.9 Hz, 1H), 7.15 (t, *J* = 8.1 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 1H), 4.15 (t, *J* = 5.8 Hz, 2H), 4.06 (t, *J* = 8.4 Hz, 2H), 3.73 (t, *J* = 6.4 Hz, 2H), 3.10 (t, *J* = 8.4 Hz, 2H), 2.16-2.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 24.3, 25.0, 32.3, 41.5, 49.2, 64.3, 106.9, 110.1, 118.6, 128.9, 144.3, 154.8, 168.6; ESIHMRS: $C_{13}H_{16}NO_2Cl$ calculated for $[M+Na]^+$: 276.0761, found 276.0763.

4-(3-azidopropoxy)-7-nitroindoline (7)

Under dark condition, to a solution of **6** (1.1 g, 4 mmol), in CH_2Cl_2/Ac_2O mixture (4:1, 40 mL) was added $Cu(NO_3)_2$-$3H_2O$ (1.16 g, 4.8 mmol). The reaction mixture was stirred overnight at room temperature, quenched with a saturated aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The

combined organic layer was dried (Na_2SO_4) and then concentrated under reduced pressure. The crude was purified on silica gel (Petroleum Ether/EtOAc, 3:2 as eluent) to give **14** as a mixture of 5 and 7-nitroindoline (890 mg, 75%, ratio ~1:1). This mixture was dissolved in DMSO-d_6 (3 mL) and NaN_3 (586.2 mg, 9 mmol) was added. The reaction mixture was heated at 100 °C and the reaction was monitored by ^{1}H-NMR in DMSO-d_6. After 2 h, the reaction was complete and the reaction was quenched with water and extracted with EtOAc. The combined organic layer was dried (Na_2SO_4) and then concentrated under reduced pressure. The crude was dissolved in a THF/MeOH/Water mixture (1:1:1, 12 mL) and then powder KOH (252.5 mg, 4.5 mmol) was added. The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure to remove THF and MeOH. A saturated aqueous NH_4Cl was added to residue and extracted with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4) and then concentrated under reduced. The crude was purified on silica (Petroleum ether/EtOAc, 3:2, as eluent) to give **7** (355.4 mg, 45%) as an orange powder: mp 69 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.84 (d, *J* = 9.5 Hz, 1H), 6.59 (s, 1H), 6.24 (d, *J* = 9.5 Hz, 1H), 4.16 (t, *J* = 6.0 Hz, 2H), 3.87 (t, *J* = 8.9 Hz, 2H), 3.51 (t, *J* = 6.6 Hz, 2H), 3.07 (t, *J* = 8.9 Hz, 2H), 1.98-2.14 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 25.8, 28.8, 47.3, 48.2, 65.2, 103.0, 116.7, 125.2, 125.6, 151.0, 159.1; ESIHMRS: $C_{11}H_{13}N_5O_3$ calculated for $[M+Na]^+$: 286.0910, found 286.0918.



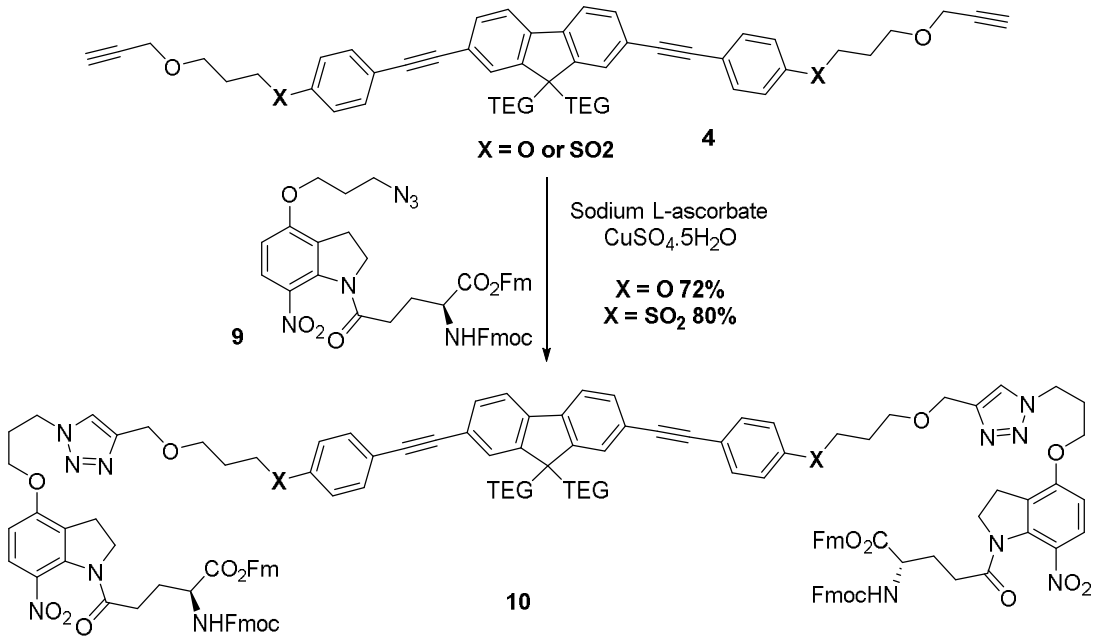
Few milligramms of **7-NAc** were obtained pur for photophysical data. ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.72 (d, *J* = 9.0 Hz, 1H), 6.62 (d, *J* = 9.0 Hz, 1H), 4.10-4.425 (m, 4H), 3.51 (t, *J* = 6.5 Hz, 2H), 3.09 (t, *J* = 8.1 Hz, 2H), 2.24 (s, 3H), 2.01-2.16 (m, 2H).

Fmoc-Glu(4-(3-azidopropoxy)-7-nitroindolinyl)-OFm (9)

Into a Schlenk tube, to a solution of Fmoc-Glu(OH)-OFm[4] **8** (438 mg, 0.8 mmol), in dry THF (8 mL), was added oxalyl chloride (freshly distilled, 69 µL, 0.8 mmol) followed by a drop of dry DMF. The resulting mixture was stirred 1 h at room temperature and concentrated under high-vacuum (on Dual bank manifolds). The white residue was dissolved with dry THF (4 mL), then **7** (105.3 mg, 0.4 mmol) and Na_2CO_3 (a little spoon) were added. The reaction mixture was stirred overnight at room temperature, quenched with a saturated aqueous NH_4Cl and then extracted with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The crude was purified on silica gel (Petroleum ether/EtOAc, 3:2 as eluent) to give **9** (180 mg, 57%) as a yellowish powder: mp 80 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.72-7.79 (m, 4H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.55-7.64 (m, 4H), 7.30-7.42 (m, 8H), 6.55 (d, *J* = 8.9 Hz, 1H), 5.66 (d, *J* = 7.7 Hz, 1H), 4.31-4.53 (m, 5H), 4.01-4.26 (m, 6H), 3.47 (t, *J* = 6.5 Hz, 2H), 3.04 (d, *J* = 8.0 Hz, 2H), 2.42-2.57 (m, 2H), 2.21-2.36 (m, 1H), 1.96-2.12 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 26.3, 27.4, 28.6, 31.6, 46.9, 47.2, 48.1, 50.0, 53.8, 65.5, 67.3, 67.4, 107.1, 120.0, 120.2, 122.9, 125.2, 125.3, 125.6, 127.2, 127.3, 127.4, 127.8, 128.0, 135.5, 136.7, 141.4, 141.5, 143.5, 143.6, 143.9, 144.0, 156.3, 157.8, 170.5, 172.0; ESIHMRS: $C_{45}H_{40}N_6O_8$ calculated for $[M+Na]^+$: 815.2799, found 815.2805.

E. Synthesis of Nl-Glu 10

Synthetic route



General procedure

To a solution of quadrupôle **4a** or **4b** (1.0 equiv) and **9** (2.5 equiv), in DMSO, was added sodium L-ascorbate (0.5 equiv) and copper sulfate (0.25 equiv). The reaction mixture was stirred overnight at room temperature, quenched with water and then concentrated by freeze-drying. The residue was diluted in CH_2Cl_2, dried (Na_2SO_4), filtrated through Celite®, and then the filtrate was concentrated under reduced pressure. The crude was purified on silica gel (EtOAc/MeCN, 4:1 for **4a** and 3:2 for **4b**, as eluent) to give expected product.

- (10a): 72%, yellow powder, mp 102 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.72-7.79 (m, 8H), 7.55-7.66 (m, 14H), 7.44-7.52 (m, 8H), 7.35-7.41 (m, 8H), 7.26-7.33 (m, 14H), 6.87 (d, *J* = 8.8 Hz, 4H), 6.48 (d, *J* = 8.9 Hz, 2H), 5.67 (d, *J* = 7.8 Hz, 2H), 4.65 (s, 4H), 4.41-4.47 (m, 10H), 4.31-4.36 (m, 4H), 4.11-4.24 (m, 8H), 3.96-4.09 (m, 8H), 3.72 (t, *J* = 6.1 Hz, 4H), 3.48-3.53 (m, 4H), 3.44-3.47 (m, 4H), 3.36-3.40 (m, 4H), 3.31 (s, 6H), 3.17-3.21 (m, 4H), 3.02-3.06 (m, 4H), 2.75-2.79 (m, 4H), 2.43-2.54 (m, 4H), 2.24-2.42 (m, 6H), 2.03-2.10 (m, 4H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 26.4, 27.4, 29.7, 29.8, 31.7, 39.8, 46.9, 47.0, 47.2, 50.0, 53.8, 59.1, 64.8, 65.2, 67.0, 67.2, 67.3, 67.4, 70.2, 70.6, 72.0, 89.0, 90.3, 107.1, 114.7, 115.5, 120.1, 120.2, 122.5, 122.7, 123.0, 125.2, 125.3, 125.4, 125.6, 126.3, 127.3, 127.3, 127.4, 127.8, 128.0, 131.1, 133.2, 135.6, 136.8, 139.8, 141.4, 141.5, 143.5, 143.6, 143.9, 144.0, 145.7, 149.5, 156.4, 157.6, 159.2, 170.5, 172.0; MALDI-MS: $C_{145}H_{142}N_{12}O_{26}$ calculated for [M]+: 2468.0, found 2468.0.

