Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Visible Light-Activatable Q-dye Molecular Beacons for Long-Term mRNA Monitoring in Neurons

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Experimental procedures

1.1. General

Milli-Q water was treated overnight with 0.1% DEPC. It was autoclaved before usage. All reagents were obtained from commercial sources and used without further purification. Reactions were performed under an argon atmosphere. For column chromatography technical grade solvents were used.

1.2. Chemical Synthesis:

The syntheses were made in a modified form following Weinrich *et al.*[1] and Weyel *et al.*[2].

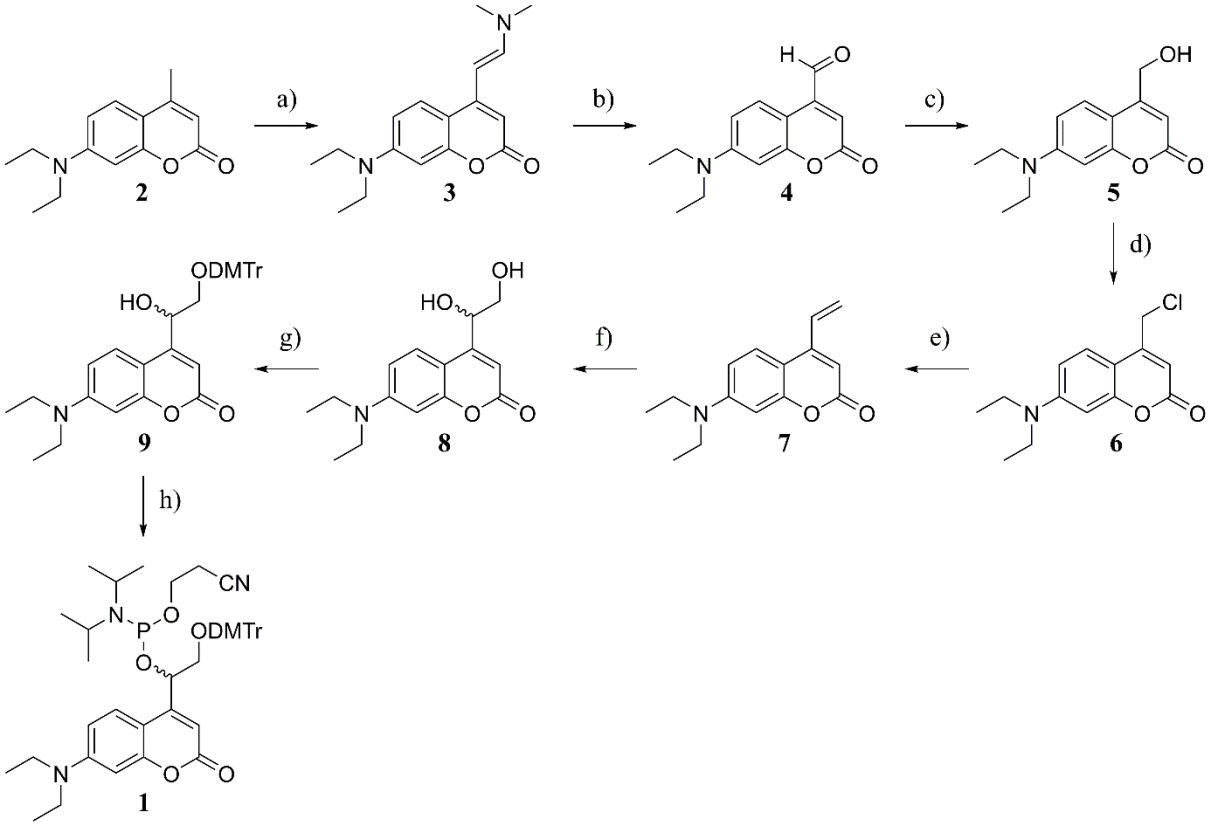

Figure S1: Chemical synthesis of phosphoroamidite **1**. a) N,N-Dimethylformamide-dimethylacetal, DMF, 94%. b) $NaIO_4$, THF/H_2O, 97%. c) $NaBH_4$, EtOH, 64%. d) 4-Toluenesulfonyl chloride, DIPEA, DCM, 81%. e) (i) Triphenylphosphine, MeCN. (ii) Formaldehyde, H_2O, 65%. f) OsO_4, NMO, acetone, 59%. g) 4,4'-dimethoxytrityl chloride, DIPEA, THF, 72%. h) 2-Cyanoethoxy-N,N-diisopropylaminochlorophosphine, DIPEA, DCM, 95%.

7-(Diethylamino)-4-(2-(dimethylamino)vinyl)-2*H*-chromen-2-on (**3**):

Compound **2** (50.0 g, 216.2 mmol, 1 eq) was dissolved in 400 mL dry DMF. N,N-Dimethylformamide-dimethylacetal (38.6 g, 43.1 mL, 324.3 mmol, 1.5 eq) was added under continuous stirring. The mixture was heated to 160 °C for 8.5 h under reflux and subsequently stirred overnight at room temperature. The precipitated yellow solid was filtered. The filtrate was added to a large volume of ice water, precipitating a yellow-brown solid. The solid fractions were combined, washed several times with distilled water and dried in vacuo.

Yield: 58.2 g (94%)

R_f (DCM 9:1 MeOH): 0.88

^{1}H NMR (400 MHz, DMSO-d_6):

δ = 7.72 – 7.66 (m, 2H), 6.59 (dd, 1H, J = 9.0 Hz, 2.5 Hz), 6.40 (d, 1H, J = 9,0 Hz), 5.83 (s, 1H), 5.28 (d, 1H, J = 13.0 Hz), 3.39 (q, 4H, J = 6.8 Hz), 3.00 (s, 6H), 1.11 (t, 6H, J = 7.0 Hz) ppm.

^{13}C NMR (100.6 MHz, DMSO-d_6):

δ = 161.53, 155.96, 152.19, 149.75, 148.41, 125.48, 107.79, 107.28, 97.01, 90.39, 85.78, 43.84, 12.35 ppm.

MALDI HRMS (m/z): calc. for $C_{17}H_{22}N_2O_2$ $[M+H]^+$ 287.17540, found 287.17537 (Δm = 0.00003, error 0.10 ppm).

7-(Diethylamino)-2-oxo-2H-chromene-4-carbaldehyde (**4**):

Compound **3** (35.57 g, 124.2 mmol, 1 eq) was suspended in 460 mL THF/H_2O (1:1). Under vigorous stirring, $NaIO_4$ (79.70 g, 372.63 mmol, 3 eq) was added and the reaction mixture turned from light yellow to dark red. After stirring for 1.5 h, the mixture was filtered over silica gel and washed with ethyl acetate until the silica gel appeared colorless. THF was removed from the filtrate in vacuo and saturated $NaHCO_3$ solution was added. The phases were separated and the aqueous phase was extracted three times with 400 mL ethyl acetate. After combining the organic phases, they were dried over Na_2SO_4 and the solvent was removed in vacuo. The product was obtained as a red resin.

Yield: 29.86 g (98%)

R_f (cyclohexane 2:1 EtOAc): 0.56

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

δ = 10.03 (s, 1H), 8.31 (d, 1H, J = 9.2 Hz), 6.63 (dd, 1H, J = 9.2 Hz, 2.6 Hz), 6.53 (d, 1H, J = 2.6 Hz), 6.45 (s, 1H), 3.43 (q, 4H, J = 7.1 Hz), 1.22 (t, 6H, J = 7.2 Hz) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$):

δ = 192.69, 162.04, 157.54, 151.18, 144.07, 127.19, 117.49, 109.67, 103.85, 97.76, 44.95, 12.58 ppm.

MALDI HRMS (m/z): calc. for $C_{14}H_{15}NO_3$ $[M+H]^+$ 246.11247, found 246.11241 (Δm = 0.00006, error 0.24 ppm).

7-(Diethylamino)-4-(hydroxymethyl)-2*H*-chromen-2-on (**5**):

Compound **4** (19.87 g, 81.0 mmol, 1 eq) was suspended in 650 mL dry EtOH. $NaBH_4$ (1.53 g, 40.5 mmol, 0.5 eq) was added and the mixture was stirred for 6 hours at room temperature. 1 M HCl was added until no gas formation was observed by further addition. EtOH was removed from the reaction mixture in vacuo and the aqueous phase was extracted three times with ethyl acetate. The organic phases were combined, concentrated to half volume in vacuo, washed two times with H_2O and dried over Na_2SO_4. The solvent was removed and the solid residue was purified by column chromatography with DCM/acetone (20:1 → 10:1). After drying in vacuo, compound **3** was obtained as a yellow-brown solid.

Yield: 13.35 g (67%)

R_f (cyclohexane 1:1 EtOAc): 0.23

^{1}H NMR (400 MHz, DMSO-d_6):

δ = 7.42 (d, 1H, J = 7.5 Hz), 6.65 (dd, 1H, J = 9.0 Hz, 2.6 Hz), 6.52 (d, 1H, J = 2.5 Hz), 6.06 (s, 1H), 5.50 – 5.48 (m, 1H), 4.66 (d, 2H, J = 5.5 Hz), 3.41 (q, 4H, J = 7.0 Hz), 1.11 (t, 6H, J = 7.0 Hz) ppm.

^{13}C NMR (100.6 MHz, DMSO-d_6):

δ = 161.11, 156.81, 155.61, 150.17, 125.05, 108.51, 105.68, 103.91, 96.77, 59.03, 43.92, 12.30 ppm.

MALDI HRMS (m/z): calc. for $C_{14}H_{17}NO_3$ $[M+H]^+$ 248.12812, found 248.12806 (Δm = 0.00006, error 0.24 ppm).

4-(Chloromethyl)-7-(diethylamino)-2*H*-chromen-2-on (**6**):

Compound **5** (3.0 g, 12.1 mmol, 1 eq) and DIPEA (2.7 ml, 15.8 mmol, 1.3 eq) were dissolved in 80 ml dry DCM. 4-Toluenesulfonyl chloride (6.94 g, 36.39 mmol, 3 eq) was added and the reaction mixture

was stirred for 72 h at room temperature. 200 ml water was added and the mixture was extracted with DCM two times. The organic phase was adsorbed on silica gel and the crude product was purified by column chromatography with cyclohexane/ethyl acetate (4:1 → 2:1).

Yield: 2.60 g (81%)

R_f (cyclohexane 2:1 EtOAc): 0.54

^{1}H NMR (600 MHz, DMSO-*d_6*):

δ = 7.57 (d, 1H, J= 9.1 Hz), 6.73 (dd, 1H, J = 9.0 Hz, 2.6 Hz), 6.54 (d, 1H, J = 2.6 Hz), 6.20 (s, 1H), 4.90 (s, 2H), 3.43 (q, 4H, J = 7.0 Hz), 1.12 (t, 6H, J = 7.0 Hz) ppm.

^{13}C NMR (162 MHz, DMSO-*d_6*):

δ = 160.6, 156.1, 151.0, 150.5, 126.0, 108.7, 108.0, 105.5, 96.9, 44.0, 41.4, 12.3 ppm.

MALDI HRMS (m/z): calc. for $C_{14}H_{16}NO_3Cl$ $[M+H]^+$ 266.09423, found 266.09415 (Δm = 0.00008, error 0.30 ppm).

7-(Diethylamino)-4-vinyl-2*H*-chromen-2-on (**7**):

Compound **6** (2.61 g, 9.84 mmol, 1 eq) and triphenylphosphine (7.74 g, 29.51mmol, 3 eq) were dissolved in 55 ml dry acetonitrile. The solution was heated to 120 °C for 14.5 h under reflux. During the reaction, a yellow solid precipitated. The cooled reaction mixture was filtered and the solid was washed with 300 mL of hot benzene. After drying in vacuo the intermediate was dissolved in 37% formaldehyde solution (55 ml, 743.7 mmol) and stirred for 15 minutes at room temperature. A 15% Na_2CO_3 solution (7 ml) was carefully added, after which the solution turned orange and became turbid. 100 mL DCM was added and the phases were separated. The aqueous phase was extracted with 100 mL DCM. After combining the organic phases the solvent was removed and the crude product was purified *via* column chromatography using cyclohexane: ethyl acetate (6:1).

Yield: 1.56 g (65%)

R_f (cyclohexane 4:1 EtOAc): 0.39

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

δ = 7.45 (d, 1H, J= 9,0 Hz), 6.93 (dd, 1H, J = 17.3 Hz, 11.0 Hz), 6.58 (dd, 1H, J = 9.0 Hz, 2.6 Hz), 6.51 (d, 1H, J = 2.6 Hz), 6.12 (s, 1H), 5.94 (d, 1H, J = 17.3 Hz), 5.63 (d, 1H, J = 11.0 Hz), 3.41 (q, 4H, J = 7.1 Hz), 1.20 (t, 6H, J = 7.1 Hz) ppm.

^{13}C NMR (162 MHz, $CDCl_3$):

δ = 162.5, 156.5, 151.1, 150.6, 130.8, 125.5, 122.2, 108.5, 104.7, 98.0, 44.7, 12.5 ppm.

7-(Diethylamino)-4-(1,2-dihydroxyethyl)-2*H*-chromen-2-on (**8**):

To a solution of compound **7** (200 mg, 0.82 mmol, 1 eq) in 50 ml acetone was added water (4.11 mmol, 5 eq). Under continuous stirring *N*-methylmorpholine-*N*-oxide (96 mg, 0.82 mmol, 1 eq) and OsO_4 (100 µl, 2.7 M in *tert*-butanol) were added subsequently. The mixture was stirred for 72 h at room temperature. The organic solvent was removed under reduced pressure and the aqueous phase was extracted with ethyl acetate. After removal of the solvent, the crude product was purified *via* flash chromatography (cyclohexane, ethyl acetate).

Yield: 134 mg (59%)

R_f (cyclohexane 1:1 EtOAc): 0.05

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39 (d, 1H, J = 9.0 Hz), 6.56 (dd, 1H, J = 9.0 Hz), 6.51-6.49 (m, 1H), 6.31 (s, 1H), 5.13 (d, 1H, J = 4.2 Hz), 3.98-3.93 (m, 1H), 3.73-3.65 (m, 1H), 3.40 (q, 4H, J = 7.0 Hz), 1.20 (t, 6H, J = 7.0 Hz) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$):

δ = 163.31, 156.35, 155.56, 150.62, 124.92, 109.00, 106.37, 105.83, 97.86, 70.64, 66.65, 44.80, 12.56 ppm.

