Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Two-Photon Uncageable Enzyme Inhibitors bearing Targeting Vectors

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Supporting Information

Instrumentation and Materials

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on Bruker Avance (400 and 500) spectrometers. ^{13}C and ^{19}F NMR measurements were carried out as ^{13}C {H} and ^{19}F {H}, respectively. ^{1}H and ^{13}C NMR spectra were referenced to the residual solvent signal, the ^{19}F spectrum to CCl_3F. IR spectra were acquired on a Perkin-Elmer Spectrum 2 FT-IR, UV/Vis spectra on a Varian Cary 100 instrument. High resolution ESI-MS spectra were measured with a Bruker ESQUIRE-LC quadrupole ion trap instrument and MALDI-MS spectra on a Bruker Autoflex I. Preparative HPLC purification was carried out with a Varian ProStar with two solvent delivery modules, an UV/Vis detector and an Agilent PrepHT 300 SB-C18 column. Analytical HPLC traces were acquired on a VWR Hitachi Chromaster with a 5110 pump system, a 5260 auto sampler, a 5310 column oven, a 5430 diode array detector, and a Macherey-Nagel EC 250/3 NUCLEOSIL 100-5 C18 column. Microwave heated reactions were carried out in an Anton Paar Monowave 300. Single-Photon uncaging was performed with an Edinburgh Instruments LP920 laser flash photolysis setup with a Continuum Surelight Nd:YAG laser, frequency-tripled to generate light with a wavelength of 355 nm. Two-photon uncaging was carried out using using a Ti:sapphire amplifier system (Spitfire Pro, 5 kHz, 90 fs, 800 µJ per pulse; Spectra-Physics). Chemicals were acquired from Sigma-Aldrich, Acros, Alfa, and TCI Europe. Celecoxib was acquired from AK Scientific, Inc. (Union City, CA, USA) and Lumiracoxib was acquired from Kemprotec Ltd. (Cumbria, UK). NHS-Fluorescein (mixed isomer, 5/6-carboxyfluorescein succinimidyl ester) was acquired from Thermo Scientific. Amino acids were acquired from Bachem (Bubendorf, Switzerland), and Iris-Biotech

(Marktredwitz, Germany). The Tentagel S Ram resin for the peptide synthesis was acquired from Rapp Polymers (Tübingen, Germany). Enzyme inhibition was tested using the "COX Fluorescent Inhibitor Screening Assay Kit" from Cayman Chemical Company (Ann Harbor, MI, USA) according to the manual. The fluorescence measurements were carried out with a Molecular Devices SpectraMax m2E microplate reader.

Methods

Single Photon Uncaging Quantum Yields

The single photon uncaging quantum yields were determined as described by us earlier.[1–3] In short, the samples were dissolved in a 1:1 mixture of acetonitrile and phosphate buffer saline (pH=7.2, 20 mM) to have an optical density OD(λ=355 nm) = 0.2. The samples were irradiated for certain intervals with an Edinburgh Instruments LP920 laser flash photolysis setup with a Continuum Surelight Nd:YAG laser, frequency-tripled to generate light with a wavelength of 355 nm. The laser was slightly misaligned, or the q-switch was delayed, to reach a suitable irradiation power. A fresh aliquot was used for each irradiation. The percentage of remaining caged compound was determined by peak integration of analytical HPLC UV-traces and setting the value in relation to the value obtained without irradiation. The percentage values were plotted against the irradiation, and the slope m_{sample} of the curve was determined for the linear range (approx. the first 20% of decomposition). As reference, *trans*-azobenzene was dissolved in methanol to the same OD(λ=355 nm) = 0.2. The irradiation was carried out as described for the uncaging samples. Subsequently, the absorbance was determined at 355 nm and the percentage of remaining *trans*-azobenzene was plotted against the irradiation. The slope $m_{reference}$ of the curve was determined for the linear range (approx. the first 20% of decomposition). Exemplary uncaging plots including linear fits are shown in the section below. Comparison of the slopes of the curves gave the uncaging quantum yields of the caged compounds under consideration of the number of molecules with the following formula:

$$\phi_{sample} = \phi_{reference} \times \frac{n_{sample,0} \times m_{sample}}{n_{reference,0} \times m_{referece}}$$

At least three independent sets of measurements were averaged to obtain the single-photon uncaging quantum yields.

Exemplary uncaging plots:

For an azobenzene-isomerization plot, the reader is referred to our recent publication on this topic.[1–3]

The data sets of the individual compounds plotted below have not been recorded on the same day and the slopes therefore cannot be compared to one another.

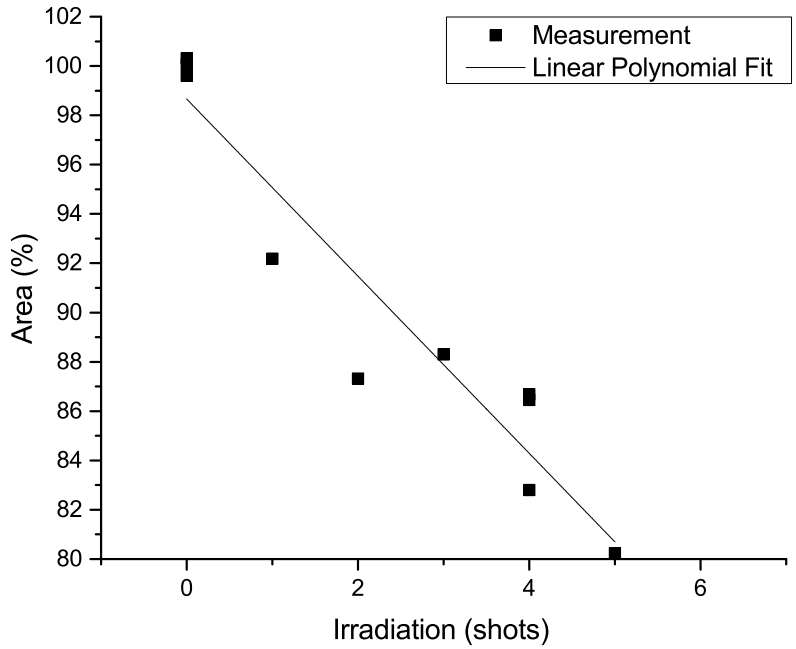


Figure S1. Single-photon uncaging of **3**. Values for the fit: y = 96.659 - 3.592 x; R^2 = 0.91.