- (10b): 80%, yellow powder, mp 115 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 7.88 (d, *J* = 8.3 Hz, 4H), 7.65 (m, 16H), 7.54-7.63 (m, 12H), 7.52 (s, 2H), 7.35-7.42 (m, 8H), 7.25-7.32 (m, 8H), 6.51 (d, *J* = 9.0 Hz, 2H), 5.69 (d, *J* = 7.9 Hz, 2H), 4.58 (s, 4H), 4.39-4.55 (m, 10H), 4.32 (d, *J* = 7.2 Hz, 4H), 4.11-4.24 (m, 8H), 3.97-4.07 (m, 4H), 3.60 (t, *J* = 5.9 Hz, 4H), 3.43-3.51 (m, 8H), 3.38 (dd, *J* = 4.9 Hz, *J* = 4.7 Hz, 4H), 3.30 (s, 6H), 3.18-3.23 (m, 8H), 3.05 (t, *J* = 8.0 Hz, 4H), 2.36-2.55 (m, 12H), 2.24-2.32 (m, 2H), 1.98-2.10 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 23.4, 26.4, 27.4, 29.8, 31.7, 39.7, 46.9, 47.1, 47.2, 50.0, 51.6, 53.4, 53.8, 59.1, 64.4, 65.1, 67.0, 67.3, 67.4, 68.0, 70.2, 70.6, 72.0, 88.7, 94.2, 107.1, 120.1, 120.2, 120.5, 121.6, 122.8, 123.0, 125.2, 125.4, 125.6, 126.9, 127.3, 127.4, 127.8, 128.0, 128.2, 129.3, 131.6, 132.3, 135.6, 136.8, 138.2, 140.7, 141.3, 141.4, 141.5, 143.5, 143.6, 143.9, 144.0, 145.2, 149.8, 156.4, 157.6, 170.5, 172.0; MALDI-MS: $C_{145}H_{142}N_{12}O_{28}S_2$ calculated for $[M+Na]^+$: 2586.9, found 2586.9.

F. Photophysical 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 spectrofluorometer. Emission spectra were obtained, for each compound, under excitation at the wavelength of the absorption maximum. Fluorescence quantum yields were measured according to literature procedures.[5]

G. Two-photon absorption

2PA cross sections (σ_2) were determined from the two-photon excited fluorescence (TPEF) cross sections ($\sigma_2\Phi$) and the fluorescence emission quantum yield (Φ). TPEF cross sections of 10^{-4} M solutions were measured relative to fluorescein in 0.01M aqueous NaOH for 700-800 nm,[6] using the well-established method described by Xu and Webb[6b] and the appropriate solvent-related refractive index corrections.[7] Reference values between 700 and 715 nm for fluorescein were taken from literature.[8] 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. This allows avoiding excited-state absorption during the pulse duration, a phenomenon which has been shown to lead to overestimated 2PA cross-section values. To span the 700-800 nm range, a Nd:YLF-pumped Ti:sapphire oscillator was used generating 150 fs pulses at a 76 MHz rate. 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.

H. Two-photon photolysis experiments

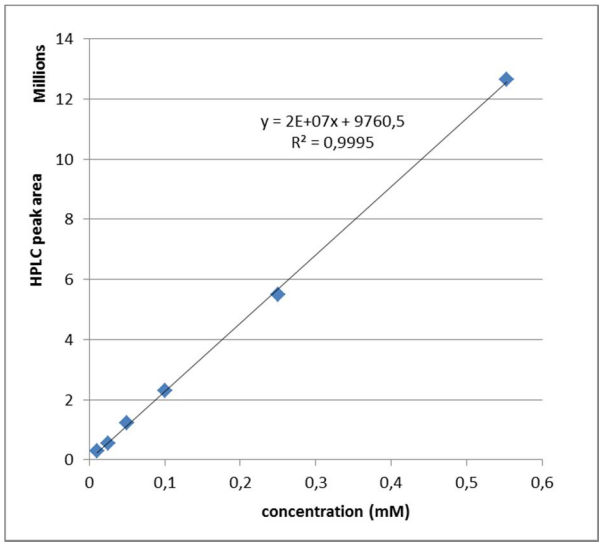
A solution of compound **10b** (2.56 mg, 1 µmol) with naproxen used as a reference (0.24 mg, 1 µmol), in 2.2 mL of a mixture 10:1 CH_3CN/water, was irradiated under slight stirring using a Nd:YLF-pumped Ti:sapphire oscillator (Coherent) focused into the cuvette. Beam parameters were 740 nm with 150 fs pulse width at 76 MHz repetition rate and 220 mW average power. Between each interval, a small aliquot (0.5 mL) of the solution was removed and the conversion of the product was assayed by reversed-phase HPLC/MS monitoring (elution with a gradient mixture of acetonitrile and water using absorbance detection at 254 nm). The ratio was estimated by analysis of HPLC chromatogram. Mass completed the analysis to confirm the authenticity of the products.

I. Quantitative two-photon photosensitivity measurements

Uncaging two photon cross-sections (δ_u) were calculated from the fractional conversion of the cage with exposures of approx. 4 hours in a 50 microlitre cuvette of 3 mm pathlength containing a solution of compound **10b** (concentration of 1 mM) in a mixture 9:1 CH_3CN/water. The expanded output of a MaiTai BB (Spectra-Physics) pulsed laser was focused with a 50 mm focal length lens into the cuvette. The two-photon excitation volume was entirely contained within the cuvette volume to obviate the need to measure the beam waist. Beam parameters were 730 nm with 1 ps pulse width at 80 MHz and 125 mW average power after the cuvette. Samples were centrifuged if necessary to remove particles if apparent in the transmitted beam. The conversion of the product was assayed by HPLC by monitoring the released Fmoc-Glu(OH)-OFm which concentration was determined by means of a calibration graph. The two-photon uncaging cross section δ_u was calculated as follows:

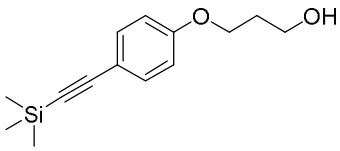
$$C/C_0 = 0.5 \,.\, \delta_u \,.\, (P.\, \lambda / hc / F)^2 / T \,.\, (\pi \,.\, n / 4\lambda / V) \,.t.\, F$$

where C/C_0 = fractional conversion; δ_u = TP uncaging cross-section (cm^4.s/photon); P = average power (W); λ= wavelength (cm); h= Plancks constant; c=velocity of light; F=pulse frequency (Hz) ; T= pulse width (s); n= refractive index; V= sample volume (cm^3); t = exposure time (s).

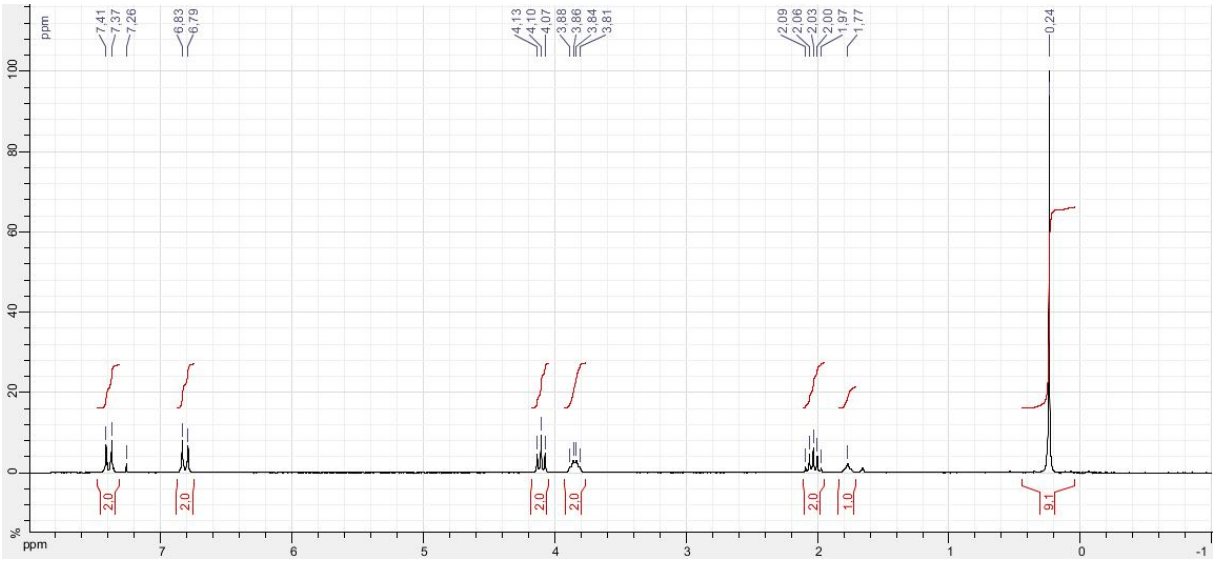


Calibration graph for the determination of the released Fmoc-Glu(OH)-OFm concentration

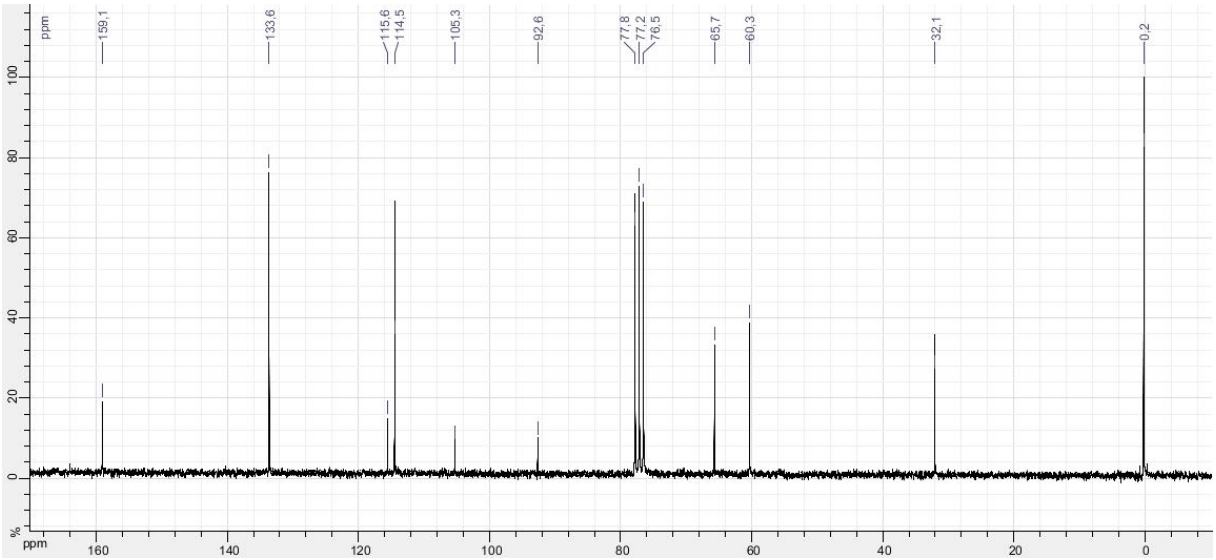
J. NMR spectra:

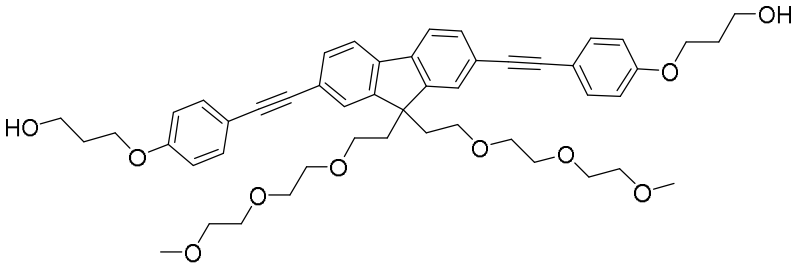


- ^{1}H NMR (200 MHz, $CDCl_3$)

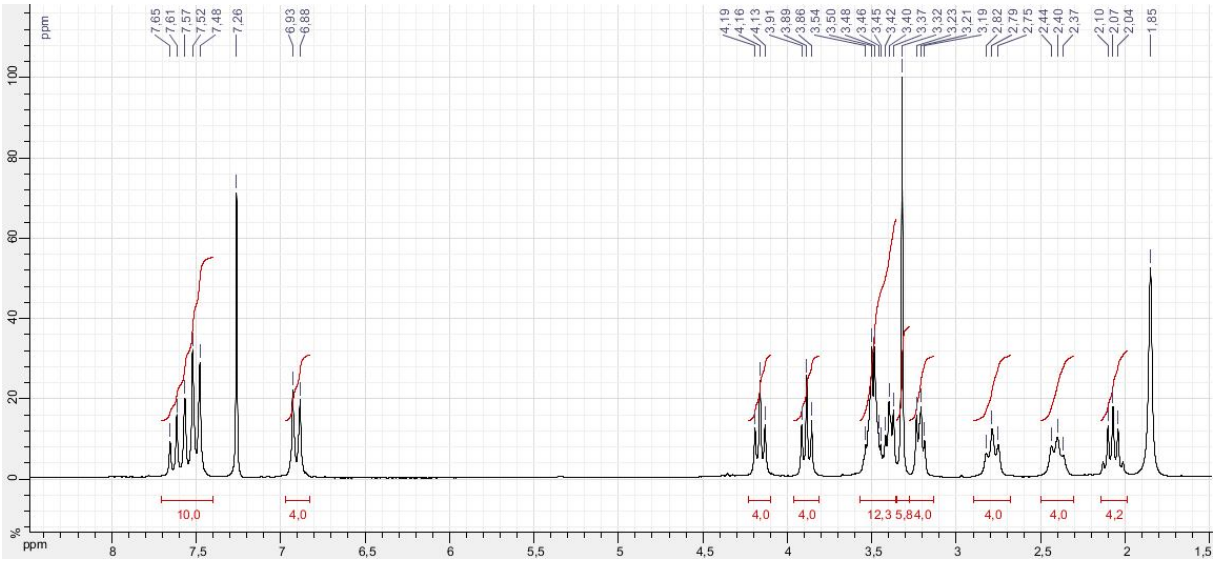


- ^{13}C NMR (50 MHz, $CDCl_3$)

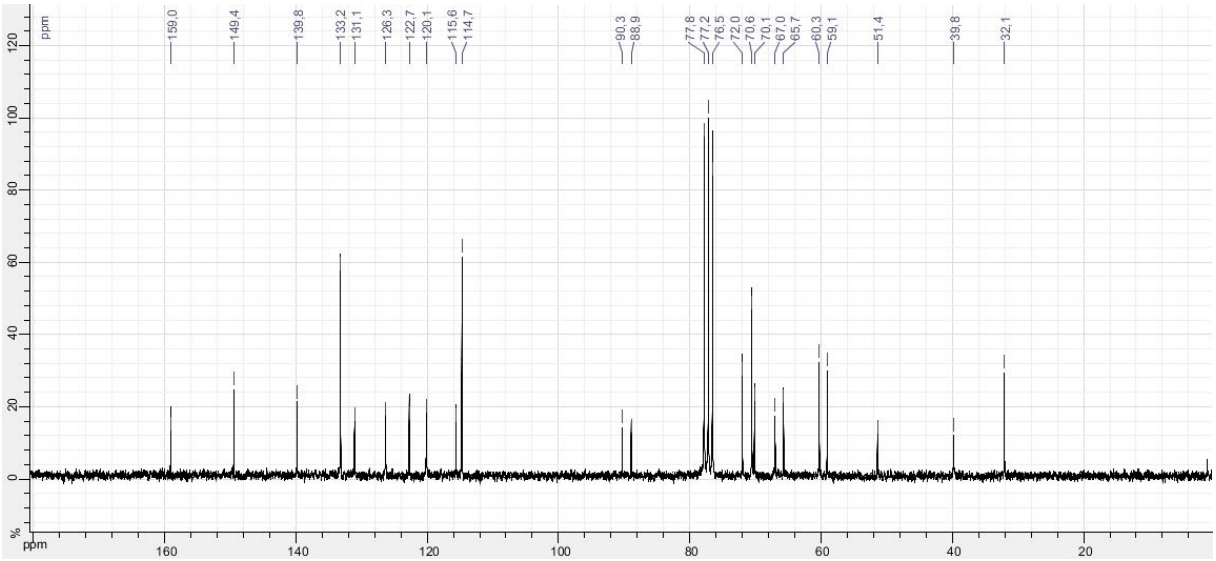


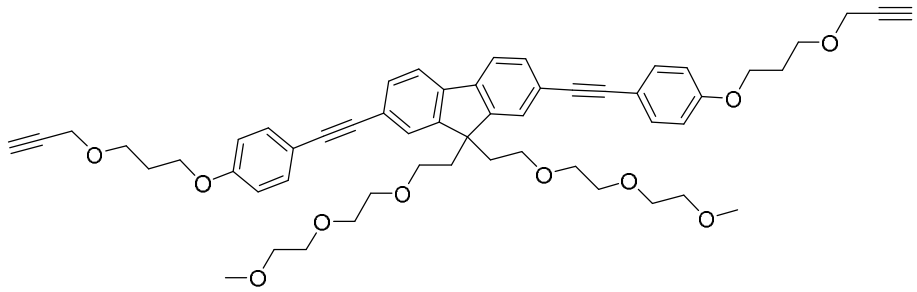


- ^{1}H NMR (200 MHz, $CDCl_3$)

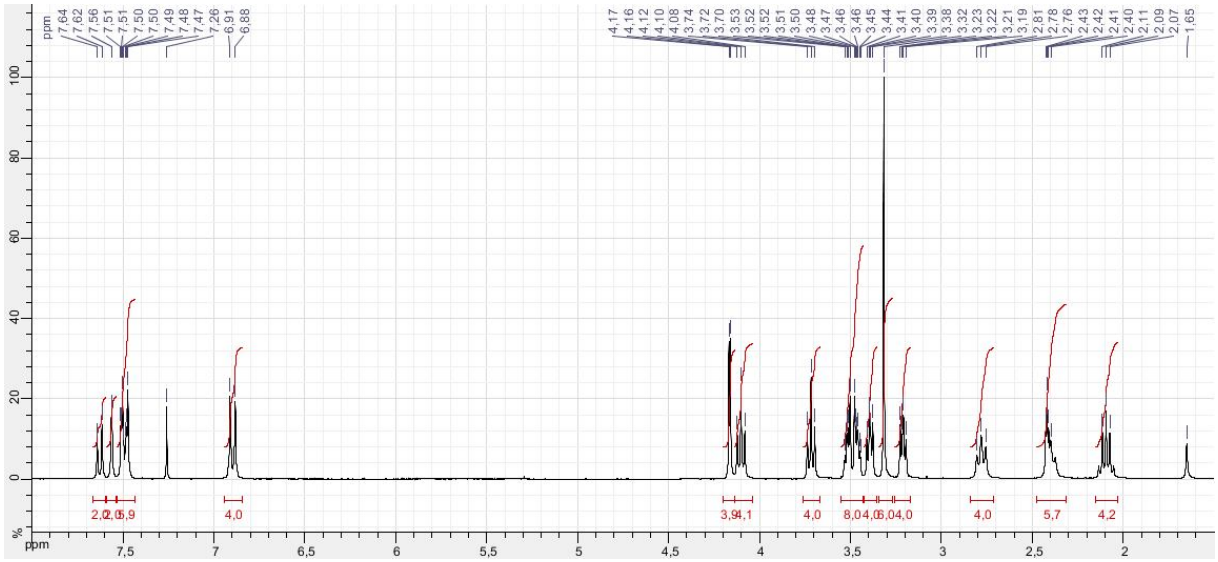


- ^{13}C NMR (50 MHz, $CDCl_3$)