MALDI HRMS (m/z): calc. for $C_{15}H_{19}NO_4$ $[M+H]^+$ 278.13868, found 278.13863 (Δm = 0.00005, error 0.18 ppm).

4-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)-1-hydroxyethyl)-7-(diethylamino)-2*H*-chromen-2-on (**9**):

Compound **8** (180 mg, 0.65 mmol, 1 eq) was dissolved in 5 ml dry THF and cooled to 0 °C. Subsequently, DIPEA (552 µl, 3.25 mmol, 5 eq) and 4,4'-dimethoxytrityl chloride (264 mg, 0.78 mmol, 1.2 eq) were added in small portions. The mixture was stirred for 12 h at room temperature. 5 ml EtOH was added. After stirring for 10 minutes the solvent was removed under reduced pressure. The crude product was purified by column chromatography using cyclohexane/ethyl acetate (2:1 → 1:1)

Yield: 271 mg (72%)

R_f (cyclohexane 2:1 EtOAc): 0.76

^{1}H NMR (600 MHz, DMSO-d_6):

δ = 7.37 (d, 1H, J = 9.1 Hz), 7.31 (d, 2H, J = 7.4 Hz), 7.23 (t, 2H, J = 7.6 Hz), 7.17 – 7.15 (m, 5H), 6.81 – 6.78 (m, 4H), 6.57 (dd, 1H, J = 9.1 Hz, 2.5 Hz), 6.51 (d, 1H, J = 2.5 Hz), 6.08 (s, 1H), 5.78 (d, 1H, J = 4.8 Hz), 5.07 (q, 1H, J = 5.0 Hz), 3.71 (s, 6H), 3.44 – 3.39 (m, 4H), 3.25 – 3.22 (m, 1H), 3.15 – 3.13 (m, 1H), 1.12 (t, 6H, J = 7.0 Hz) ppm.

^{13}C NMR (162 MHz, DMSO-d_6):

δ = 161.0, 158.0, 157.4, 155.8, 150.0, 144.8, 135.5, 135.4, 129.7, 127.7, 126.6, 126.0, 113.0, 108.4, 106.1, 105.4, 96.8, 85.5, 68.1, 67.6, 55.0, 43.9, 12.3 ppm.

MALDI HRMS (m/z): calc. for $C_{36}H_{37}NO_6$ $[M+H]^+$ 580.26937, found 580.27193 (Δm = 0.00256, error 4.41 ppm).

2-(Bis(4-methoxyphenyl)(phenyl)methoxy)-1-(7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)ethyl(2-cyanoethyl)diisopropylphosphoramidite (**1**):

Compound **9** (300 mg, 0.52 mmol, 1 eq), was dissolved in 12 ml dry DCM under an argon atmosphere. DIPEA (0.54 ml, 401 mg, 3.11 mmol, 6 eq) was added and the mixture was stirred for 5 minutes. 2-Cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine (367 mg, 1.55 mmol, 3 eq) was added and the mixture was stirred for 16 h under an argon atmosphere at room temperature. 10 ml sat. $NaHCO_3$ solution was added and the phases were separated. The aqueous phase was extracted with DCM two times. The combined organic phases were dried in vacuo and the crude product was purified *via* flash chromatography (cyclohexane/ethyl acetate). The residue was dissolved in 10 ml acetone. 90 ml *n*-hexane (prechilled to -20 °C) was added and the mixture was stored at -20 °C overnight. The precipitant was filtered, washed with cool *n*-hexane and dried under reduced pressure.

Yield: 381 mg (95%)

R_f (cyclohexane 1:1 EtOAc): 0.73

^{1}H NMR (500 MHz, DMSO-d_6):

δ = 743-7.16 (m, 10H), 6.83 – 6.78 (m, 4H), 6.56 (dd, 1H, J = 9.1 Hz, 2.4 Hz), 6.58 – 6.54 (m, 1H), 6.54-6.50 (m, 1H), 6.07 (d, 1H, J = 2.5 Hz), 5.23 – 5.21 (m, 1H), 3.85 – 3.78 (m, 1H), 3.71 – 3.70 (m, 6H), 3.65 – 3.49 (m, 2H) 3.43 – 3.34 (m, 5H), 3.28 – 3.17 (m, 1H), 2.76 (t, 1H, J = 5.8 Hz), 2.67 (t, 1H, J = 5.8 Hz), 1.16 – 1.11 (m, 12H), 0.99 (d, 3H, J = 6.7 Hz), 0.86 (t, 3H, J = 6.8 Hz) ppm.

^{13}C NMR (120 MHz, DMSO-d_6):

δ = 161.1, 158.5, 156.3, 154.7, 150.7, 145.2, 135.8, 135.6, 130.1, 128.2, 128.1, 127.1, 126.0, 119.4, 113.6, 109.0, 106.6, 105.8, 97.4, 86.2, 67.2, 59.3, 55.5, 44.4, 43.2, 31.4, 12.8 ppm.

^{31}P NMR (162 MHz, DMSO-d_6): 148.62, 148.57 ppm.

MALDI HRMS (m/z): calc. for $C_{45}H_{54}N_3O_7P$ $[M+Na]^+$ 802.35916, found 802.36245 (Δm = 0.329 error 4.10 ppm).

1.3. Solid-phase synthesis

Solid-phase synthesis was performed on an ABI392 instrument. Pac_2O (Merck) was used as capping reagent and 0.3 M BTT (emp Biotech) as activator. Coupling time for A, C, G, U and the amino-modifier was 6 minutes, for BHQ-2 and compound (**1**) 15 minutes. Synthesis was performed in DMTr-Off mode. The cyanoethyl groups were removed with 20% diethylamine (emp Biotech) for 10 minutes. Cleavage from the solid phase was performed at room temperature with aqueous ammonia (32%) (Merck) for 4 hours. After spin filtration, the solvent was removed at 4 °C using a vacuum concentrator (SpeedVac™, Thermo Fischer). The oligonucleotides were purified on an Agilent 1200 equipped with a reversed phase waters XBridge BEH C18 OBD column (300 Å, 5 µm, 19x250 mm, 4 mL/min, 60 °C). As solvents 400 mM hexafluoroisopropanol (Fluorochem), 16.3 mM Et_3N (Merck), pH 8.3 and MeOH (Fluka) were used with a gradient from 5% to 100% MeOH in 30 minutes.

The following oligonucleotides were synthesized:

Sequence	mass calc. [M-H]	mass found [M-H]
MB1 (without fluorophore) 5' Q_1**1**MCACGAGGUAAAAACUUCCCCUCACUCCUUCCUCGUGQ$_2$ 3'	14052.6	14053.2
MB2 (without fluorophore) 5' Q_1**1**MCACGACAAAACAAAACAAAAAAACUUAAAAAAAAUCGUGQ$_2$ 3'	14350.8	14349.7
MB3 (without fluorophore) 5' Q_1NMCACGACAAAACAAAACAAAAAAACUUAAAAAAAAUCGUGQ$_2$ 3'	14268.8	14267.7
MB1 5' Q_1**1**FCACGAGGUAAAAACUUCCCCUCACUCCUUCCUCGUGQ$_2$ 3'	14545.8	14541.1
MB2 5' Q_1**1**FCACGACAAAACAAAACAAAAAAACUUAAAAAAAAUCGUGQ$_2$ 3'	14844.1	14840.8
MB3 5' Q_1NFCACGACAAAACAAAACAAAAAAACUUAAAAAAAAUCGUGQ$_2$ 3'	14763.0	14761.8
MB4 (without fluorophore) Q_1**1**MCACGAUCGCCUUAAUCCCGUUCUUCUGCUUGUCUCGUGQ$_2$ 3'	14041.5	14041.5
MB4 Q_1**1**FCACGAUCGCCUUAAUCCCGUUCUUCUGCUUGUCUCGUGQ$_2$ 3'	14535.7	14539.5

A = 2’-OMe-Pac-A-CE Phosphoramidite (*LinkTech*)

C = 2’-OMe-Ac-C-CE Phosphoramidite (*Linktech*)

G = 2’-OMe-*i*Pr-Pac-G-CE Phosphoramidite (*LinkTech*)

U = 2’-OMe-U-CE Phosphoramidite (*LinkTech*)

Q_1 = 5’-BHQ-2-CE Phosphoramidite (*LinkTech*)

1 = Compound (**1**)

N = PC Linker-CE Phosphoramidite (*LinkTech*)

M = Fmoc-Amino-DMT C-3 CED phosphoramidite (*ChemGenes*)

F = Fmoc-Amino-DMT C-3 CED phosphoramidite (*ChemGenes*) labeled with ATTO565 NHS ester (*ATTO-Tec*)

Q_2 = BHQ-2 CPG (*Primetech*)

1.4. Fluorophore labeling and preparation for live cell use

5 nmol of each unlabeled oligonucleotide was dissolved in 150 µL borate-buffer (0.1 M sodium tetraborate (Merck), pH 8.4) and incubated with 100 nmol ATTO565 NHS (ATTO-TEC), dissolved in 50 µL DMF (lumiprobe, labeling grade), for 4 hours at 37 °C. Buffer and the excess of fluorophore were removed by size exclusion chromatography (NAP 25, GE Healthcare). The residue was purified by RP-HPLC on an Agilent 1200 equipped with an Xbridge BEH C18 OBD (300 Å, 3.5 µm, 4.6x250 mm, 1 mL/min, 60 °C). As solvents 400 mM hexafluoroisopropanol, 16.3 mM Et_3N, pH 8.3 and MeOH were used with a gradient from 5% MeOH to 100% MeOH in 50 minutes.

For use in living cells, remaining HPLC buffer ions had to be removed. Therefore, the oligonucleotides were dissolved in 0.3 M NaOAc (Merck) (10 µL per 1 nmol RNA). EtOH (Sigma-Aldrich), prechilled to -20 °C, 40 µL per 1 nmol RNA) was added. The mixture was cooled to -20 °C for at least 6 hours. The precipitant was pelletized by centrifugation at 4 °C, 20000 g for 20 minutes. The residue was redissolved in 0.3 M NaOAc and the precipitation steps were repeated 3 times. To remove sodium ions, the oligonucleotides were desalted using a 1k cut-off membrane filter (Microsep Advance Centrifugal Devices with Omega Membrane 1K, PALL). Before adding the oligonucleotides, each filter was washed 5 times with DEPC water at 15000 g, 15 °C for 20 minutes. The desalting step was repeated 3 times.

1.5. Fluorescence assay

Antisense oligonucleotides for MB1-MB3 were obtained from Biomers. For a single measurement 100 pmol of desalted oligonucleotide was dissolved in 100 µL buffer (135 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4) on a black 96-well fluorescence plate (Corning). Measurements were

performed on a Plate Reader Infinite M-200 Pro (Tecan). Atto565 was excited with 525 nm. Every experiment was repeated 3-5 times.

1.6. Hippocampal neurons

Dissociated rat hippocampal neuron cultures were prepared and maintained as described previously.[3] Cells were plated at a density of $30 - 40 \times 10^3$ cells/cm² on poly-D-lysine coated glass-bottom Petri dishes (MatTek). Hippocampal neurons were maintained and matured in a humidified atmosphere at 37°C and 5% CO_2 in growth medium (Neurobasal-A supplemented with B27 and GlutaMAX-I, life technologies) for 18-21 days in vitro (DIV) to ensure synapse maturation. All experiments complied with national animal care guidelines and the guidelines issued by the Max Planck Society and were approved by local authorities.

1.7. Transfection and imaging of MBs

For transfection of molecular beacons, DIV17-19 neurons were transfected with Attractene (Qiagen). For each Mattek dish, 20pmol of molecular beacon was resuspended in 75 µL of buffer EC (Qiagen) along with 2 µL of Attractene. The beacon-attractene mix was incubated for 20 minutes at room temperature before being added to neurons. Samples were imaged 1-hour post transfection. Prior to imaging, samples were washed in fresh media to remove non-transfected beacons.

Investigation of the mRNA dynamics was carried out using a Leica DMi8 TIRF microscope. Differential interference contrast (DIC) microscopy was used to identify neurons with well-isolated dendrites. Photoactivation was achieved through the use of a 405 nm laser (7.78 mW/cm^2 intensity), targeting the soma. 10-20 pulses illumination were performed for up to 30 seconds to photoactive the MBs. mRNA dynamics were recorded for 16 hours with a single frame acquired every 10 minutes in epi-fluorescence mode. ATTO565 fluorophores were excited using a 561 nm diode laser which provided 1.8 kW/cm^2 of intensity at the sample plane. The fluorescence was recorded with a scientific-CMOS camera (Leica-DFC9000GT). The exposure time was fixed to 200 ms and 2x2 camera binning and set the digitalization to 12 bit (low noise) was used to limit the data volume. A 100x oil objective (HC PL APO 100x/1.47 OIL) was used to record a field-of-view of 133 µm x 133 µm. With these settings our pixel size was 130 nm, matching the Nyquist sampling frequency. Neurons were left in their glia-conditioned neuro-basal, B27 and glutamax media owing to a Pecon TempController 2000-1 and a Pecon CO2-Controller 2000 which kept the samples at 37°C in a 5% CO_2 atmosphere.

1.8. Tracking the mRNA movement post photoactivation

We tracked the mRNAs post photoactivation for 14+ hours using a custom MATLAB script. First, we carry out an orthogonal projection (maximum) to include signal collected from all imaging planes. Then we use Differential interference contrast (DIC) microscopy to check the absence of significant drift during imaging time. We also use DIC as basis to manually segment the neuronal soma. We extract the mean intensity of the soma and normalize it in order to measure the relative fluorescence before and after photoactivation and check there is no significant photobleaching during the several hours of imaging time. Using the same MATLAB script we manually draw the neuronal dendrite in the projected time series and extract the cumulative intensity along the dendrite over time for each pixel along the dendrite. We use this to calculate the half time for populating the dendrite, $t_{1/2}$.