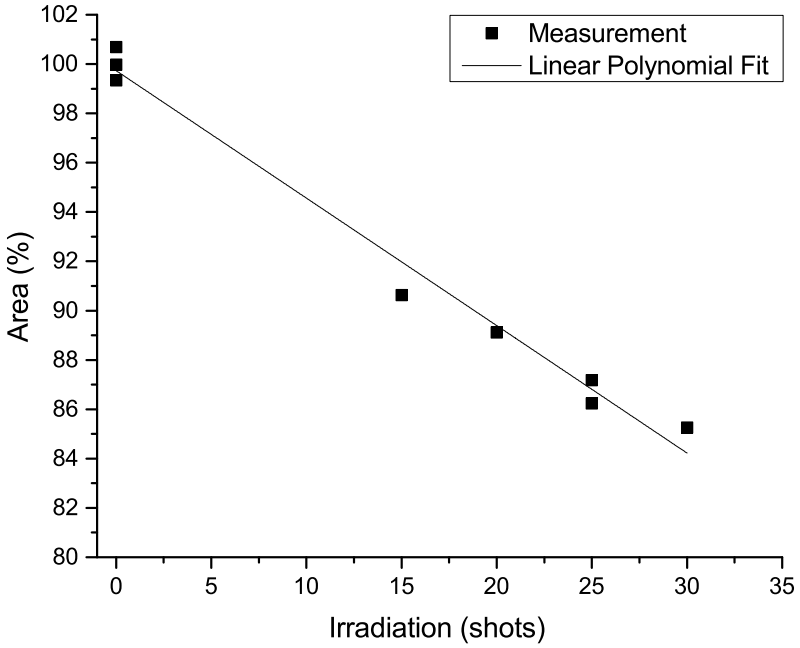


Figure S2. Single-photon uncaging of **4**. Values for the fit: y = 99.732 - 0.5170 x; R^2 = 0.99.

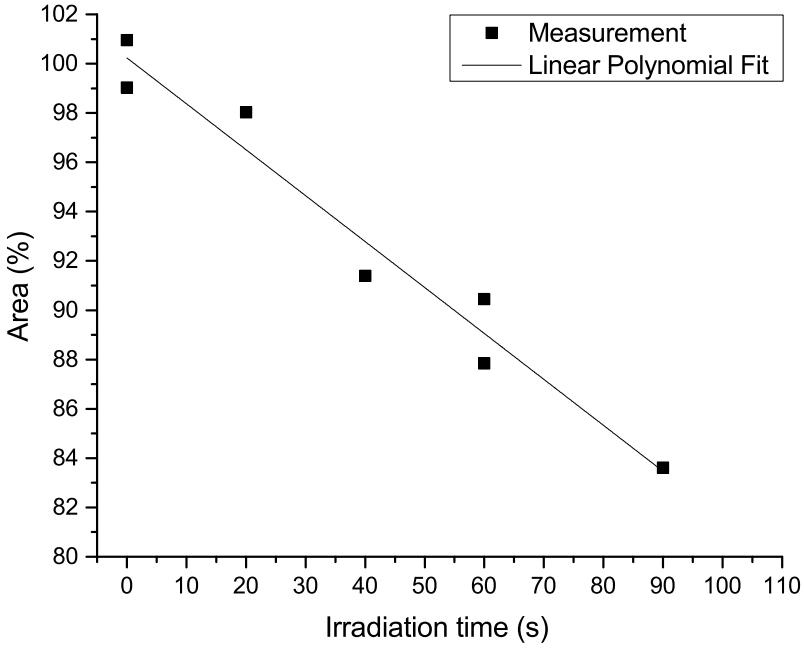


Figure S3. Single-photon uncaging of **5**. Values for the fit: y = 100.24 - 0.1863 x; R^2 = 0.96.

Two-Photon Uncaging Action Cross-Sections

The two-photon uncaging action cross-sections were determined as previously described.[4,5] In short, the samples were dissolved in a 1:1 mixture of acetonitrile and phosphate buffer saline (pH=7.2, 20 mM) to have an optical density OD(λ=400 nm)=0.2. As reference, 7-hydroxycoumarin-4-ylmethyl acetate[6] was dissolved in a 1:1 mixture of acetonitrile and phosphate buffer saline (pH=7.2, 20 mM) to have OD(λ=400 nm)=0.2. The respective solutions were separately illuminated for certain time intervals in Hellma 105.202-QS cuvettes (window size 2.5x2 mm², 50 µL volume, 10 mm light path). A fresh aliquot was used for each irradiation. An amplified Ti:sapphire laser system was used to obtain 90 fs pulses centered at 800 nm with a 5 kHz repetition rate. The intensity was reduced to 40 to 100 µJ using a neutral density filter. The beam diameter was reduced to only illuminate the cuvette window by the use of a telescope. The disappearance of the caged compounds was detected by integration of the peaks in the UV traces of analytical HPLC measurements and plotted against the irradiation time. Exemplary plots including linear fits are shown in the section below. The slopes m of these curves were used to calculate the two-photon uncaging action cross-sections, according to the following formula:

$$\phi_{sample} = \phi_{reference} \times \frac{m_{sample}}{m_{reference}}$$

At least three independent sets of measurements were averaged to obtain the two-photon uncaging action cross-section.

Exemplary uncaging plots:

The data sets of the individual compounds plotted below have not been recorded on the same day and the slopes therefore cannot be compared to one another.

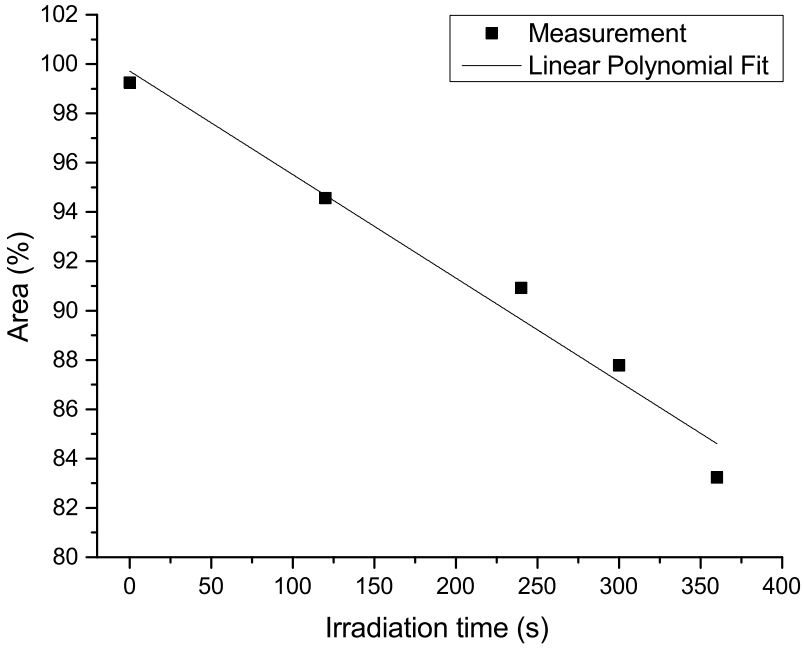


Figure S4. Two-photon uncaging of **CouOAc**. Values for the fit: y = 99.71 - 0.04197 x; R^2 = 0.97.