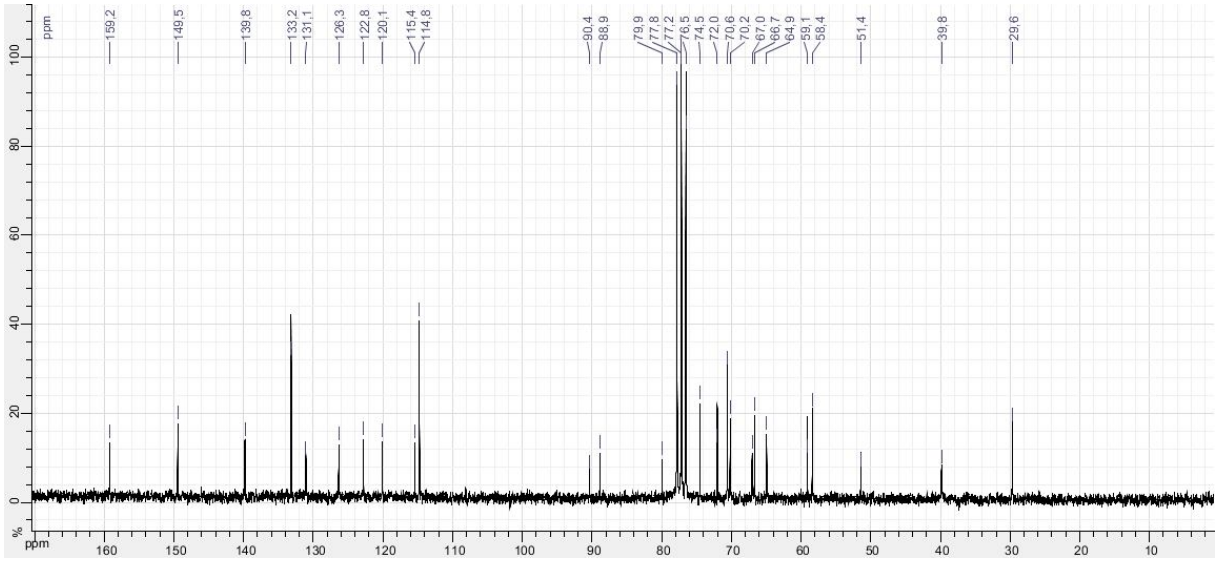


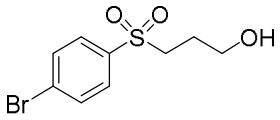


- ^{1}H NMR (300 MHz, $CDCl_3$)

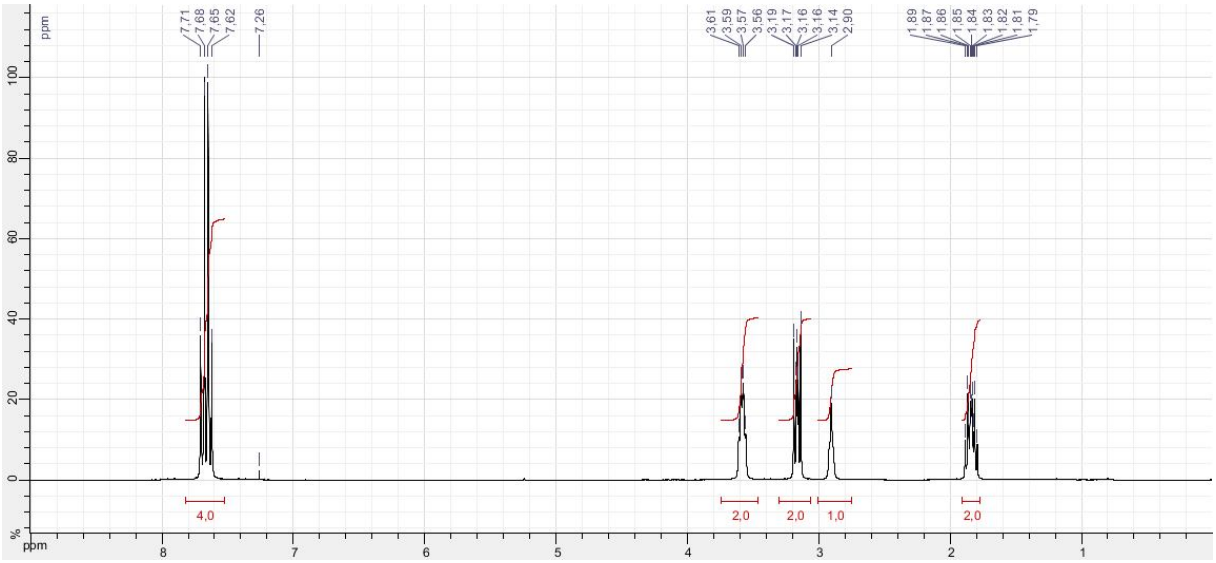


- ^{13}C NMR (50 MHz, $CDCl_3$)

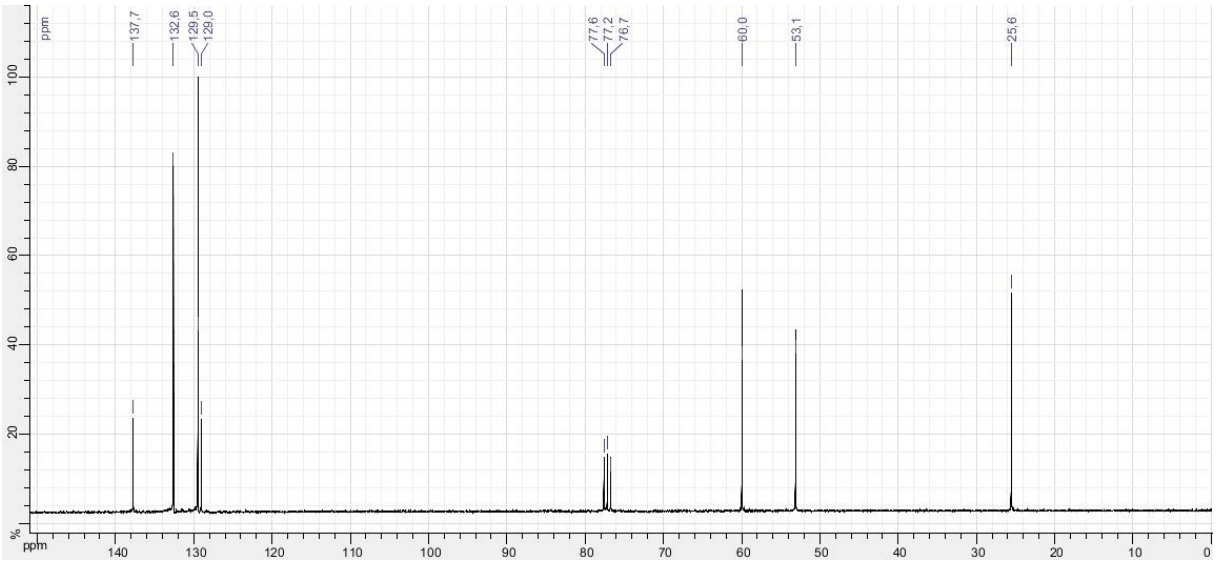


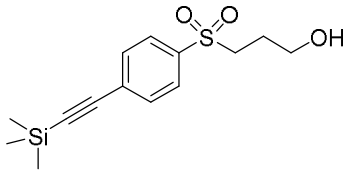


- ^{1}H NMR (300 MHz, $CDCl_3$)

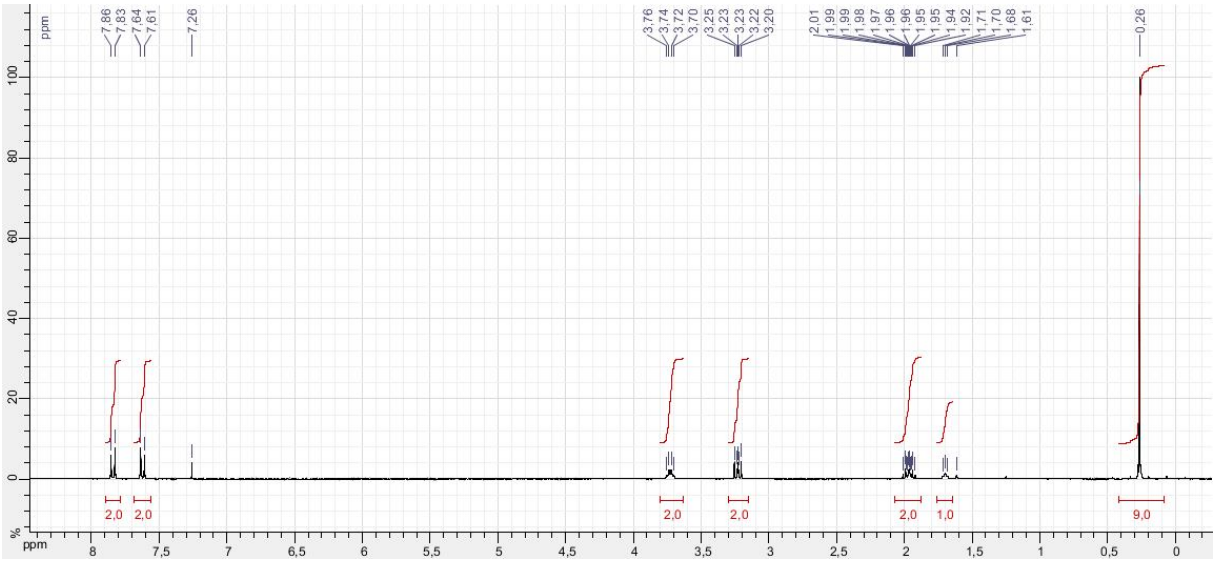


- ^{13}C NMR (75 MHz, $CDCl_3$)