NMR spectra

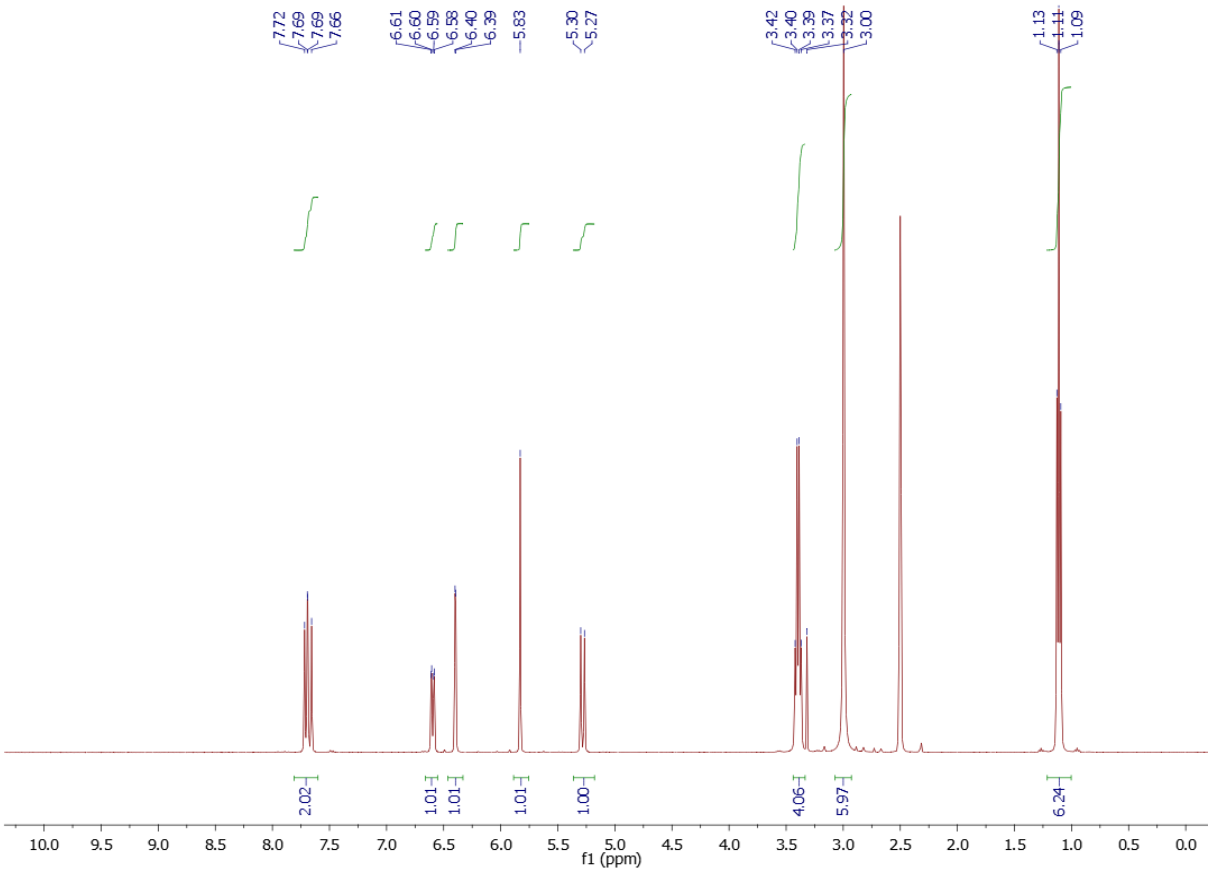

Figure S2: ^{1}H-NMR spectrum of compound **3** in DMSO-d_6.

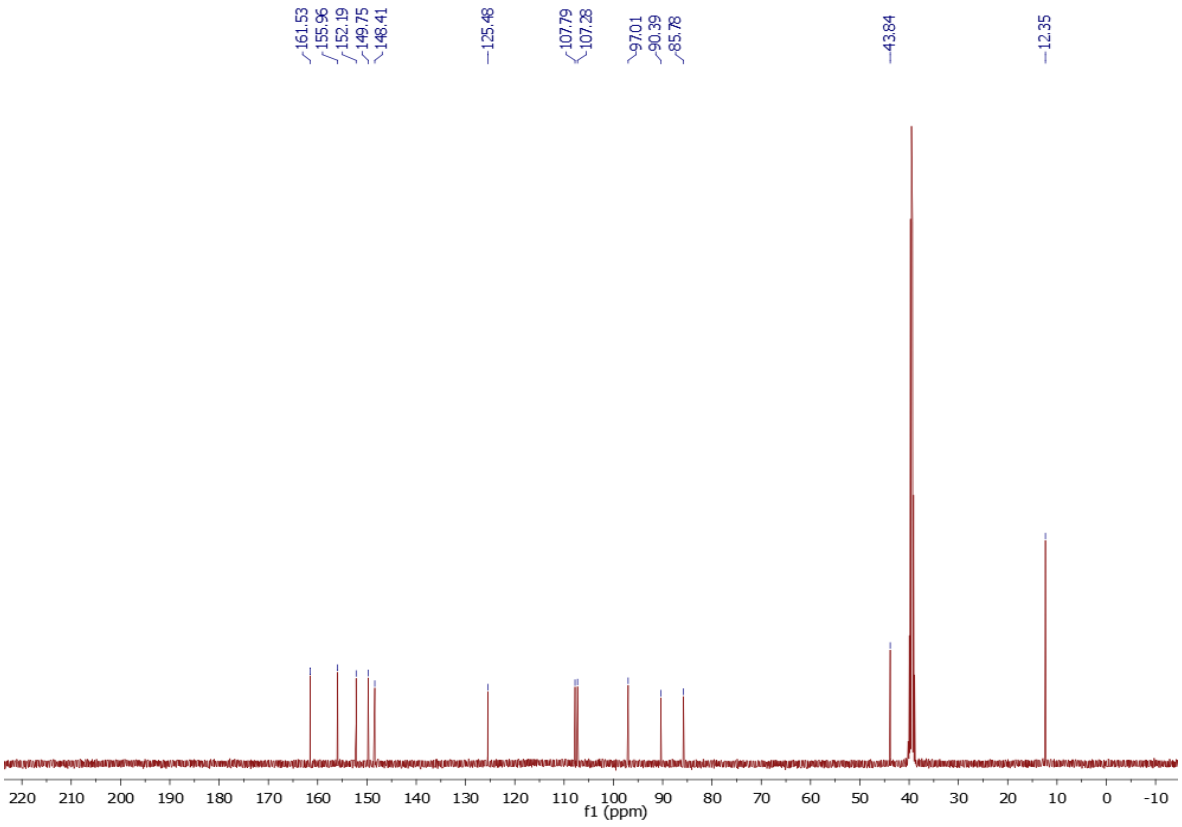

Figure S3: ^{13}C-NMR spectrum of compound **3** in DMSO-d_6.

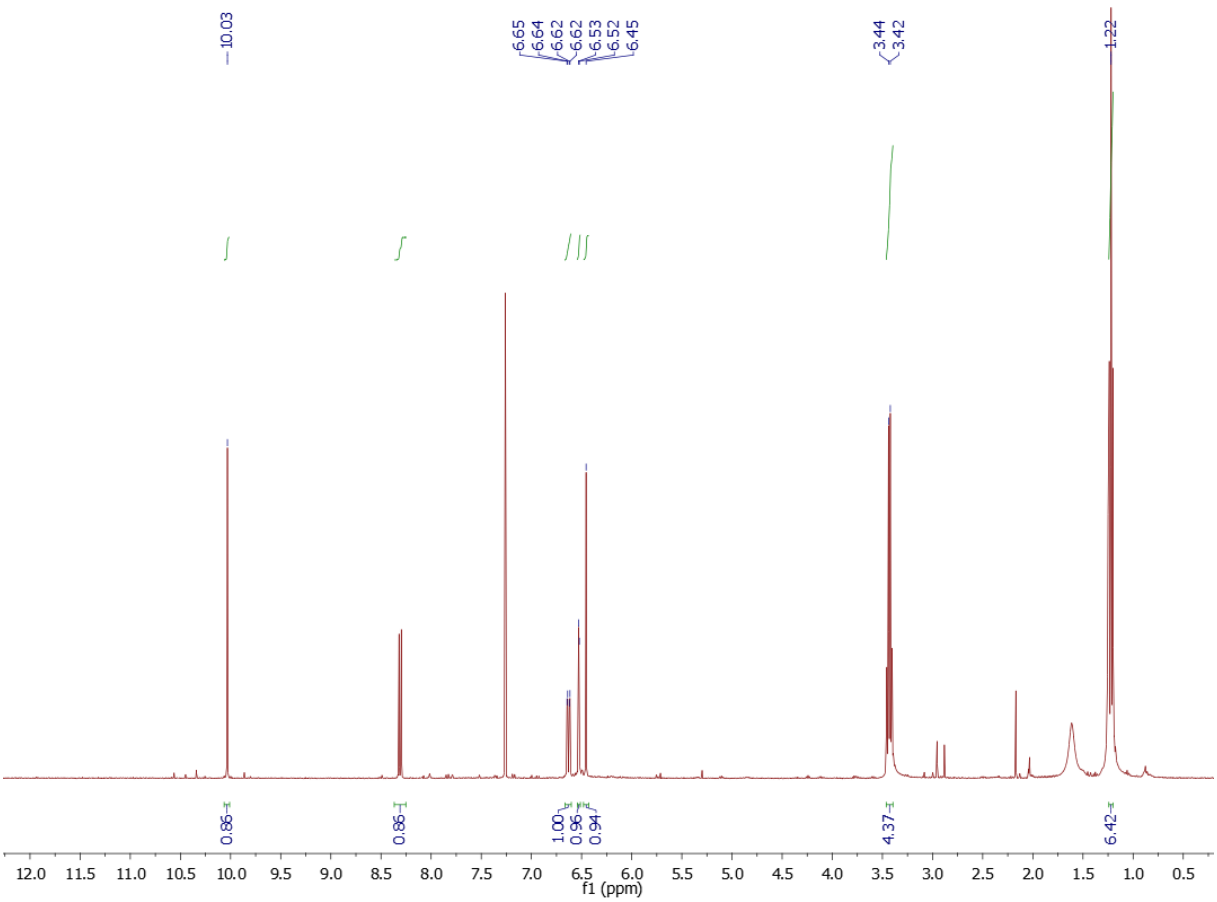

Figure S4: ^{1}H-NMR spectrum of compound **4** in $CDCl_3$.

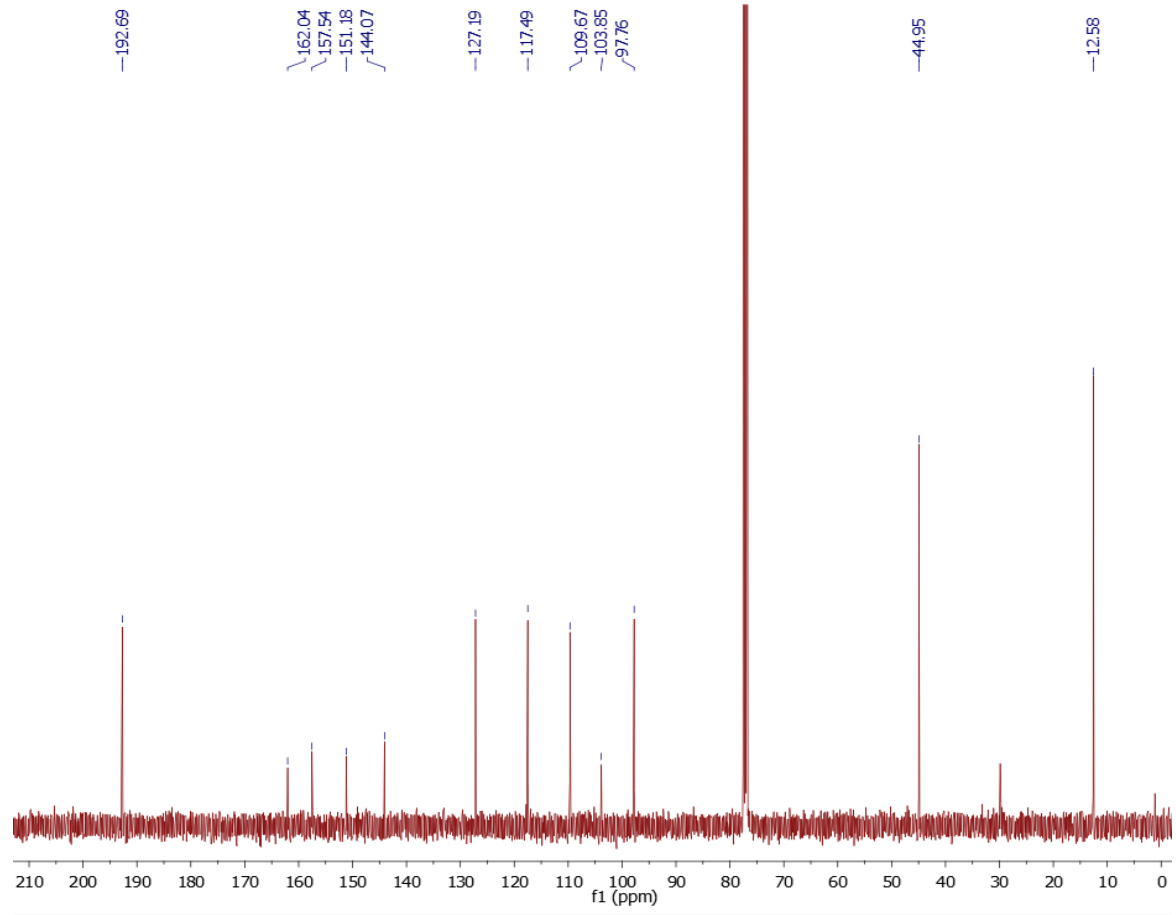

Figure S5: ^{13}C-NMR spectrum of compound **4** in $CDCl_3$.