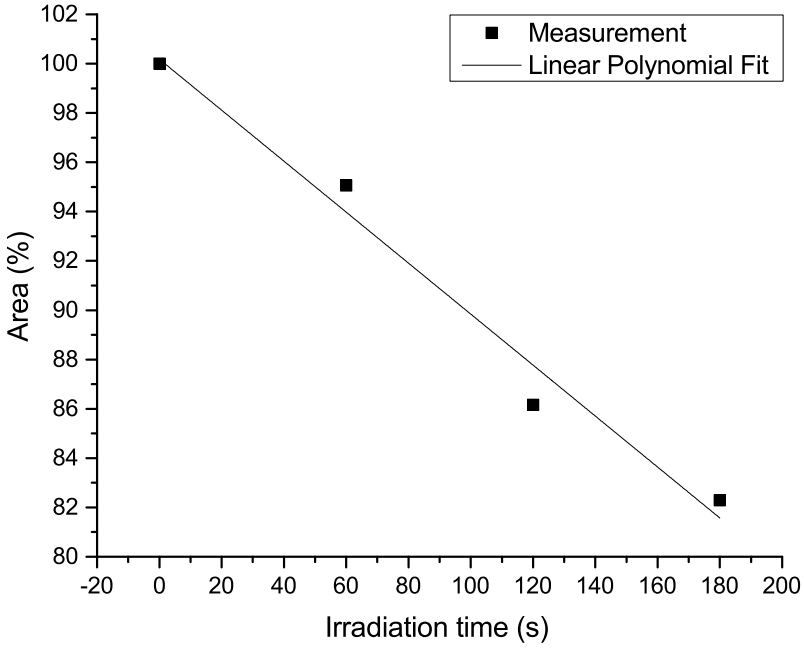


Figure S5. Two-photon uncaging of **3**. Values for the fit: y = 100.18 - 0.1034 x; R^2 = 0.98.

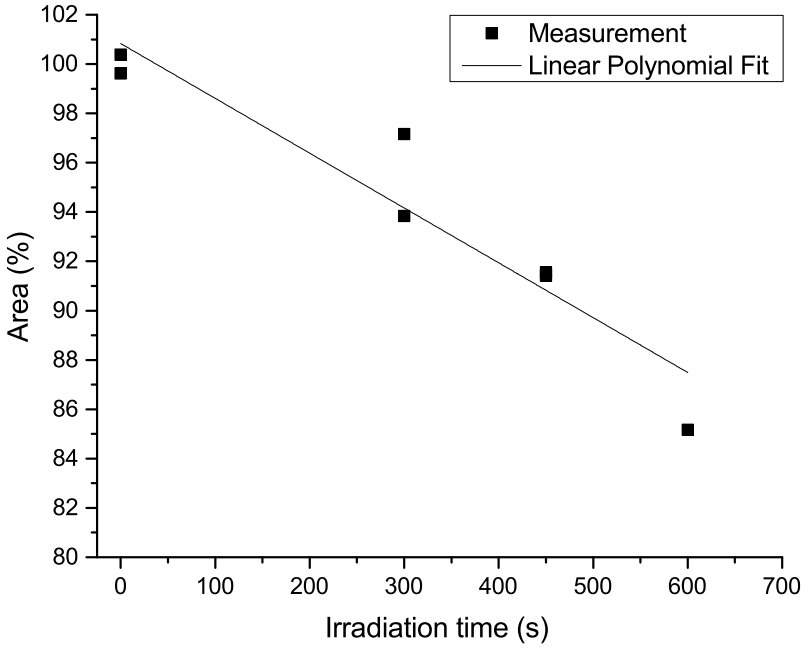


Figure S6. Two-photon uncaging of **4**. Values for the fit: y = 100.83 - 0.0222 x; R^2 = 0.90.

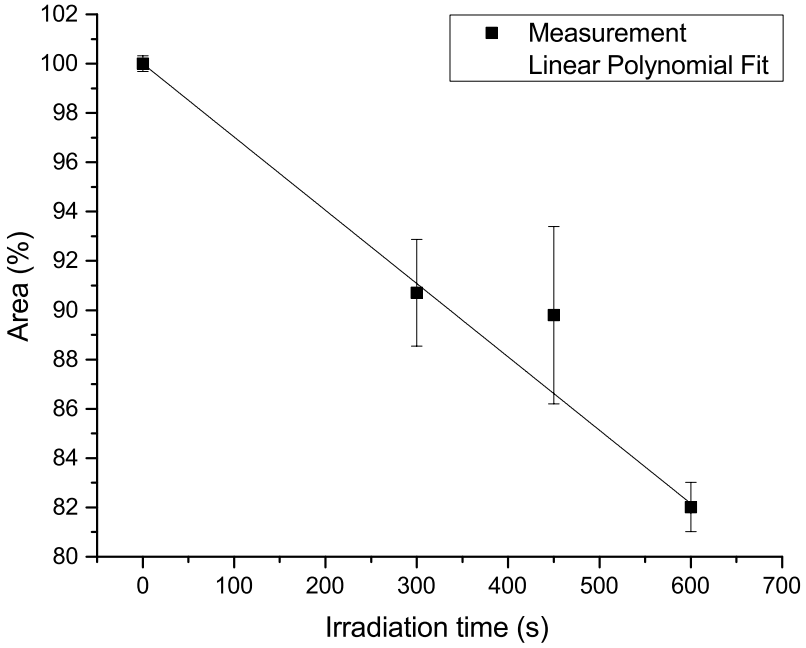


Figure S7. Two-photon uncaging of **5**. Values for the fit: y = 100.02 - 0.0297 x; R^2 = 0.99.