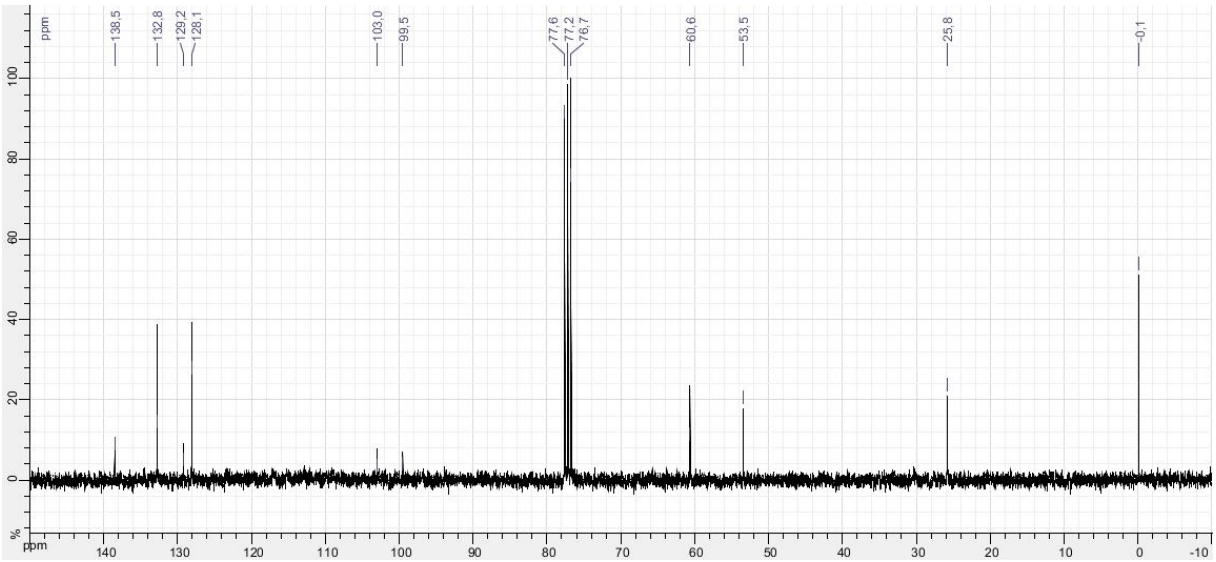


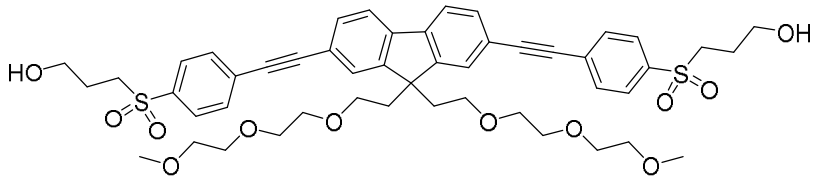


- ^{1}H NMR (300 MHz, $CDCl_3$)

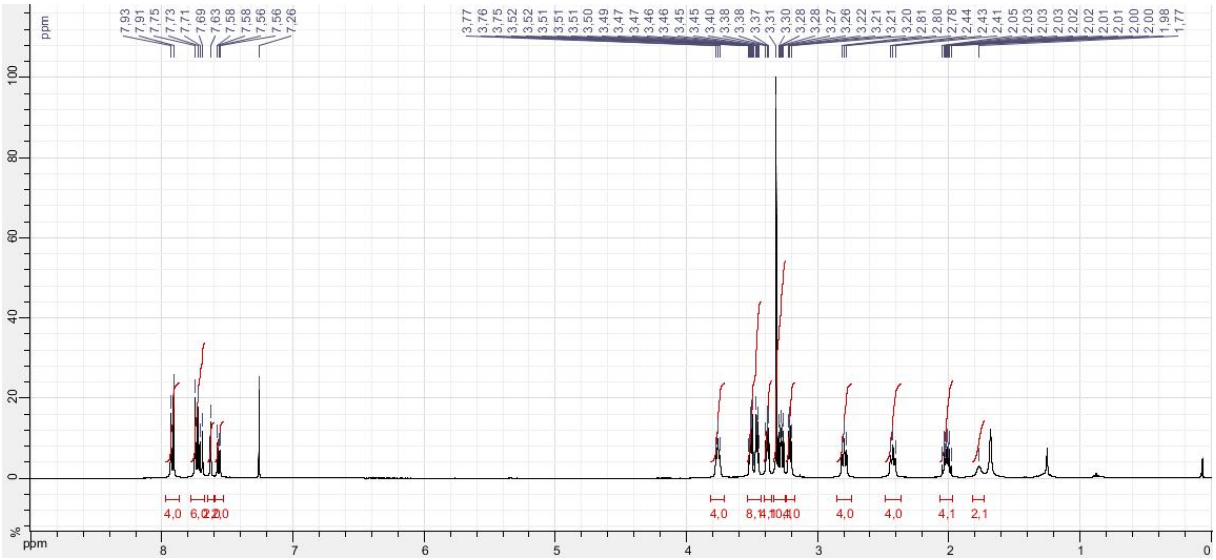


- ^{13}C NMR (75 MHz, $CDCl_3$)

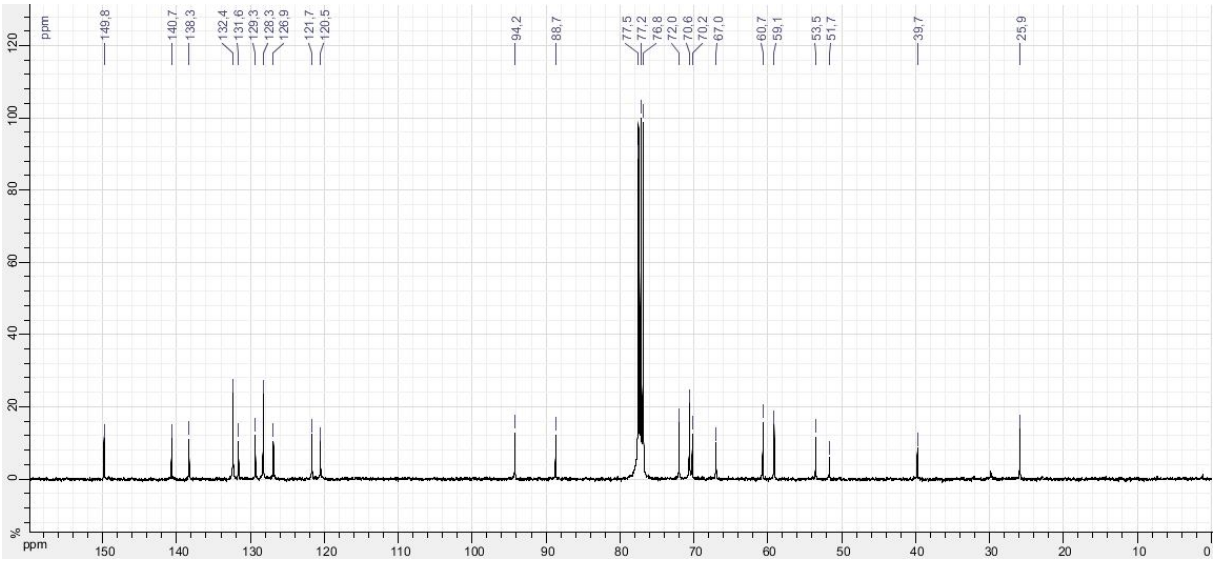


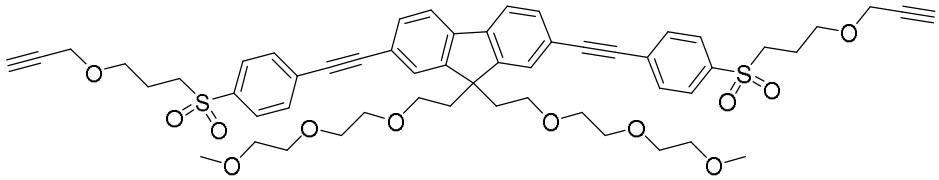


- ^{1}H NMR (400 MHz, $CDCl_3$)

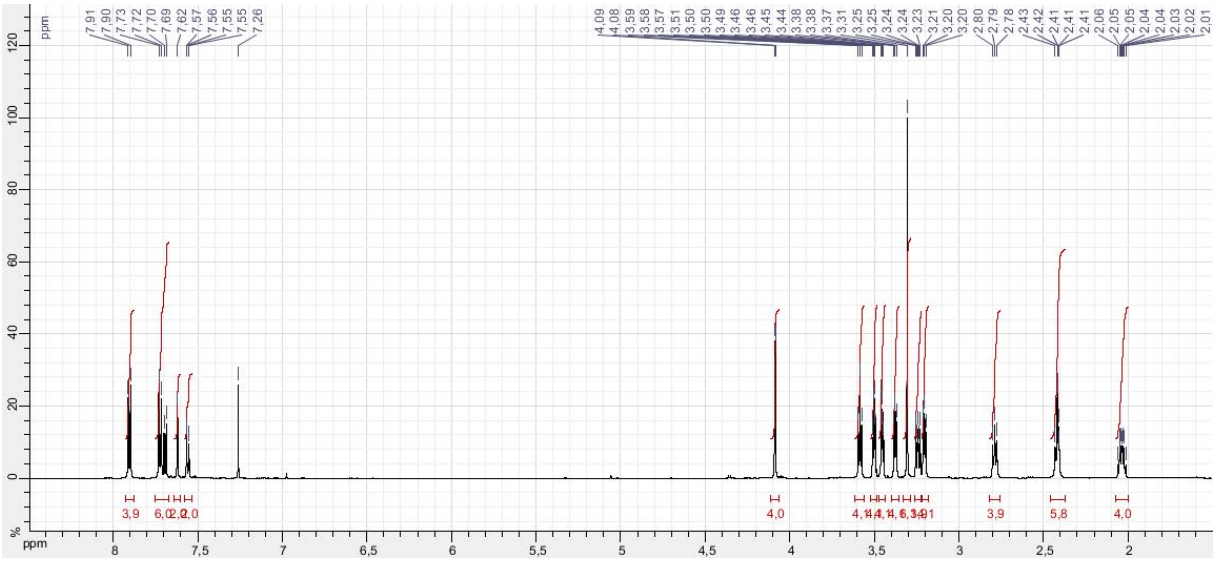


- ^{13}C NMR (100 MHz, $CDCl_3$)