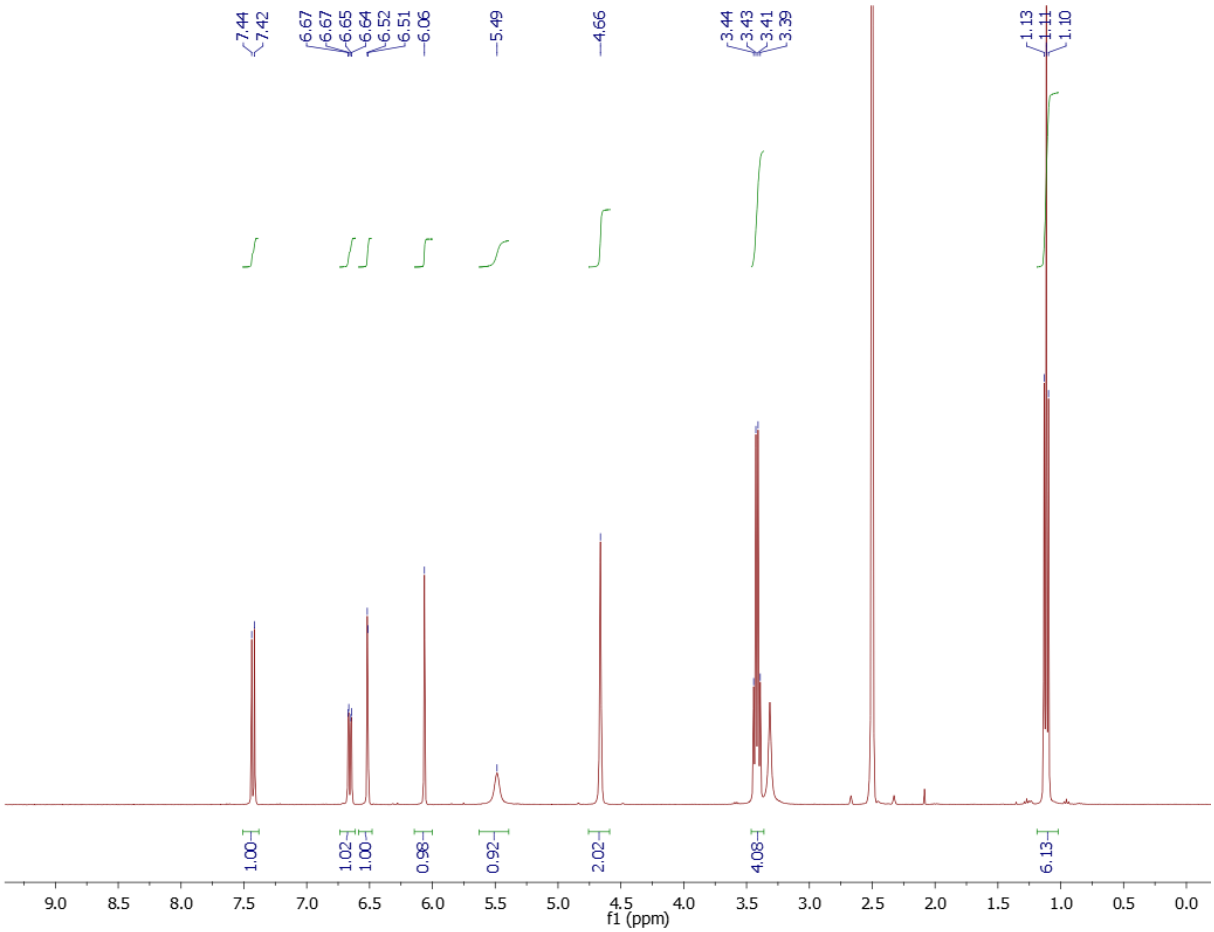

Figure S6: ^{1}H-NMR spectrum of compound **5** in DMSO-d$_6$.

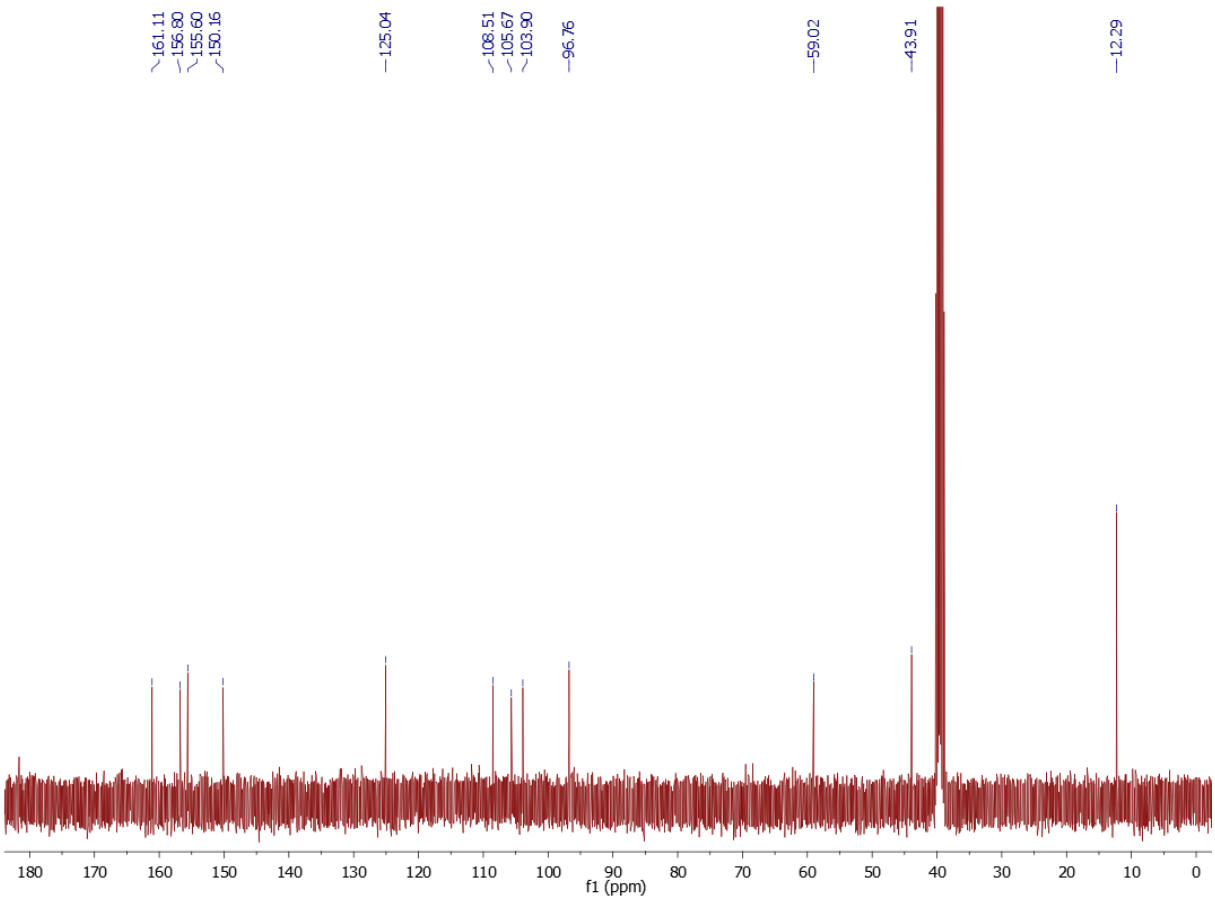

Figure S7: ^{13}C-NMR spectrum of compound **5** in DMSO-d$_6$.

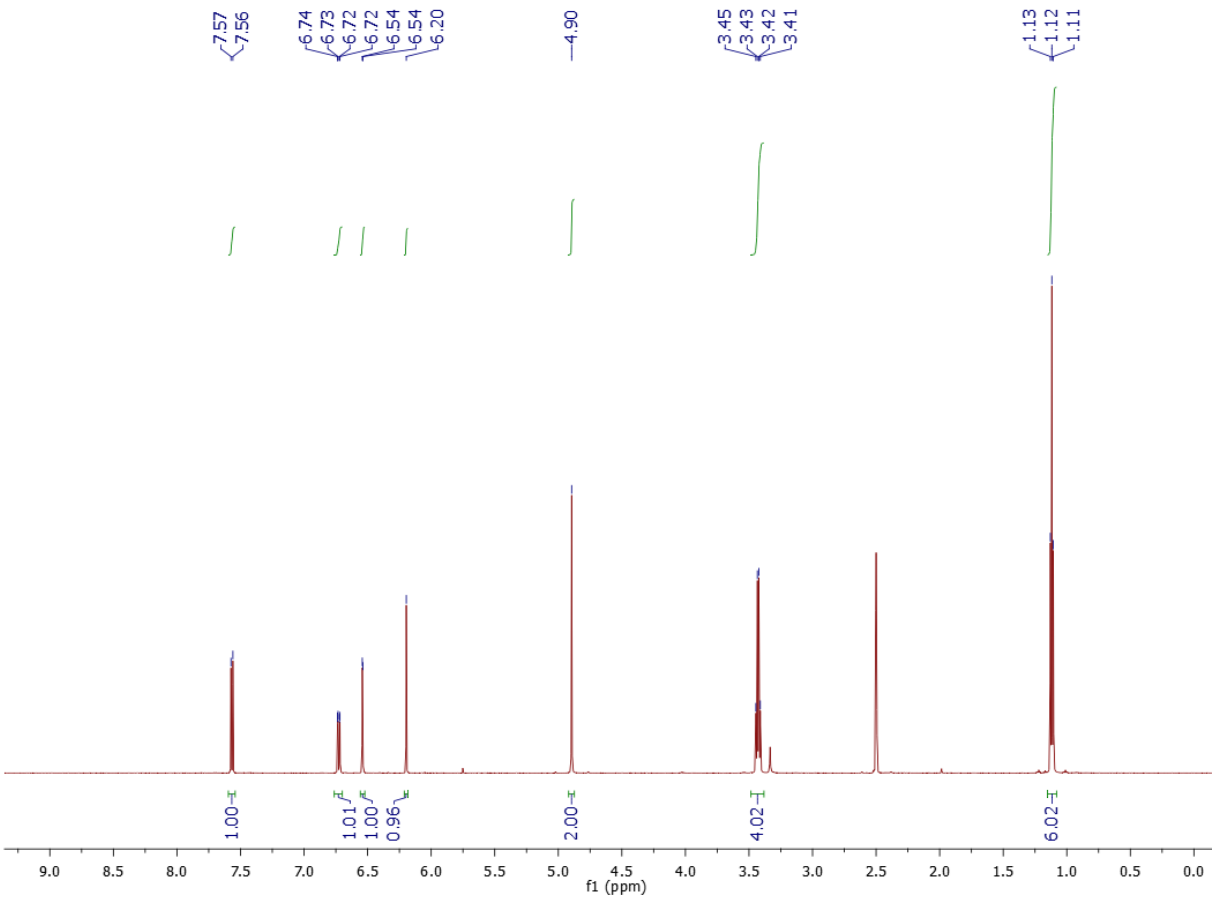

Figure S8: ^{1}H-NMR spectrum of compound **6** in DMSO-d_6.

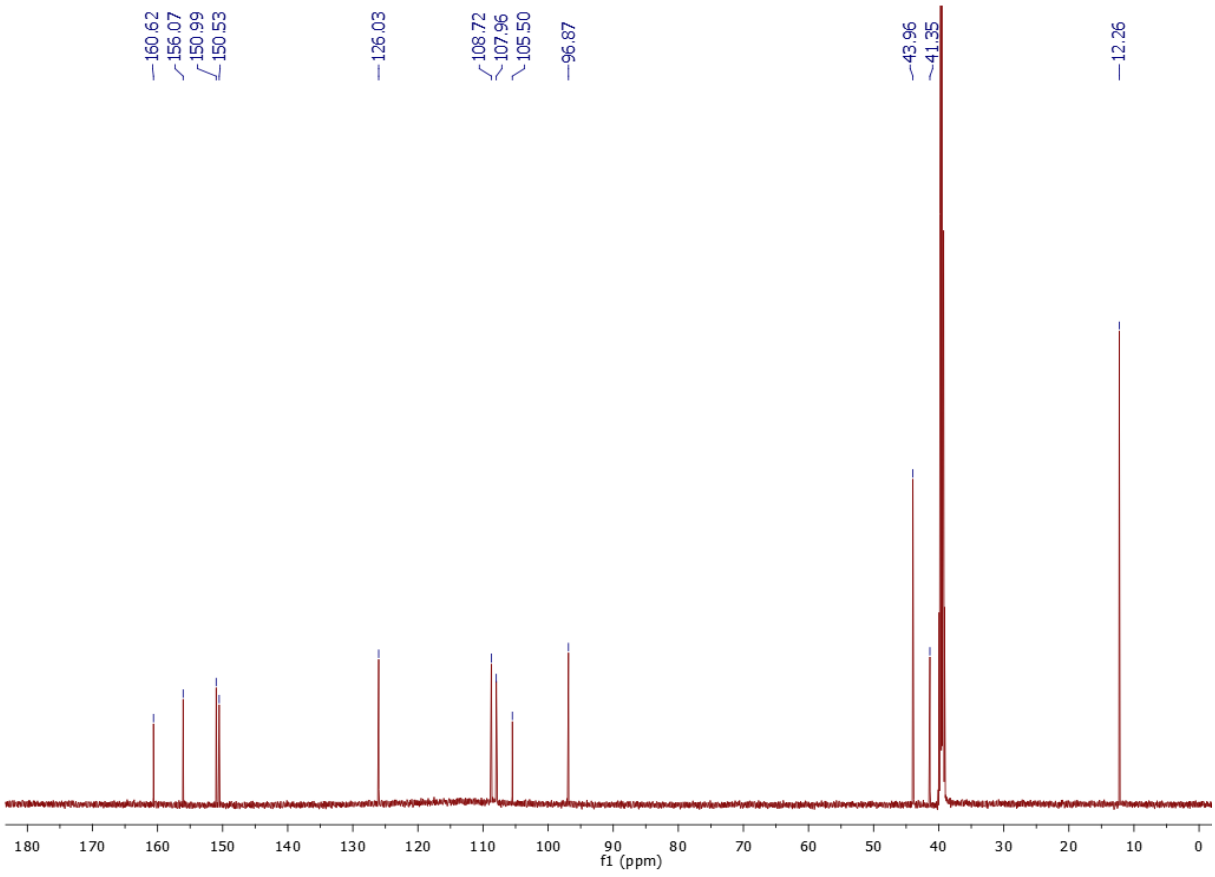

Figure S9: ^{13}C-NMR spectrum of compound **6** in DMSO-d_6.