Cell culture

A549 human lung adenocarcinoma epithelial cells were cultured in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (FCS) (Gibco). HEK 293 human embryonic kidney

cells were maintained in DMEM containing 10% FCS. All cell lines were complemented with penicillin (100 U/mL), and streptomycin (100 µg/mL) and stored in a humidified incubator containing 5% CO2 at 37 °C.

Fluorescence quantification by ELISA READER

A549 and HEK 293 cells were seeded at a density of 3 x 10^5 cells/mL in a 6 cm dish in 3 mL medium. The next day, cells were treated for 4 h with **15** (1 µM). After trypsinisation and 3 washes in 1x PBS, 1.8 x 10^5 cells were seeded in triplicate in a 96-well plate and fluorescence quantified with 510 nm emission and 395 nm excitation wavelength in a SpectraMax M5 microplate reader.

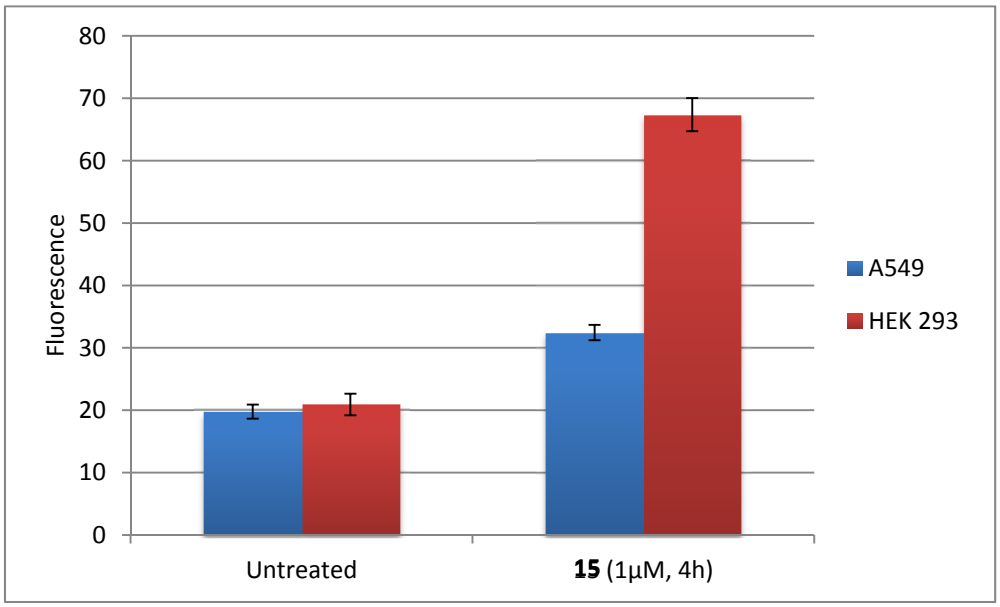


Figure S8. Fluorescence of cells with and without treatment with fluorescent

In Vitro Fluorescence Evaluation.

Cellular localization of **15** was assessed by fluorescence microscopy. Cells were grown on 35 mm Cellview glass bottom dishes (Greiner) at a density of 2.5 × 10^5 cells/mL and incubated for 1, 2 or 4 h with the complex at 10 µM at 37 °C. Cells were washed 3x in PBS 1x and fixed in 4% formaldehyde solution prior visualization on an inverted Olympus IX microscope. The GFP was visualized using the FITC channel settings.

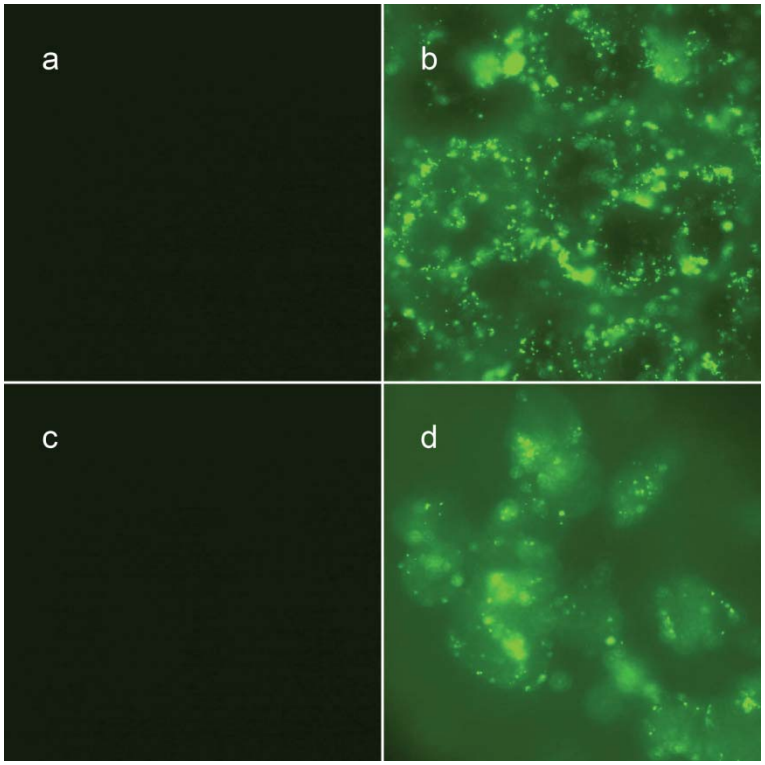


Figure S9. Fluorescence microscopy of A549 (a, b) and HEK 293 (c, d) cells, untreated (a, c) and after 4 h of incubation with **15** (b, d).

Enzyme Inhibition Assays

Enzyme inhibition was tested using the "COX Fluorescent Inhibitor Screening Assay Kit" from Cayman Chemical Company (Ann Harbor, MI, USA). All experiments were performed at least as duplicates of duplicates. The assay was carried out as described in the manufacturer's manual, with the following adjustments.

The incubation time for all samples was 20 min, as opposed to the 5 min period recommended in the manual.