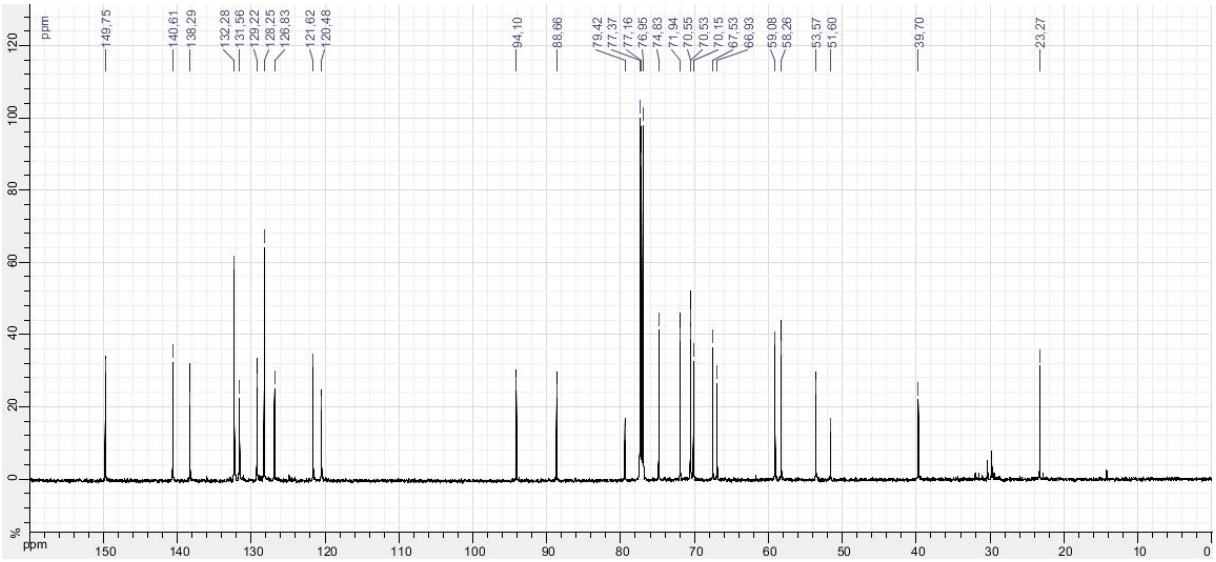


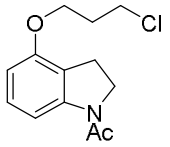


- ^{1}H NMR (600 MHz, $CDCl_3$)

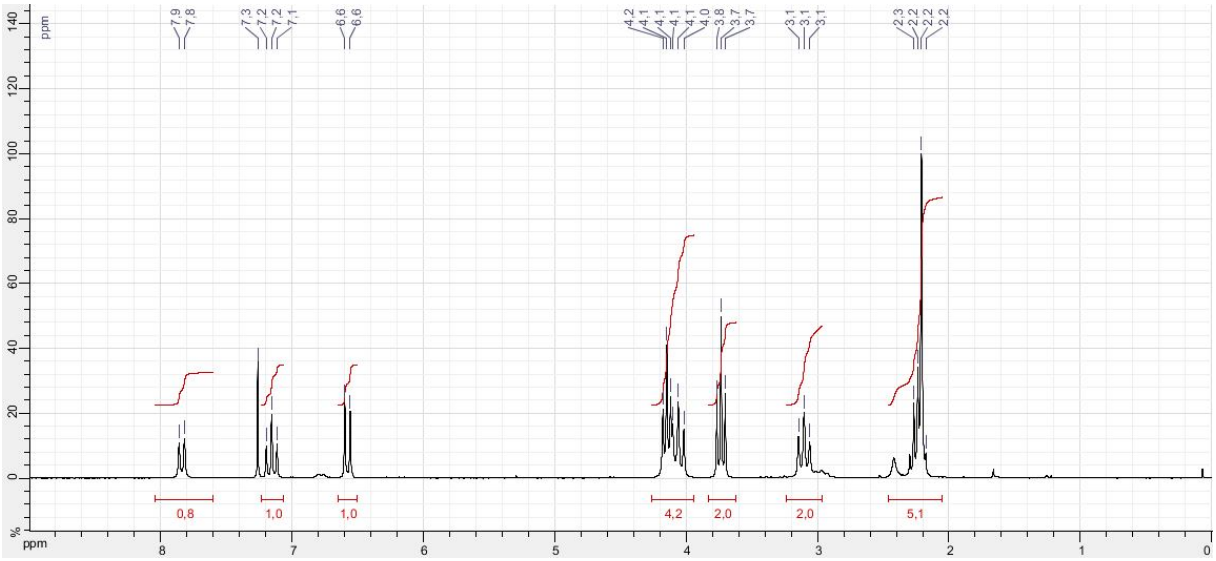


- ^{13}C NMR (150 MHz, $CDCl_3$)

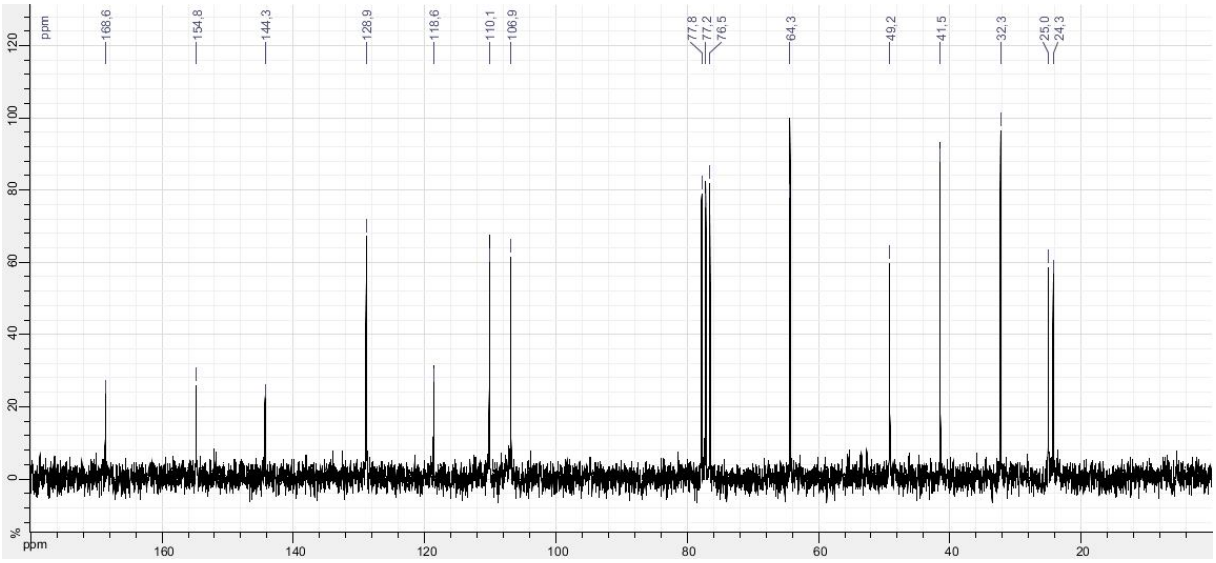


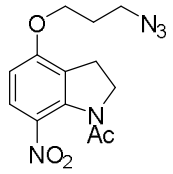


- ^{1}H NMR (200 MHz, $CDCl_3$)

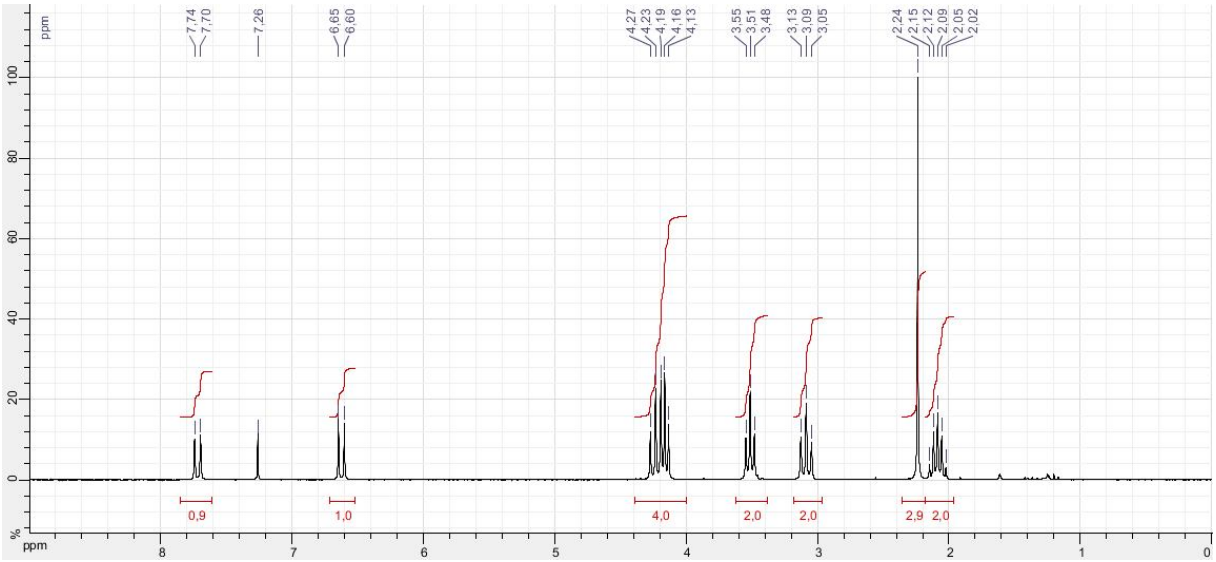


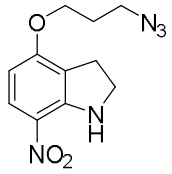
- ^{13}C NMR (50 MHz, $CDCl_3$)



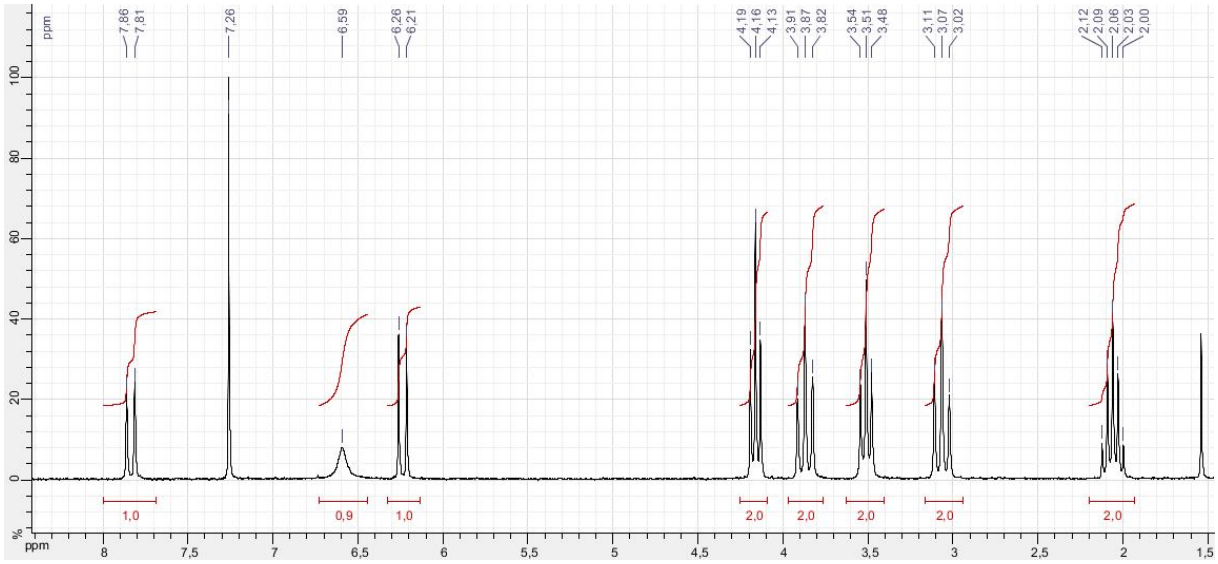


- ^{1}H NMR (200 MHz, $CDCl_3$)