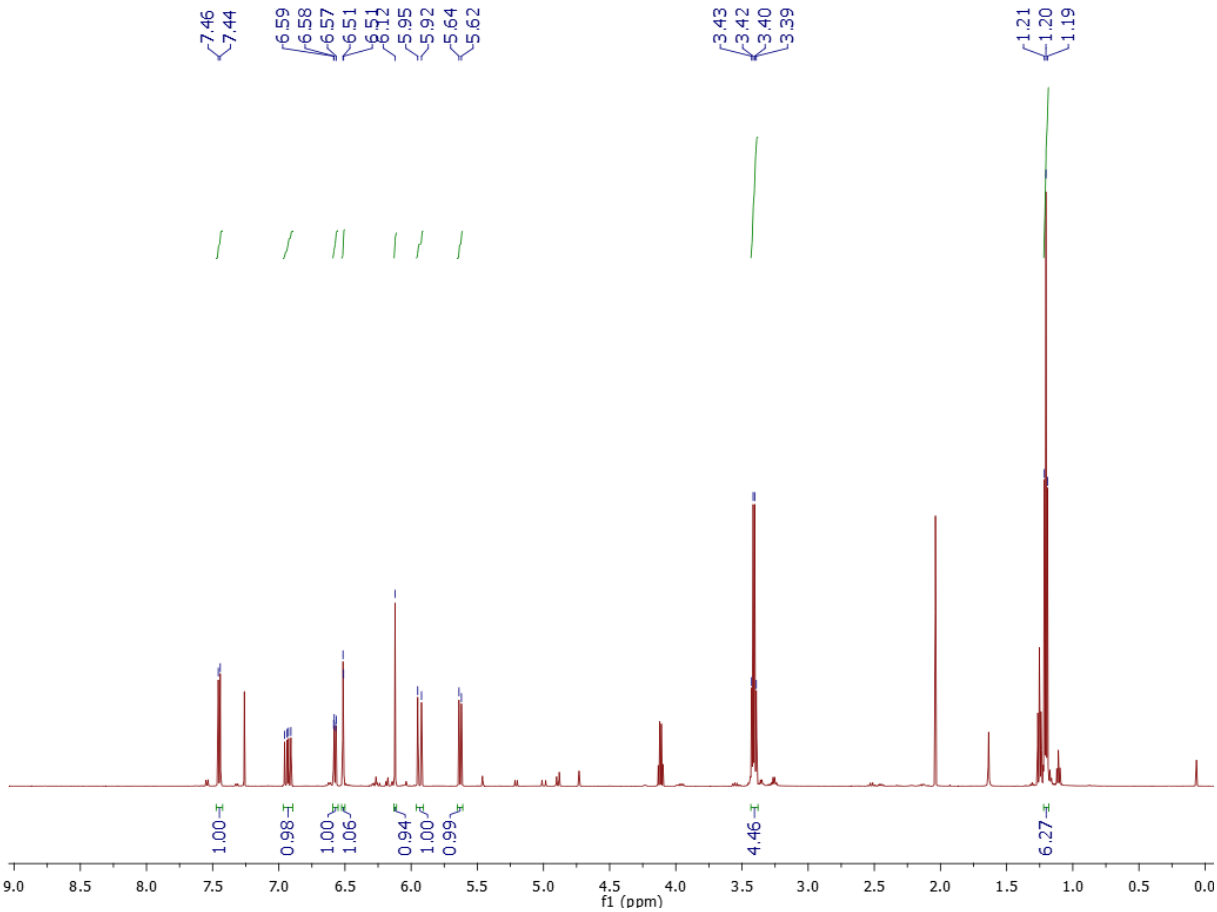

Figure S10: ^{1}H-NMR spectrum of compound **7** in $CDCl_3$.

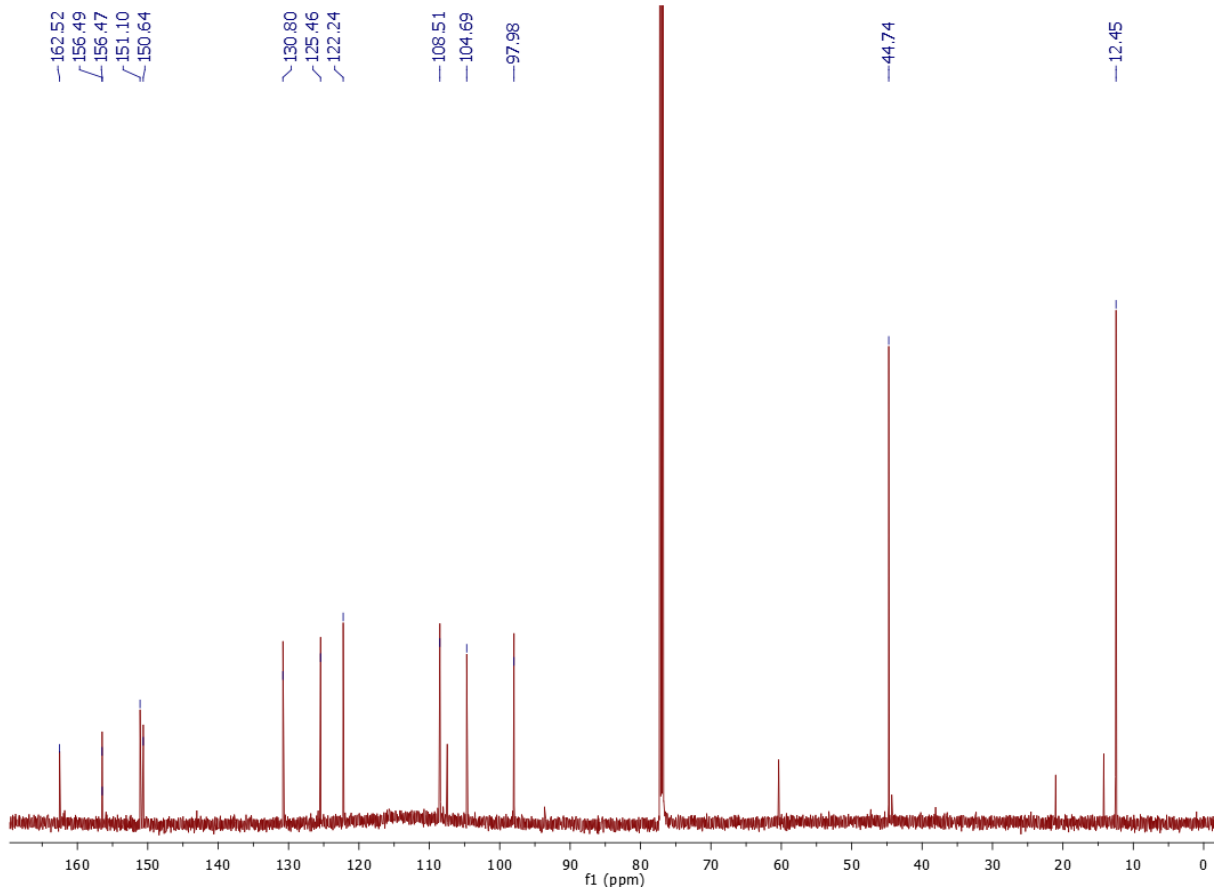

Figure S11: ^{13}C-NMR-spectrum of compound **7** in $CDCl_3$.

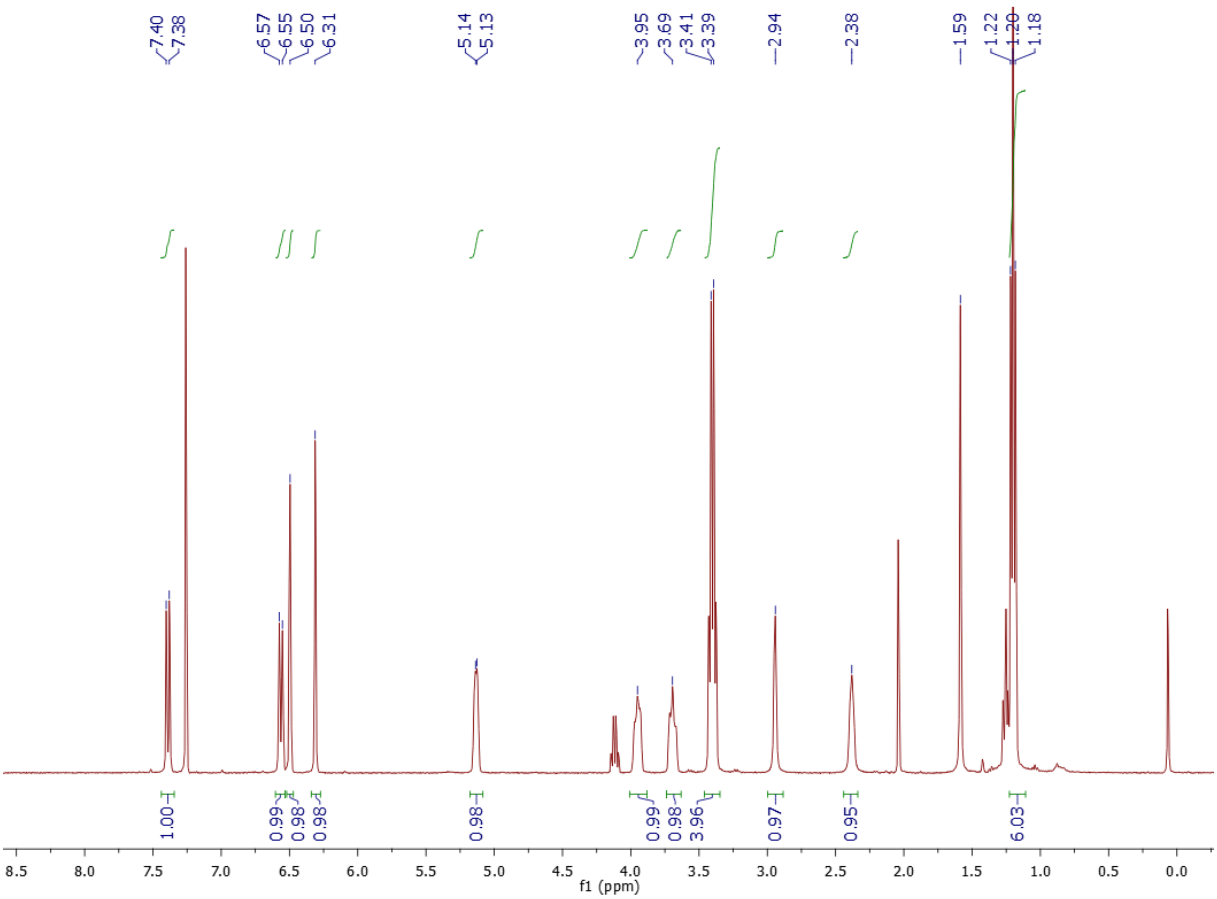

Figure S12: ^{1}H-NMR spectrum of compound **8** in $CDCl_3$. Residual EtOAc signals visible.

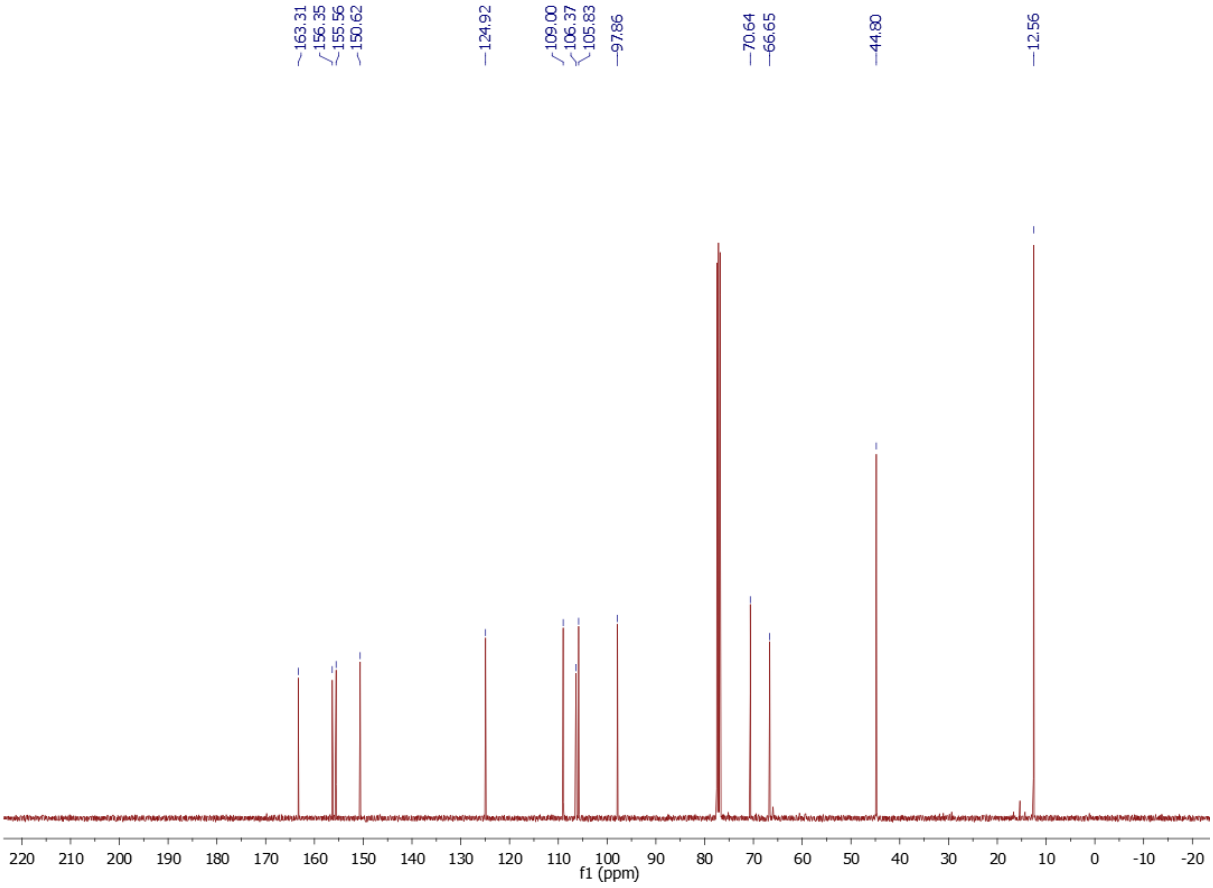

Figure S13: ^{13}C-NMR-spectrum of compound **8** in $CDCl_3$.