For the experiments in which the COX-2 enzyme was irradiated, 10 µL of ready-to-use diluted COX-2 solution was further diluted with COX-FIS Assay Buffer 1x diluted to 50 µL and subsequently irradiated in a Hellma 105.202-QS cuvette as described above for the acquisition of two-photon uncaging cross-sections. The power was adjusted to values of 180-200 µJ with neutral density filters. The solution was then transferred to the 96-well plate. The amount of buffer used for this determination was reduced by 40 µL to compensate for the additional volume contributed by the diluted enzyme solution. As reference, the same procedure was done without irradiating. A decrease in COX-2 activity was observed for both the irradiated and non-irradiated samples, which could be due to aggregation effects in the

cuvette. The effect was identical for both the irradiated and the non-irradiated enzyme samples.

Exemplary Enzyme Activity Plots with Irradiated and Non-Irradiated Caged Compounds

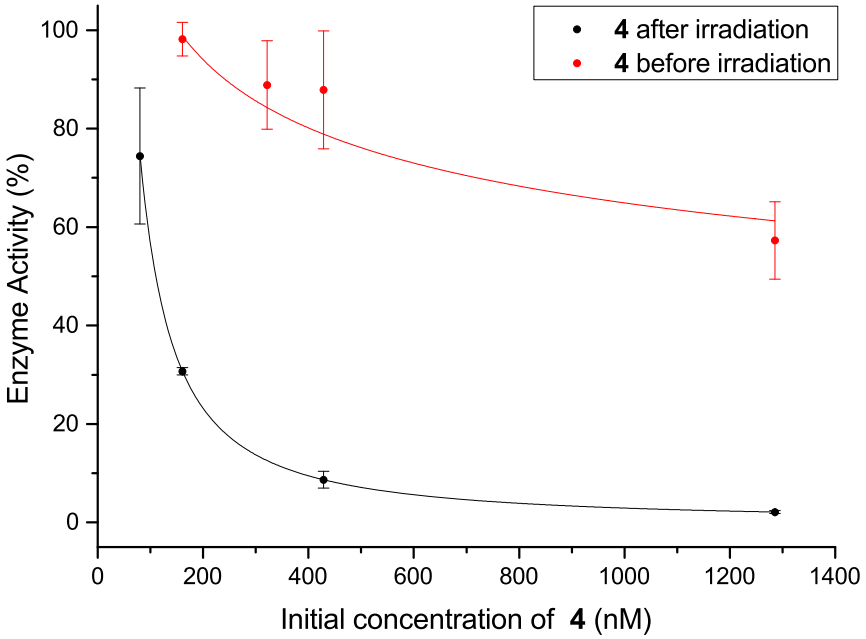


Figure S10. Enzyme inhibition of caged Celecoxib **4** before and after irradiation at 800 nm with a pulsed laser.

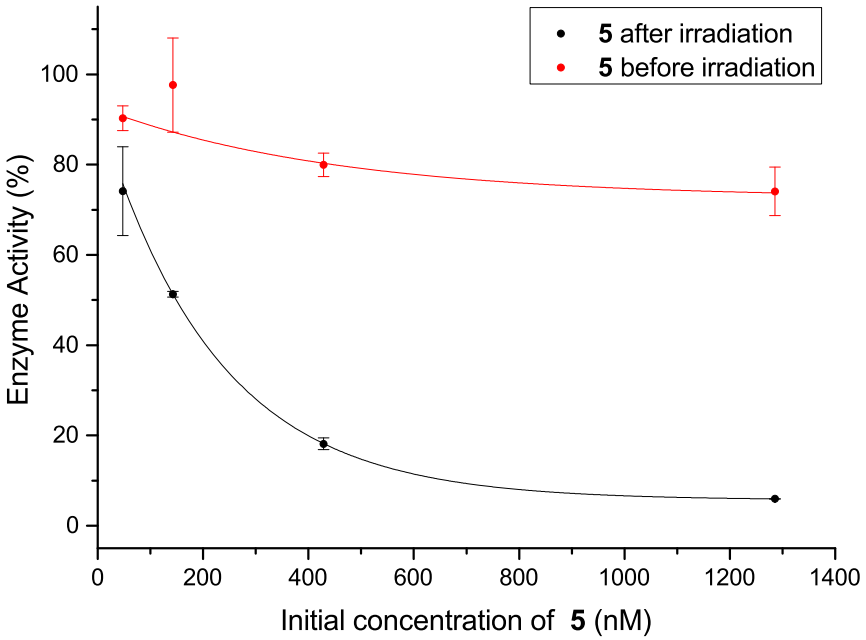
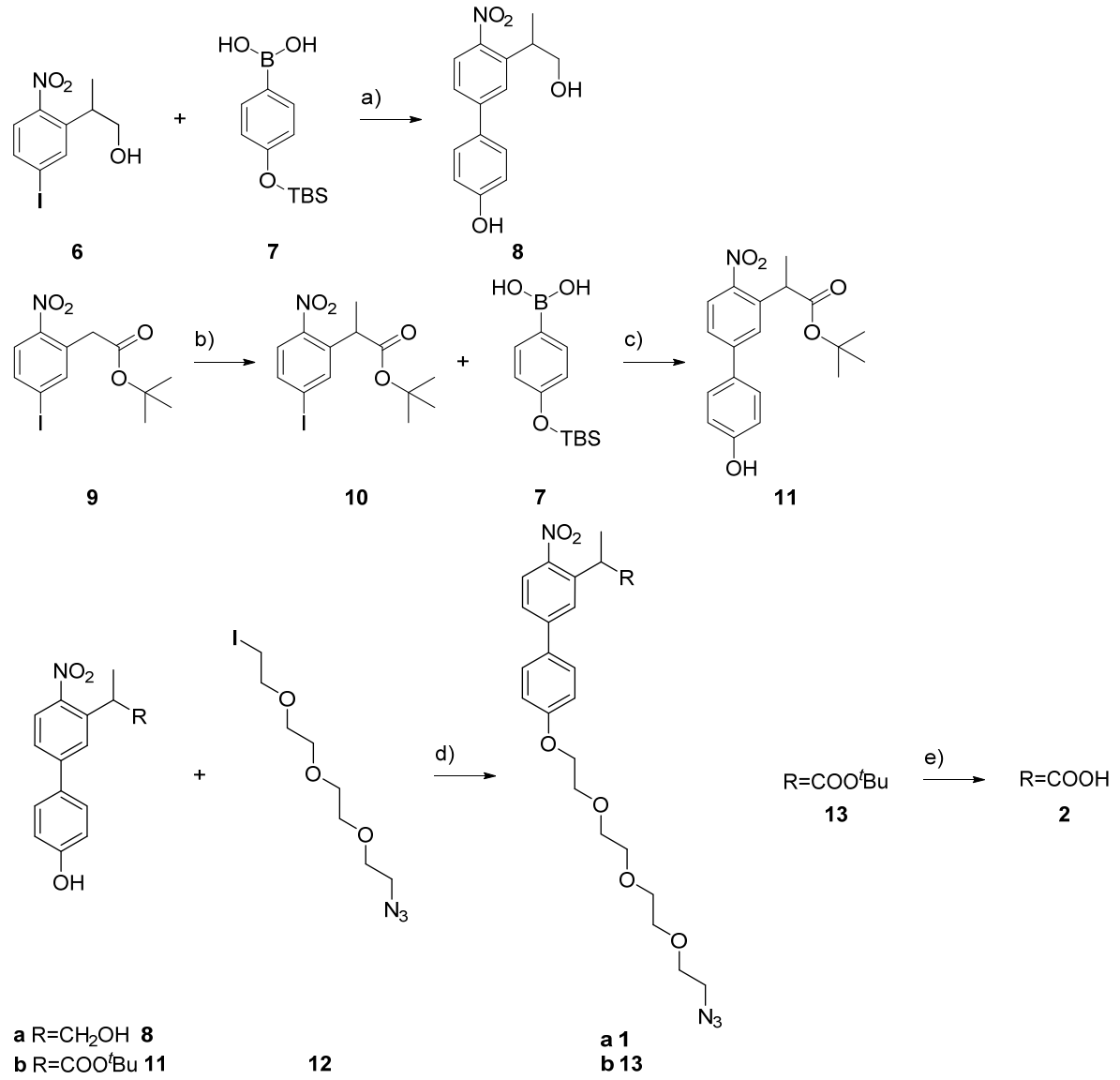