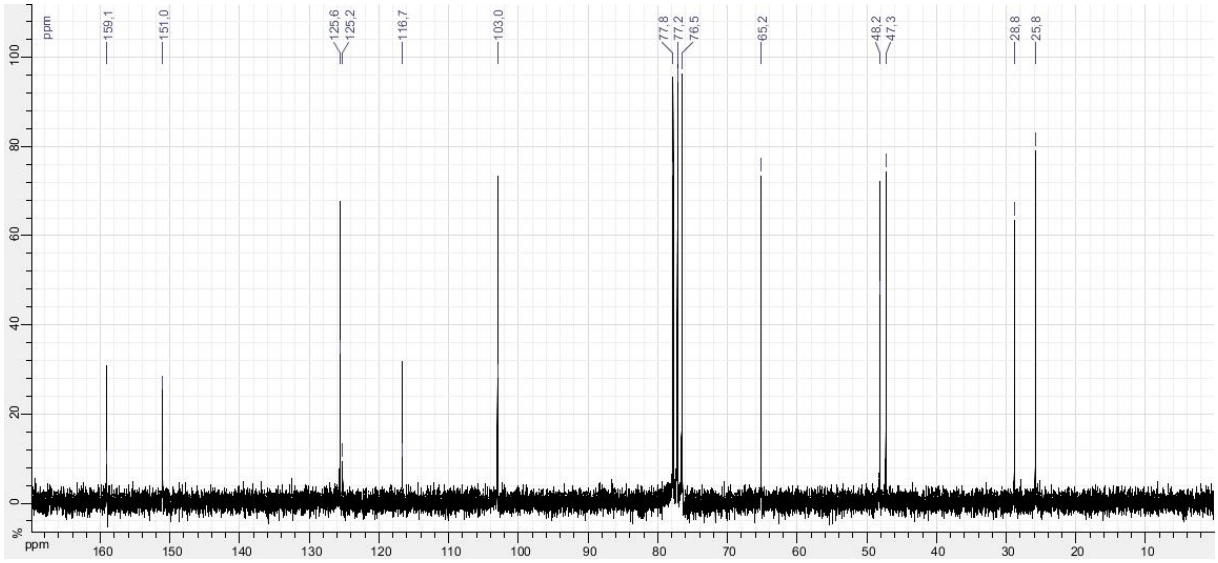


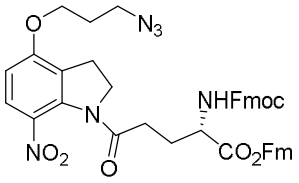


- ^{1}H NMR (200 MHz, $CDCl_3$)

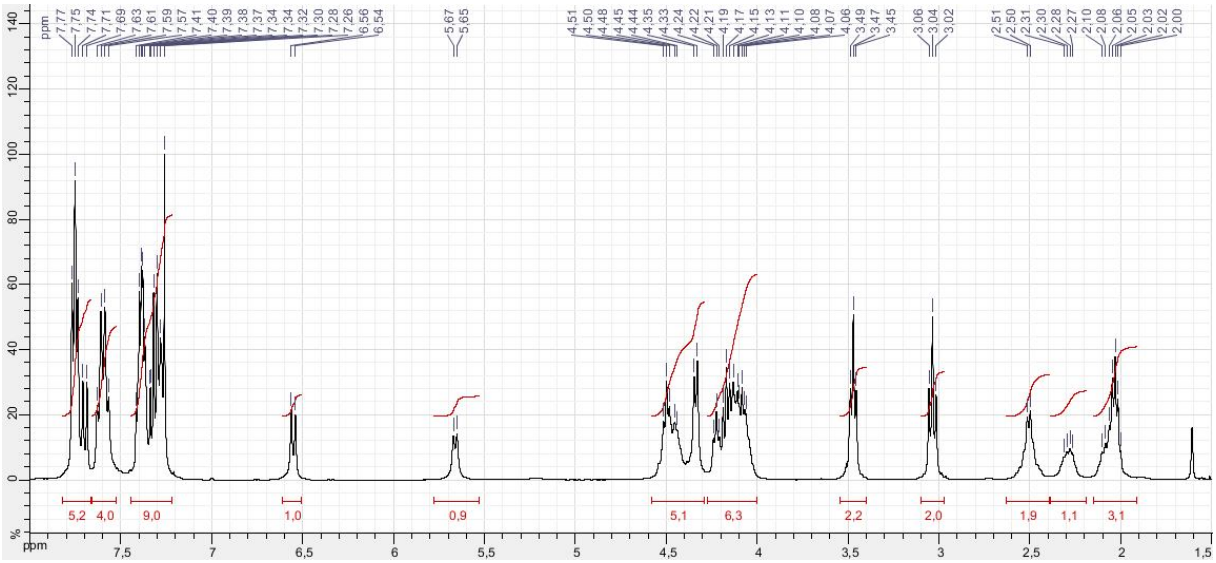


- ^{13}C NMR (50 MHz, $CDCl_3$)

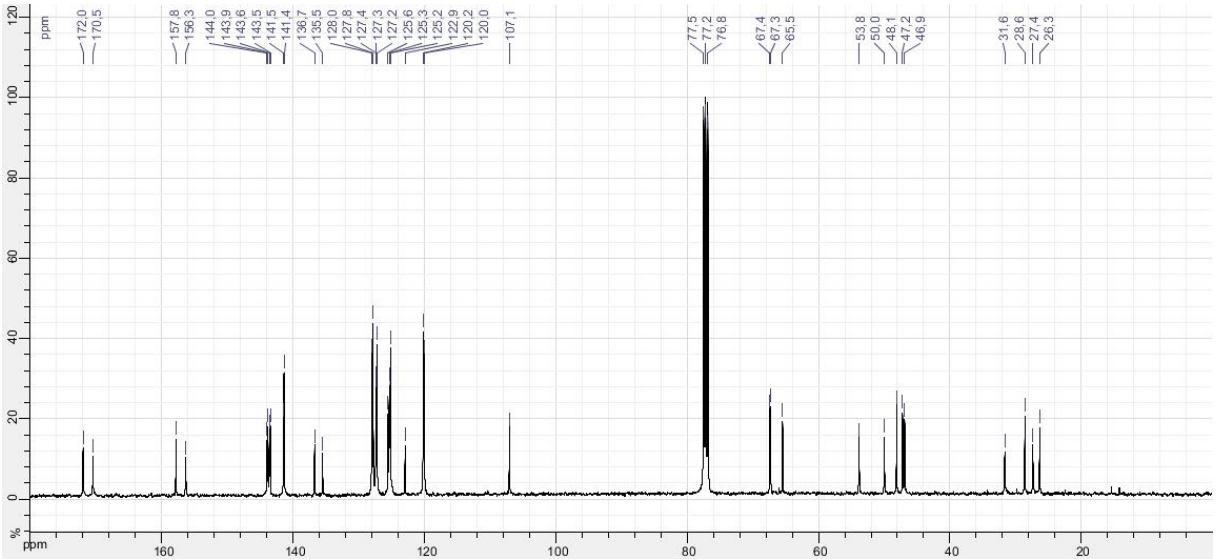


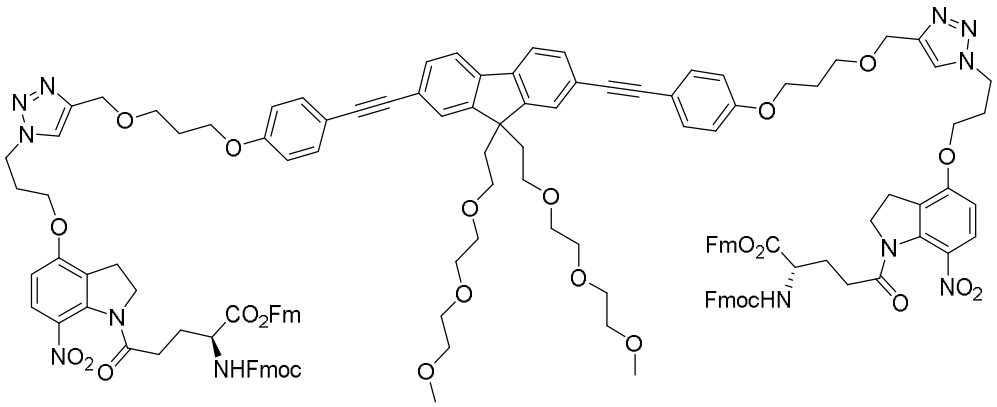


- ^{1}H NMR (400 MHz, $CDCl_3$)