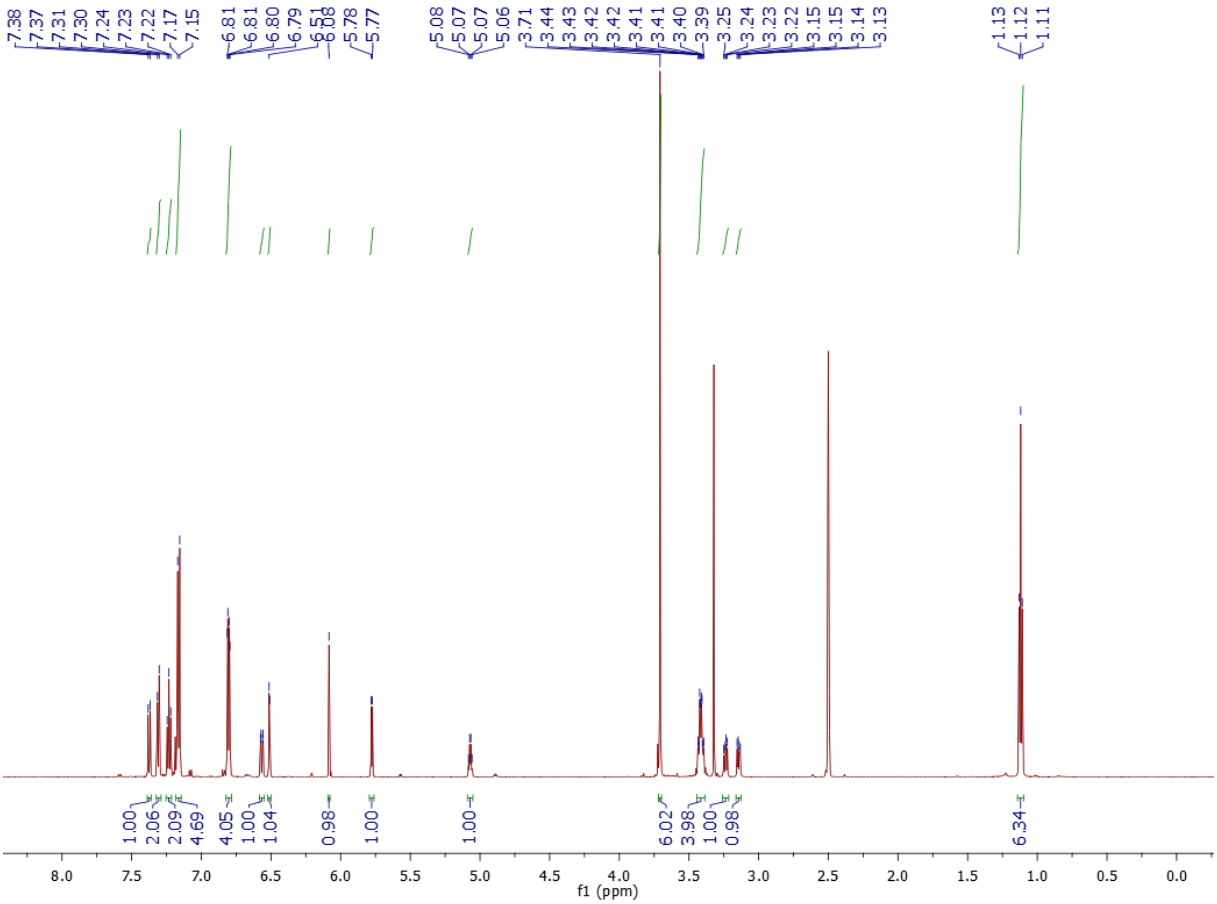

Figure S14: ^{1}H-NMR spectrum of compound **9** in DMSO-d_6.

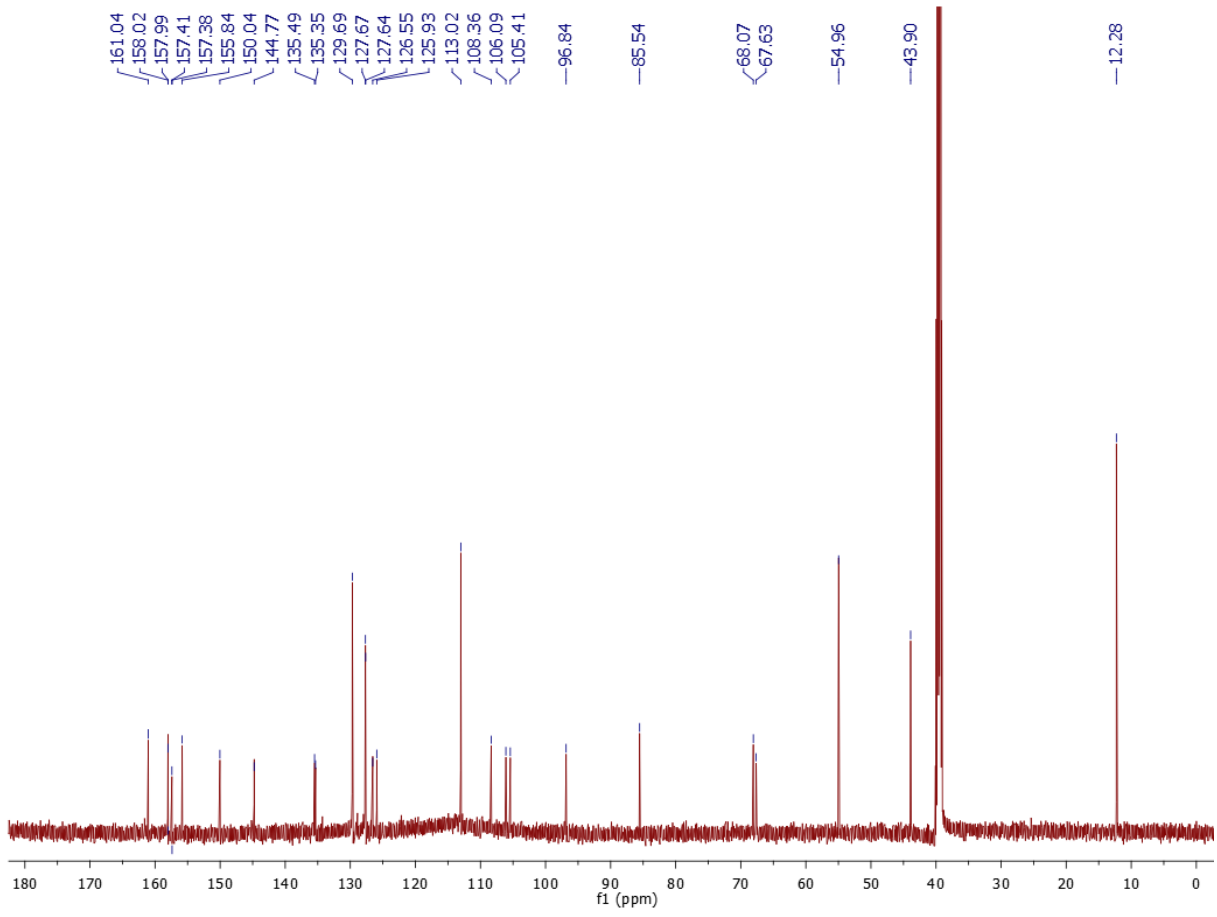

Figure S15: ^{13}C-NMR spectrum of compound **9** in DMSO-d_6.

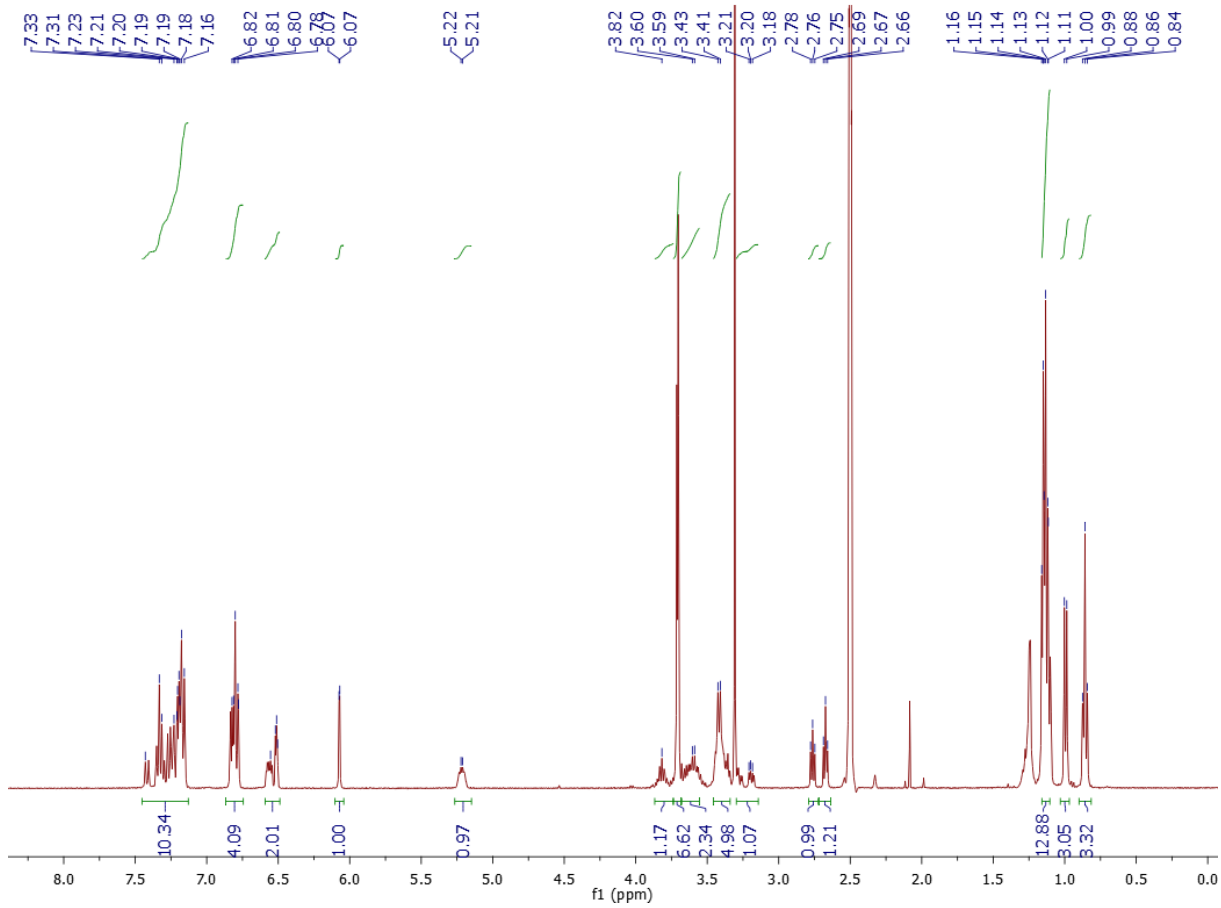

Figure S16: ^{1}H-NMR spectrum of compound **1** in DMSO-d_6.

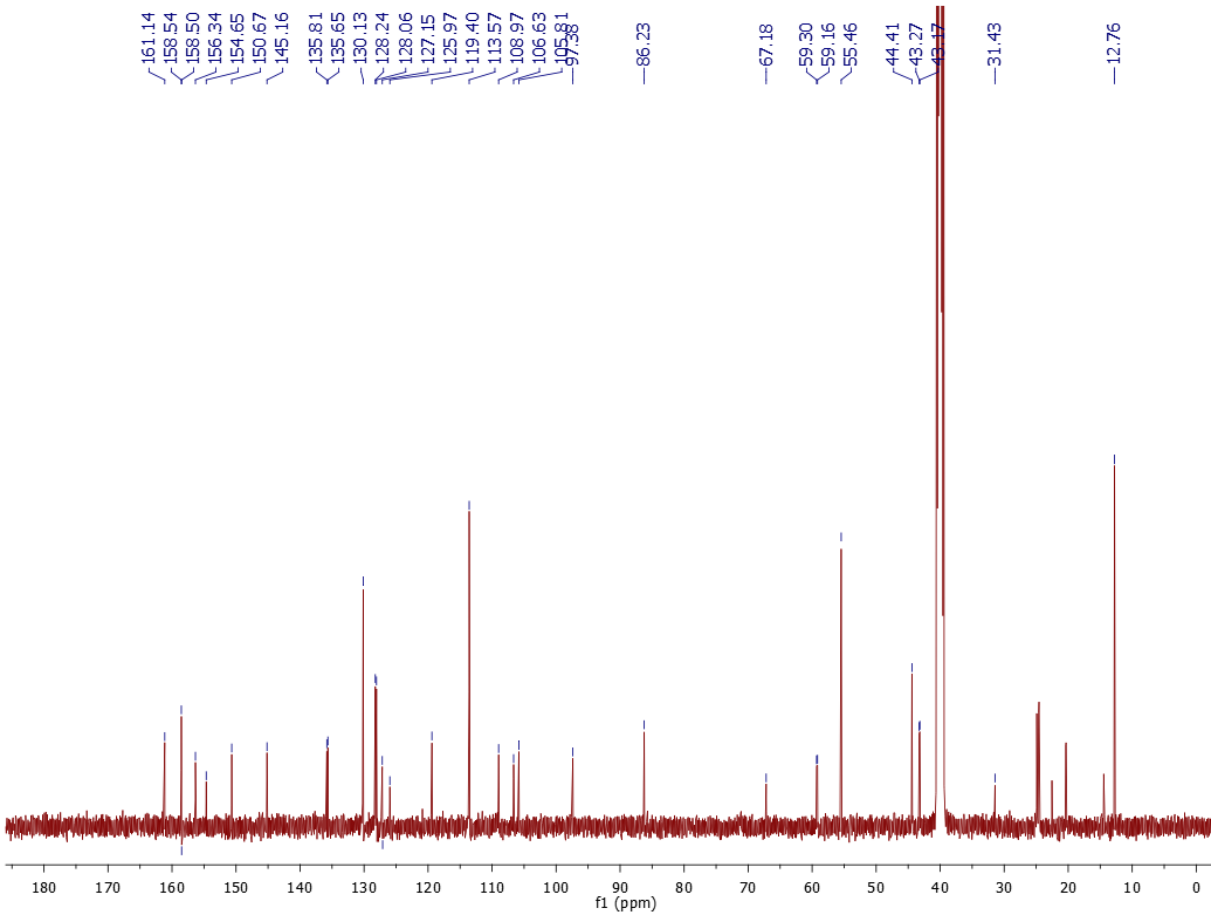

Figure S17: ^{13}C-NMR spectrum of compound **1** in DMSO-d_6.