Figure S11. Enzyme inhibition of caged Celecoxib **4** before and after irradiation at 800 nm with a pulsed laser.

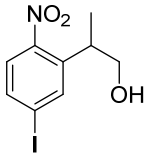
Syntheses

Overview over Synthetic Steps not Depicted in the Article



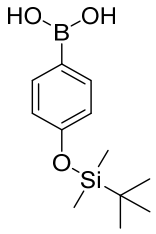
Scheme S1. Synthetic steps not depicted in the main article; procedures: a) 1. $Pd(PPh_3)_4$, Na_2CO_3, toluene, 90 °C, 16 h, 2. Tetrabutylammonium fluoride, THF, rt, 2 h, 98%; b) CH_3I, NaH, THF, 0 °C → rt, 64 h, 45%; c) $Pd(PPh_3)_4$, Na_2CO_3, water, toluene, 95 °C, 17 h, 2. Tetrabutylammonium fluoride, THF, rt, 3 h, 84%; d) K_2CO_3, MeOH, microwave, 100 °C, 10 h, a: 78%, b: 51%; e) Trifluoroacetic acid, CH_2Cl_2, rt, 20 h, 99%.

Compound 6



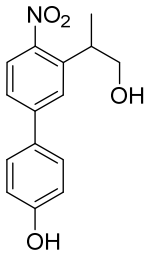
The title compound was prepared as described by Bühler *et al*.[7] The analytical data matched what was reported there.

Compound 7



The title compound was prepared as described by Jeanjot *et al*.[8] The analytical data matched what was reported there.

Compound 8



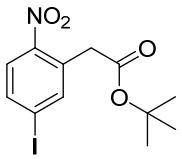
Under an atmosphere of N_2, **6** (307 mg, 1 mmol), **7** (303 mg, 1.19 mmol), toluene (20 mL), saturated Na_2CO_3 solution (10 mL), and water (3 mL) were mixed and degassed. $Pd(PPh_3)_4$ (116 mg, 0.10 mmol) was added and the mixture was stired at 105 °C for 20 min, and 90 °C for 3.5 h. The reaction mixture was allowed to cool down to rt. Water was added and extracted with diethyl ether (3x). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*.

Under exclusion of light and moisture, the residue (710 mg) was dissolved in THF (8 mL), Tetrabutylammonium fluoride (1 M in THF, 2 mL) was added and the mixture was stirred for 2 h at rt. Afterwards, saturated NH_4Cl solution was added. The organic layer was diluted with diethyl ether, separated and washed with water and brine. Drying (Na_2SO_4), filtration, and

concentration afforded the crude product. Column chromatography (silica gel, hexanes/EtOAc 7:4) afforded **8** as a yellow solid (98%, relative to **6**).

R_f = 0.09 (Hexanes/EtOAc 7:3). IR (KBr) 3469 (s), 2952 (w), 2925 (w), 1635 (m), 1612 (s), 1517 (m), 1349 (m), 823 (s) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) ∂ 7.84 (d, J = 8.5 Hz, 1 H), 7.58 (d, J = 1.9 Hz, 1 H), 7.53–7.40 (m, 3 H), 6.91 (d, J = 8.6 Hz, 2 H), 5.11 (bs, 1 H), 3.90–3.75 (m, 2 H), 3.72–3.58 (m, 1 H), 1.36 (d, J = 7.0 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): ∂ 156.3, 149.0, 145.4, 138.9, 131.7, 128.8, 126.3, 125.3, 125.0, 116.0, 68.0, 36.4, 17.6. HRMS m/z calcd. for $C_{15}H_{15}NNaO_4$ ($[M+Na]^+$) 296.08933, found 296.08886.

Compound 9

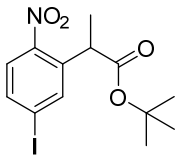


The title compound was synthesized as described by Katayama *et al*.[9] The analytical data matched what was reported there.

Additional analytical data:

R_f = 0.13 (hexanes/EtOAc 40:1). ^{13}C NMR (100 MHz, $CDCl_3$): ∂ 168.5, 148.5, 142.1, 137.6, 132.1, 126.4, 100.8, 82.2, 40.6, 27.9. HRMS m/z calcd. for $C_{12}H_{14}INNaO_4$ ($[M+Na]^+$) 385.98597, found 385.98598.