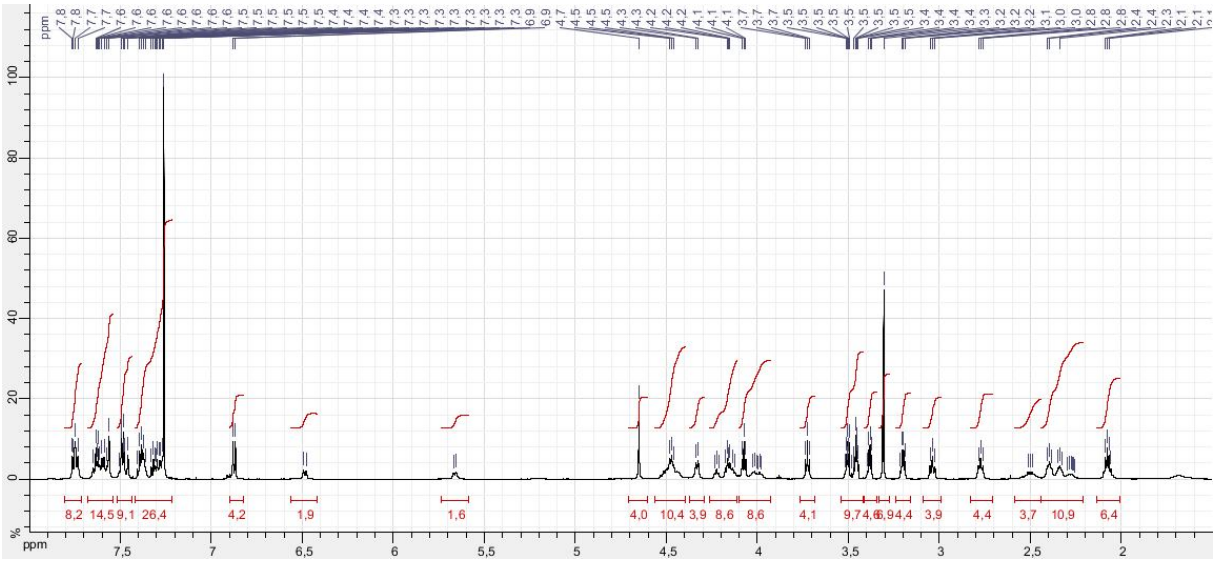


- ^{13}C NMR (100 MHz, $CDCl_3$)

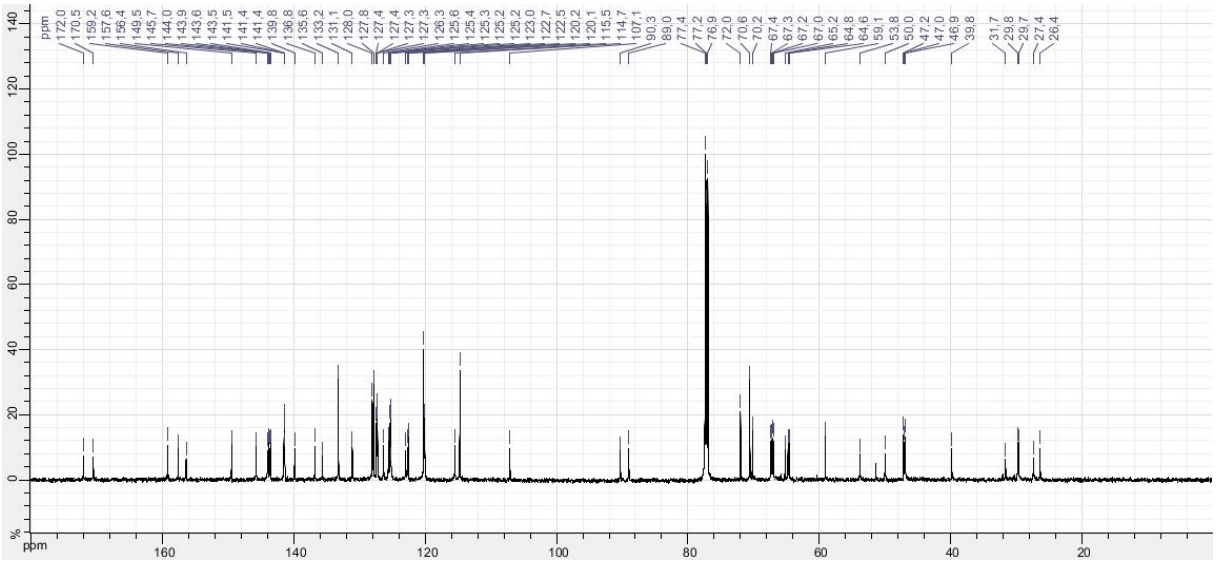


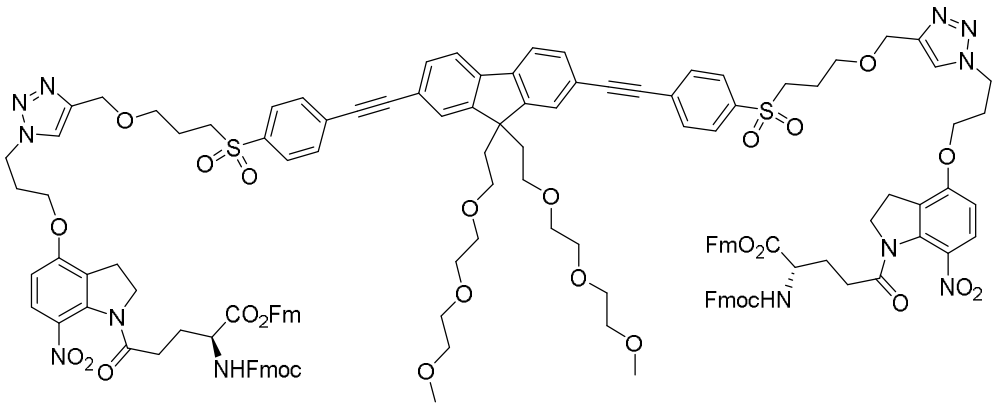


- ^{1}H NMR (600 MHz, $CDCl_3$)

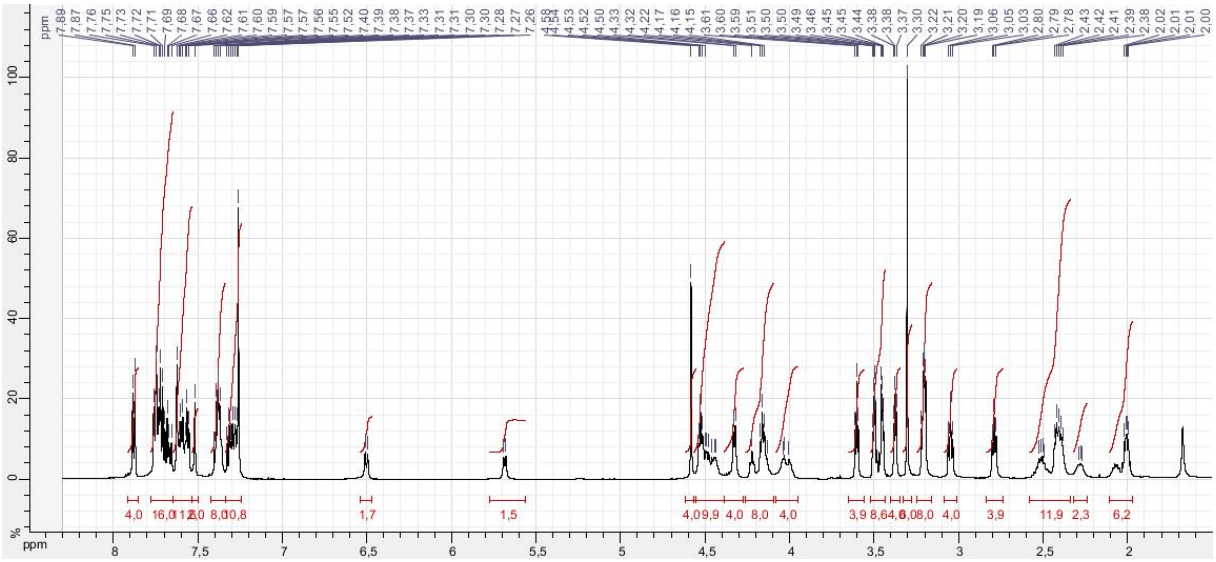


- ^{13}C NMR (150 MHz, $CDCl_3$)

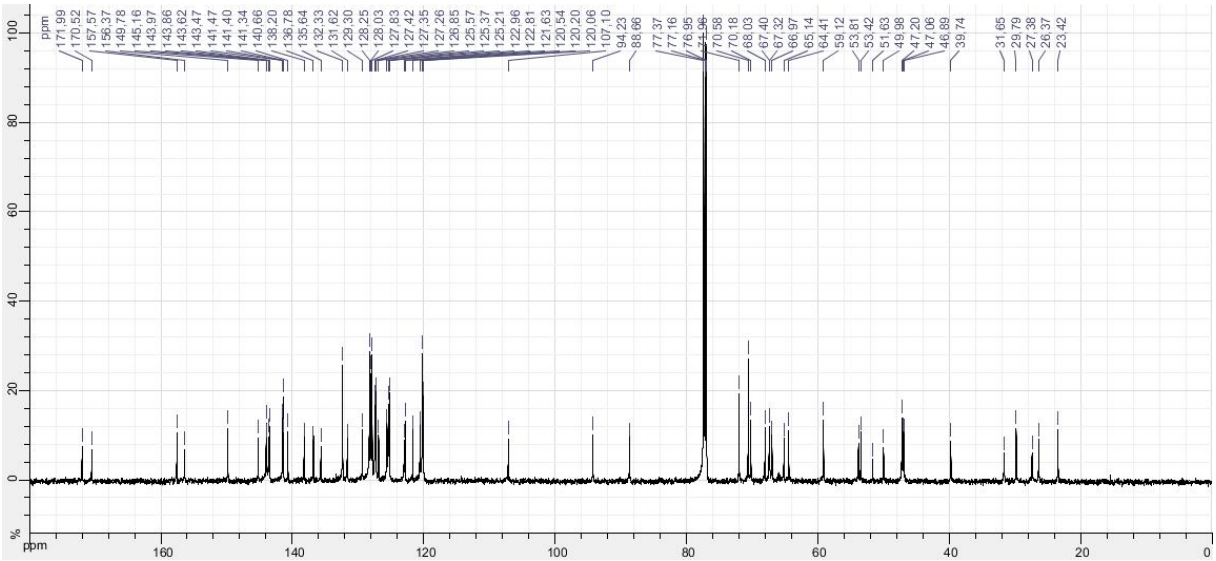




- ^{1}H NMR (600 MHz, $CDCl_3$)



- ^{13}C NMR (150 MHz, $CDCl_3$)



K. References:

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