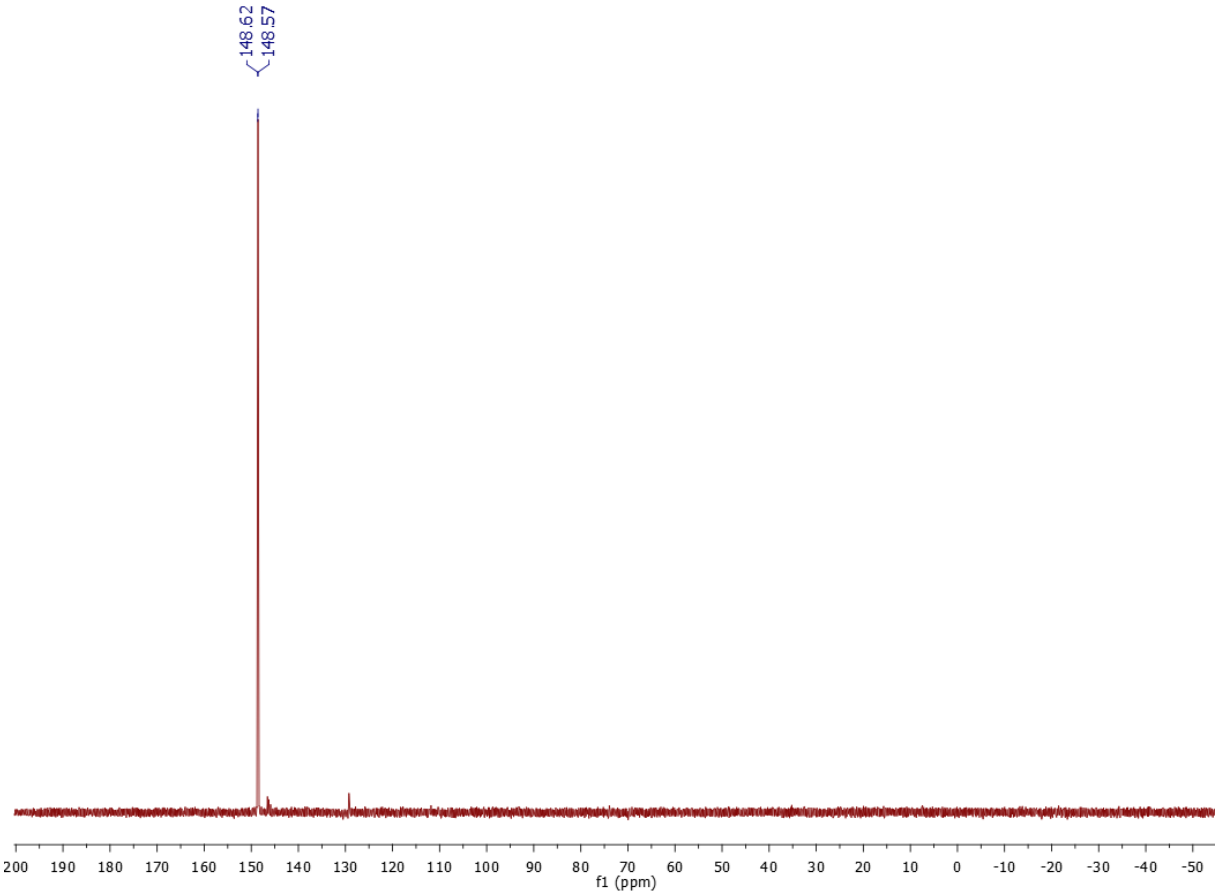

Figure S18: ^{31}P-NMR spectrum of compound **1** in DMSO-d_6.

Mass-spectra

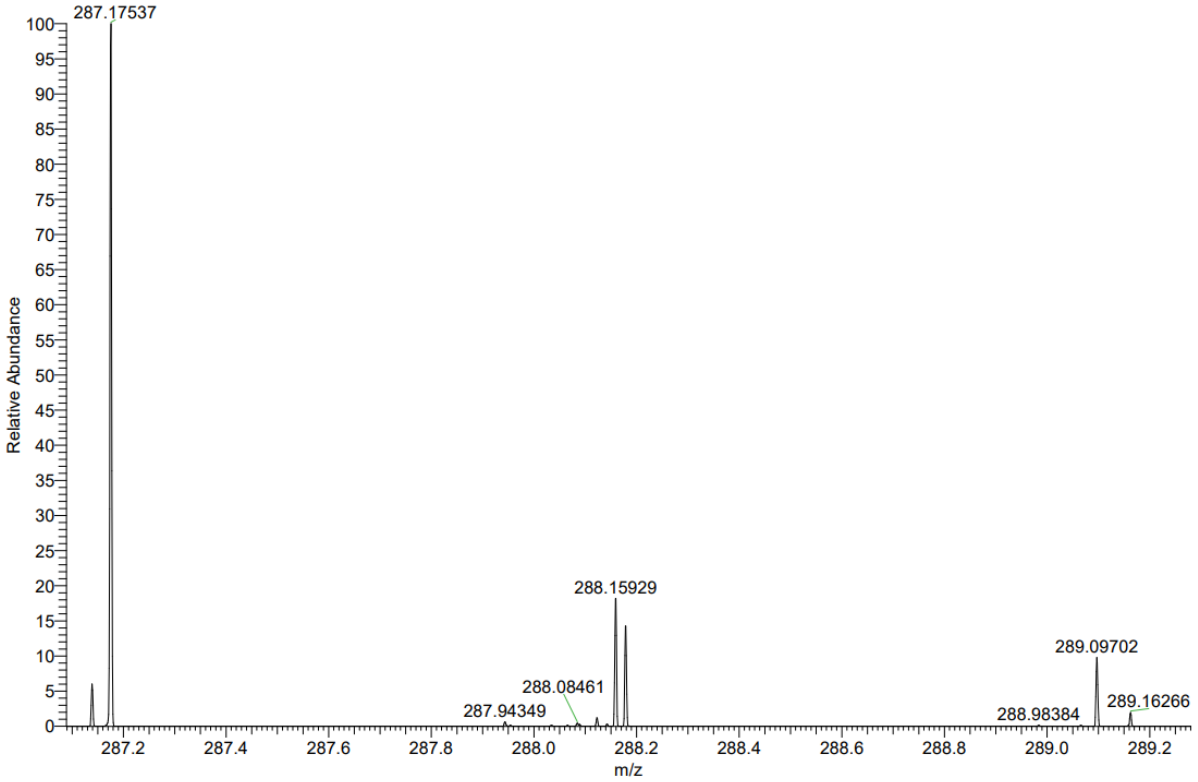

Figure S19: MALDI-HRMS spectrum of compound **3**.

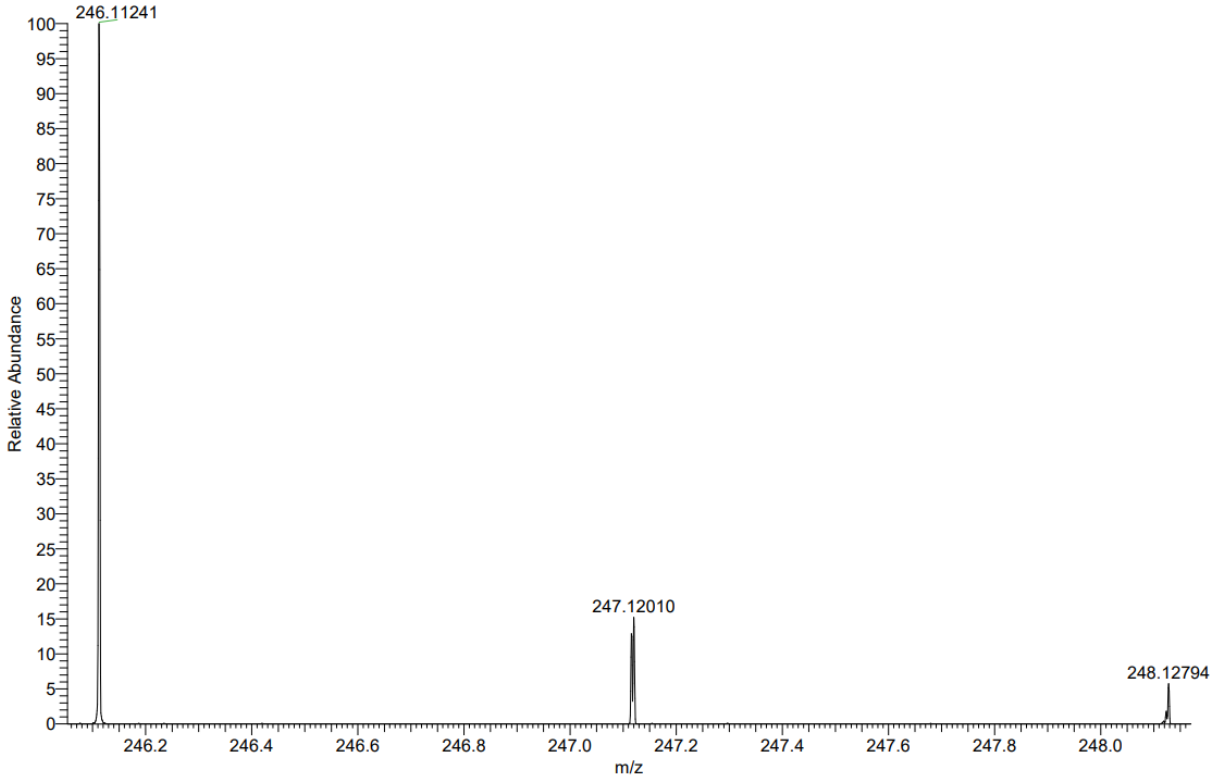

Figure S20: MALDI-HRMS spectrum of compound **4**.

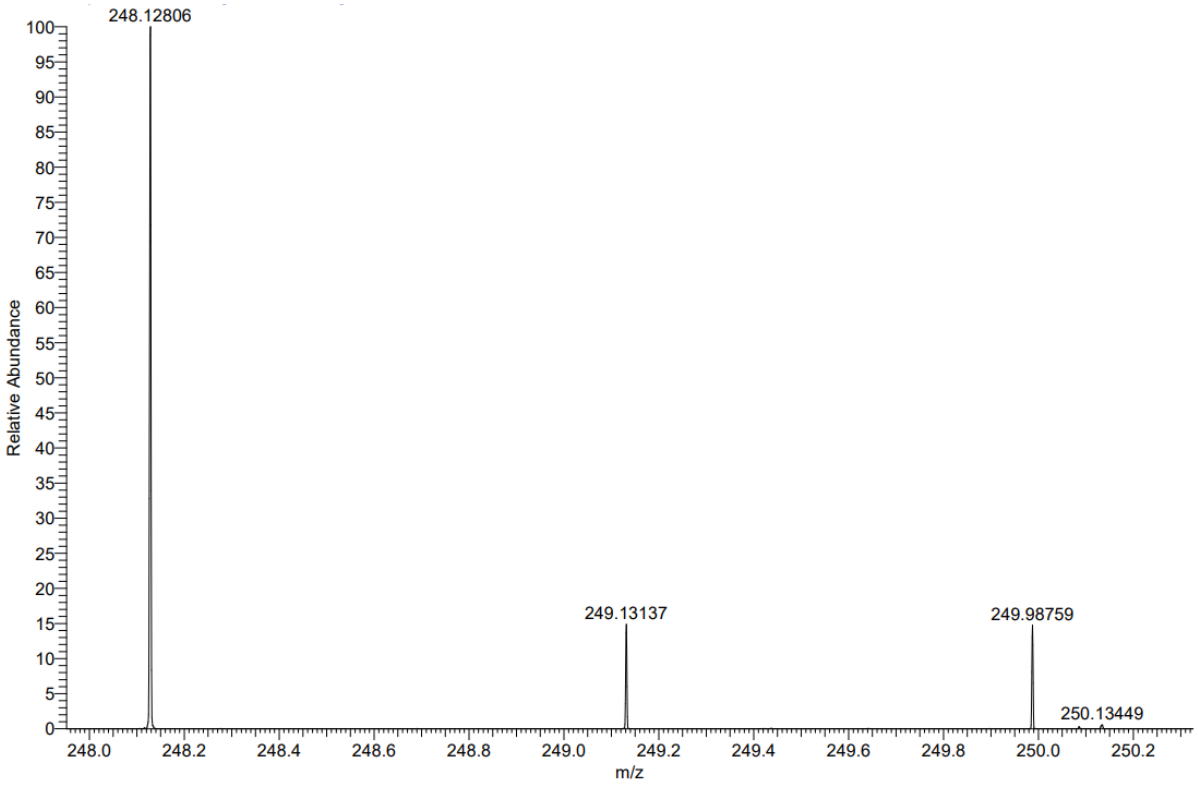

Figure S21: MALDI-HRMS spectrum of compound **5**.

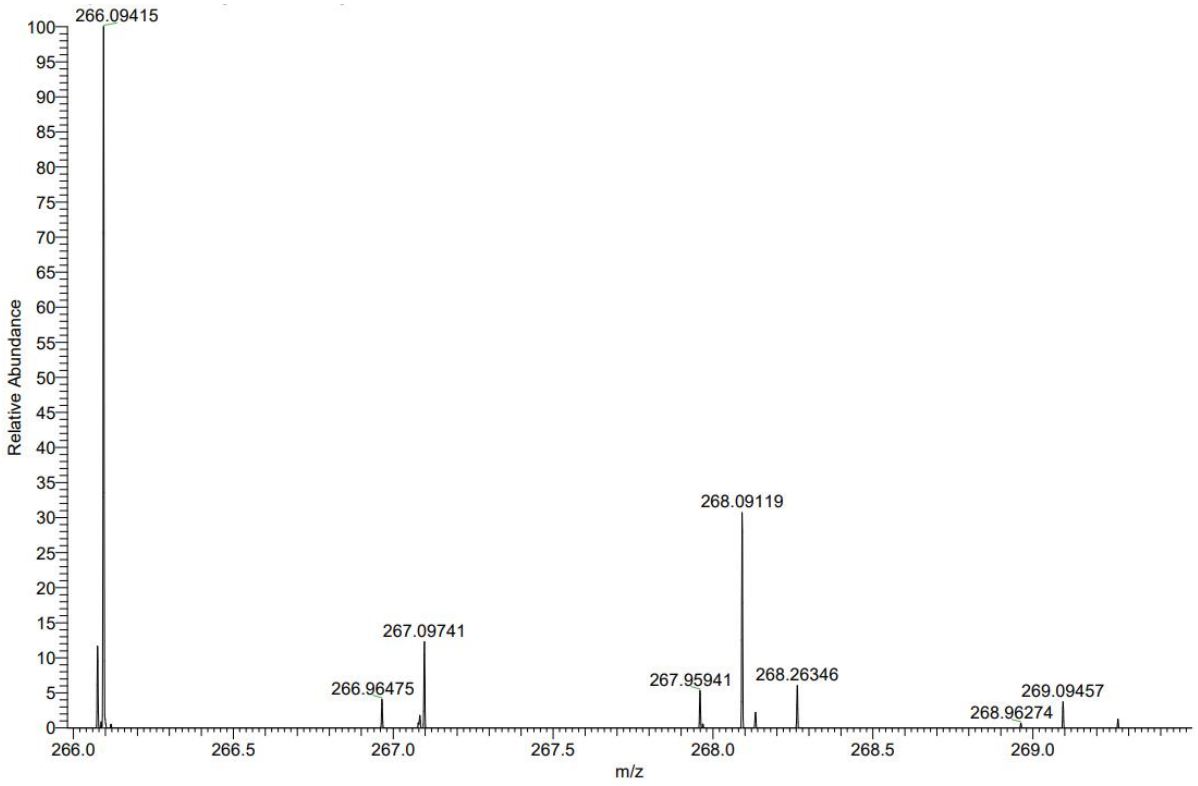

Figure S22: MALDI-HRMS spectrum of compound **6**.