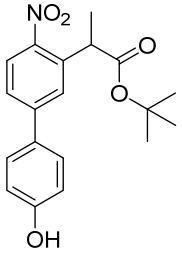
Compound 10



Under an atmosphere of N_2 **9** (1.00 g, 2.75 mmol) was added to a suspension of NaH (60% in oil, 110 mg, 2.75 mmol) in dry THF (10 mL). The purple mixture was cooled to 0 °C and methyl iodide (343 µL, 782 mg, 5.51 mmol) was added dropwise. The mixture was stirred first for 2 h at 0 °C, then for 62 h at rt. The reaction was quenched by adding water and HCl (1 M, 5 mL) at 0 °C. The mixture was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexanes/EtOAc 42:1) to yield **10** as a light yellow solid (468 mg, 45%).

R_f = 0.19 (hexanes/EtOAc 38:3). IR (neat) 2975 (w), 1721 (s), 1598 (w), 1556 (w), 1514 (m), 1335 (m), 1243 (s), 1151 (s), 858 (m), 844 (m), 832 (m) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) ∂ 7.80 (d, J = 1.9 Hz, 1 H), 7.74 (dd, J = 1.9 Hz, 8.5 Hz, 1 H), 7.62 (d, J = 8.5 Hz, 1 H), 4.15 (q, J = 7.2 Hz, 1 H), 1.54 (d, J = 7.2 Hz, 3 H),1.38 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): ∂ 171.6, 148.7, 138.4, 137.4, 137.0, 126.1, 100.4, 81.8, 42.1, 27.8, 17.4. ESI-MS m/z 400.00 (100, $[M+Na]^+$) 343.94 (10, $[M-^tBu+Na]^+$). HRMS m/z calcd. for $C_{13}H_{16}INNaO_4$ ($[M+Na]^+$) 400.00162, found 400.00153.

Compound 11

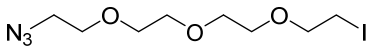


10 (250 mg, 663 µmol) and **7** (201 mg, 694 µmol) were mixed in toluene (10 mL), saturated Na_2CO_3 solution (6.4 mL) and water (2 mL). N_2 was bubbled through the bi-layered solution for 20 min. $Pd(PPh_3)_4$ (76.6 mg, 66.3 µmol) was added and the mixture stirred at 95 °C for 17 h. The solution was allowed to cool to rt. Water and Et_2O were added and the two layers were separated. The aqueous layer was extracted wit Et_2O (3x). The combined organic fractions were dried ($MgSO_4$), filtered, and concentrated.

Tetra-*n*-butylammonium fluoride (1 M in THF, 1.33 mL, 1.33 mmoL) was added to a solution of the crude residue (289 mg) in THF (8 mL). The solution was stirred for 3 h at rt. Saturated NH_4Cl solution and Et_2O were added and the two layers were separated. The aqueous layer was extracted wit Et_2O (3x). The combined organic fractions were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (hexanes/EtOAc 7:3), yielding pure **11** (191 mg, 84% relative to **10**) as a colorless solid.

R_f = 0.65 (hexanes/EtOAc 3:7). IR (neat) 3317 (w), 2989 (w), 1725 (s), 1604 (m), 1579 (m), 1514 (s), 1332 (s), 1304 (m), 1220 (m), 1152 (m), 830 (s) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) ∂ 8.01 (d, J = 8.6 Hz, 1 H), 7.60 (d, J = 2.0 Hz, 1 H), 7.54 (dd, J = 8.6 Hz, 2.0 Hz, 1 H), 7.48 (d, J = 8.6 Hz, 2 H), 6.92 (d, J = 8.6 Hz, 2 H), 5.12 (bs, 1 H), 4.32 (q, J = 7.2 Hz, 1 H), 1.62 (d, J = 7.2 Hz, 3 H), 1.41 (s, 9 H). ^{13}C NMR (125 MHz, $CDCl_3$) ∂ 172.7, 156.5, 147.2, 145.8, 136.3, 131.2, 128.7, 127.6, 125.7, 125.6, 116.0, 81.6, 42.6, 27.9, 17.6. HRMS m/z calcd. for $C_{19}H_{21}NNaO_5$ ($[M+Na]^+$) 366.13119, found 366.13131.

Compound 12

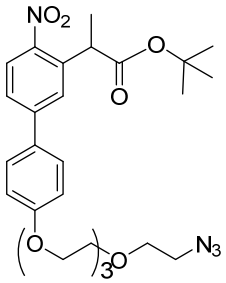


The title compound was prepared as described in the literature. The analytical data matched what was reported before,[10] except for the 13C{1H} NMR spectrum in which 8 instead of 7 signals were observed (see below).

Additional/deviating analytical data:

^{13}C NMR (125 MHz, $CDCl_3$): *∂* 72.0, 70.74, 70.71, 70.3, 70.1, 70.0, 50.7, 2.9. HRMS m/z calcd. for $C_8H_{16}IN_3NaO_3$ ($[M+Na]^+$) 352.01286, found 352.01288.

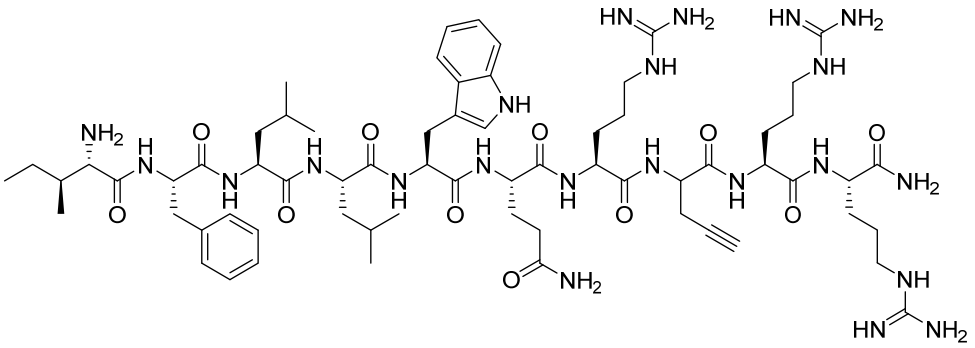
Compound 13



11 (300 mg, 0.874 mmol), **12** (261 mg, 0.794 mmol) and K_2CO_3 (121 mg, 0.874 mmol) were suspended in MeOH (2 mL). The mixture was stirred and heated with a microwave reactor for 12 h at 100 °C. The solvent was removed and the red residue was taken up in saturated NH_4Cl solution and CH_2Cl_2. The two layers were separated. The organic layer was washed with saturated NH_4Cl solution (2x) and water. The combined aqueous layers were extracted with CH_2Cl_2 (3x). The combined organic layers were washed with water, dried ($MgSO_4$), filtered, and concentrated. Purification with column chromatography (hexanes/EtOAc 8:1 → 1:1) gave pure **13** as yellow oil (245 mg, 51%).