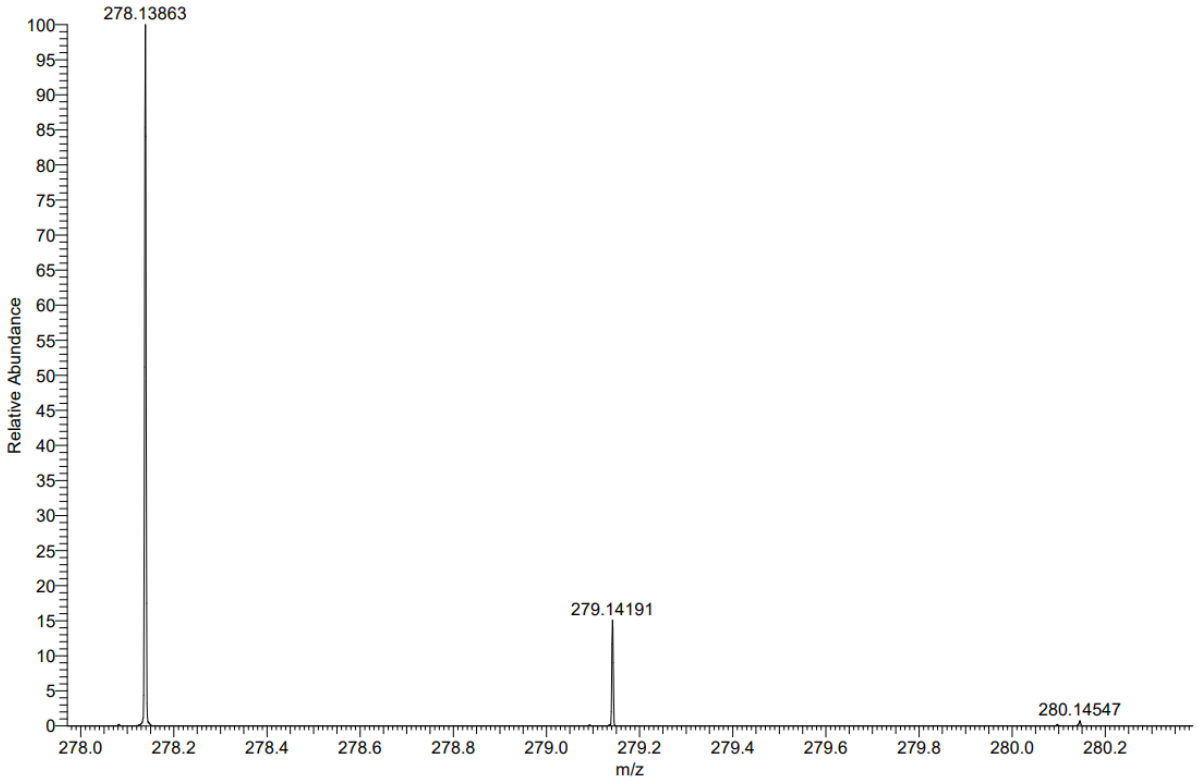

Figure S24: MALDI-HRMS spectrum of compound **8**.

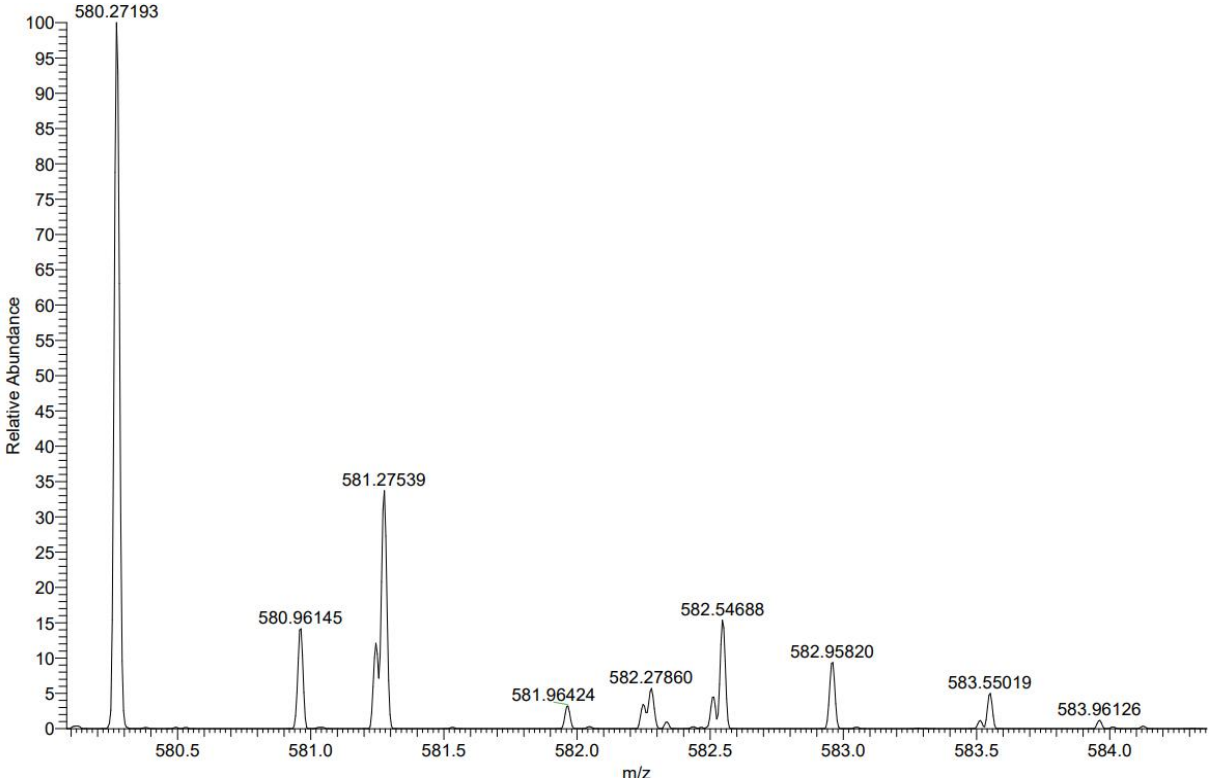

Figure S25: MALDI-HRMS spectrum of compound **9**.

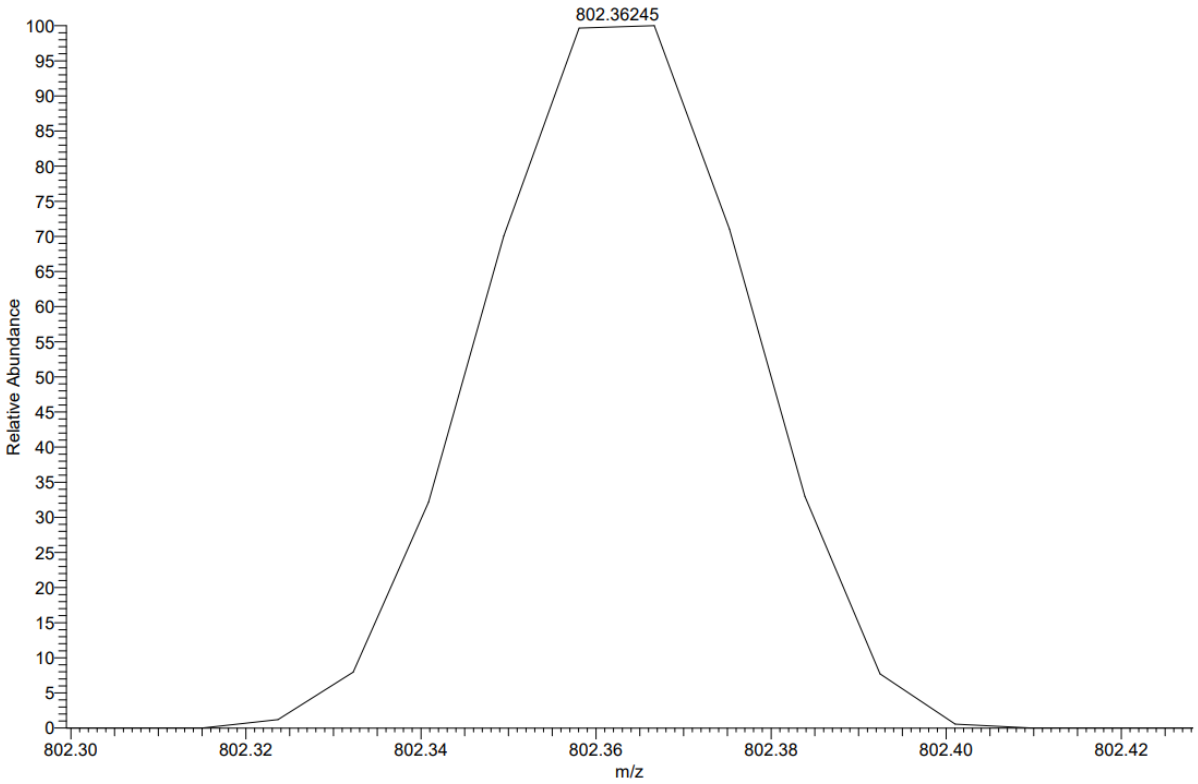

Figure S26: MALDI-HRMS spectrum of compound **1**.

Absorption spectra

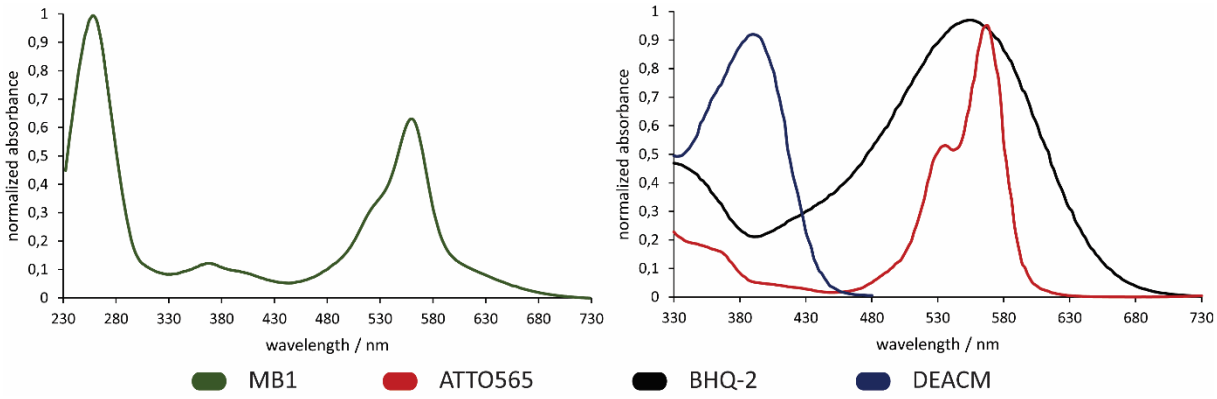

Figure S27: Absorption spectra of **MB1** (left) and the individual chromphores ATTO565, BHQ-2 and DEACM (right).

List of available videos

Video 1: Hippocampal neuronal soma transfected with **MB1**. Photo-activation was achieved using a 405 nm laser (7.78 mW/cm^2 intensity), targeting the soma. 20 frames in total, each frame acquired every 10 seconds. First 10 frames before activation, second 10 frames after activation.

Video 2: Hippocampal neuronal soma transfected with **MB2**. Photo-activation was achieved using a 405 nm laser (7.78 mW/cm^2 intensity), targeting the soma. 20 frames in total, each frame acquired every 10 seconds. First 10 frames before activation, second 10 frames after activation.

Video 3: Hippocampal neuronal soma transfected with **MB1**. Photo-activation was achieved using a 405 nm laser (7.78 mW/cm^2 intensity), targeting the soma. 20 frames in total, each frame acquired every 10 seconds. First 10 frames before activation, second 10 frames after activation. The same cell was used for long-term mRNA monitoring in Videos 4 & 5.

Video 4: Same hippocampal neuronal soma transfected with **MB1** as shown in **Video 3**. mRNA-beacon hybrids were monitored post photo-activation for 14 hours. 85 frames in total, each frame acquired every 10 minutes.

Video 5: DIC image of the same hippocampal neuron shown in **Videos 3&4**. Overnight imaging of **MB1** resulted in no overtly observable stress to the cell. Imaged for 14 hours, 85 frames in total, each frame acquired every 10 minutes.

Video 6: Two adjacent hippocampal neuronal somas transfected with **MB3**. Photo-activation was achieved using a 405 nm laser (7.78 mW/cm^2 intensity), targeting the soma. 20 frames in total, each frame acquired every 10 seconds. First 10 frames before activation, second 10 frames after activation.

Video 7: The same hippocampal neurons transfected with **MB3** shown in **Video 6**. mRNA-beacon hybrids were monitored post photo-activation for 2.8 hours. 17 frames in total, each frame acquired every 10 minutes.

Video 8: DIC image of the same hippocampal neurons shown in **Videos 6&7**. Observable cell stress and eventual death noticeable for both cells within the first 40 minutes of imaging. Imaged for 2.8 hours, 17 frames in total, each frame acquired every 10 minutes.

Video 9: Hippocampal neuronal soma transfected with **MB4**. Photo-activation was achieved using a 405 nm laser (7.78 mW/cm^2 intensity), targeting the soma. 20 frames in total, each frame acquired every 10 seconds. First 10 frames before activation, second 10 frames after activation.

References

(1) Weinrich, T.; Gränz, M.; Grünewald, C.; Prisner, T. F.; Göbel, M. W. Synthesis of a Cytidine Phosphoramidite with Protected Nitroxide Spin Label for EPR Experiments with RNA. *European J. Org. Chem.* **2017**, *2017* (3), 491–496. https://doi.org/10.1002/ejoc.201601174.

(2) Weyel, X. M. M.; Fichte, M. A. H.; Heckel, A. A Two-Photon-Photocleavable Linker for Triggering Light-Induced Strand Breaks in Oligonucleotides. *ACS Chem. Biol.* **2017**, *12* (8), 2183–2190. https://doi.org/10.1021/acschembio.7b00367.

(3) Aakalu, G.; Smith, W. B.; Nguyen, N.; Jiang, C.; Schuman, E. M. Dynamic Visualization of Local Protein Synthesis in Hippocampal Neurons. *Neuron* **2001**. https://doi.org/10.1016/S0896-6273(01)00295-1.