R_f = 0.43 (hexanes/EtOAc 3:7). IR (neat) 2880 (w), 2103 (s), 1726 (m), 1605 (s), 1516 (s), 1344 (s), 1248 (s), 1149 (s), 827 (m) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) *∂* 8.01 (d, *J* = 8.5 Hz, 1 H), 7.61 (d, *J* = 1.9 Hz 1 H), 7.58–7.49 (m, 3 H), 7.04–6.99 (m, 2 H), 4.31 (q, *J* = 7.2 Hz, 1 H), 4.23–4.14 (m, 2 H), 3.94–3.82 (m, 2 H), 3.79–3.63 (m, 10 H), 3.44–3.33 (m, 2 H), 1.61 (d, *J* = 7.2 Hz, 3 H), 1.40 (s, 9 H). ^{13}C NMR (125 MHz, $CDCl_3$) *∂* 172.4, 159.5, 147.3, 145.7, 136.5, 131.3, 128.5, 127.6, 125.6, 125.5, 115.2, 81.4, 70.9, 70.73, 70.72, 70.69, 70.0, 69.7, 67.6, 50.7, 42.5, 27.9, 17.6. HRMS m/z calcd. for $C_{27}H_{36}N_4NaO_8$ ($[M+Na]^+$) 567.24254, found 567.24254.

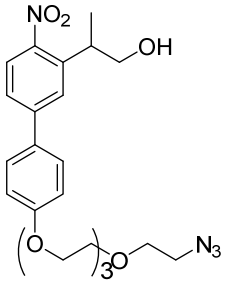
Targeting Peptide IFLLQR-Pra-RR 14



The peptide synthesis was performed according to previously published procedures.[11] In short, solid-phase peptide synthesis was carried out manually in a single-use polypropylene syringe with a filter frit. The syringe was filled with 515 mg Tentagel S Ram (0.24 mmol/g). The resin was swollen in DMF for 1 h before use. All reactions were performed on a mechanical shaker with 600 rpm, soaking approximately 2.5–3.5 mL of freshly prepared solutions into the syringe. Fmoc deprotection steps were performed with piperidine in DMF (2:8, v/v; two runs of 2 min and 10 min, repsectively).Fmoc/Pbf protected amino acids (4 equiv.) were pre-activated in Eppendorf tubes before every coupling step for 5 min with TBTU (3.9 equiv.) and DIPEA (10 equiv.) in DMF under sonication. For each coupling step the resin beads were treated with the activated acid and subsequently washed with DMF (5x) and CH_2Cl_2 (5x). The coupling step was monitored with the Kaiser test. The resin beads were then washed again with DMF (5x). The whole procedure (deprotection, coupling, monitoring) was repeated for every monomer until the sequence was completed. The resin was then shrunk with methanol (30 min) and dried under vacuum. Final cleavage of the peptide from the resin and deprotection of the Pbf side chain protecting group were simultaneously performed in TFA/triisopropylsilane (TIS)/H_2O (38:2:1, v/v/v; 3x 2 h). Following the removal of all volatiles under reduced pressure, the crude product was purified by preparative HPLC (20 mL/min, H_2O+0.1% TFA : acetonitrile = 0 min 95:5, 31 min 0:100, 33 min 0:100, 36 min 95:5, 37 min 95:5).

MALDI-MS calcd. for $C_{66}H_{105}N_{22}O_{11}$ ($[M+H]^+$) 1381.8, found 1381.6. HPLC (1 mL/min, acetonitrile: H_2O+0.1%TFA = 0 min 0:100, 30 min 100:0, 35 min 100:0, 36.5 min 0:100): t_R = 12.0 min.

Compound 1

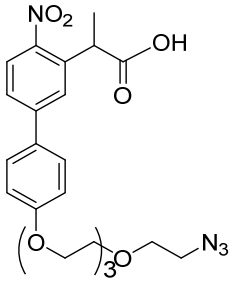


8 (583 mg, 2.14 mmol), **12** (787 mg, 1.95 mmol) and K_2CO_3 (296 mg, 2.14 mmol) were suspended in MeOH (4 mL). The mixture was stirred and heated with a microwave reactor for 10 h at 100 °C. The solvent was removed and the red residue was taken up in NaOH (1 M) and CH_2Cl_2. The two layers were separated. The organic layer was washed with NaOH (1 M). The combined aqueous layers were extracted with CH_2Cl_2 (3x). The combined organic layers were washed with water, dried ($MgSO_4$), filtered, and concentrated. Purification with column chromatography gave pure **1** as yellow oil (726 mg, 78%).

The combined aqueous layers were acidified with conc. HCl and subsequently extracted with CH_2Cl_2 (3x). The combined organic extracts were washed with water, filtered, and concentrated. The residue was pure **8** (119 mg, 20% of what had been used).

R_f = 0.32 (CH_2Cl_2/MeOH 95:5). IR (KBr) 3446 (w), 2873 (w), 2102 (m), 1604 (m), 1583 (w), 1515 (s), 1478 (w), 1348 (w), 1292 (m), 1248 (s), 1116 (m), 1059 (m), 827 (m), 632 (m), 536 (m) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): ∂ 7.85 (d, J = 8.5 Hz, 1 H), 7.61 (s, 1 H), 7.55–7.45 (m, 3 H), 7.02 (d, J = 8.8 Hz, 2 H), 4.21–4.16 (m, 2 H), 3.94–3.58 (m, 15 H), 3.37 (t, J = 5.0 Hz, 2 H), 1.81 (bs, 1 H), 1.38 (d, J = 6.9 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): ∂ 159.4, 148.8, 145.4, 140.0, 131.6, 128.4, 126.2, 125.2, 125.0, 115.2, 70.8, 70.68, 70.67, 70.6, 70.0, 69.6, 67.9, 67.5, 50.6, 36.4, 17.6. ESI-MS m/z 497.20 (100, $[M+Na]^+$), 513.18 (10, $[M+K]^+$).HRMS m/z calcd. for $C_{23}H_{30}N_4NaO_7$ ($[M+Na]^+$) 497.20067, found 497.20128.

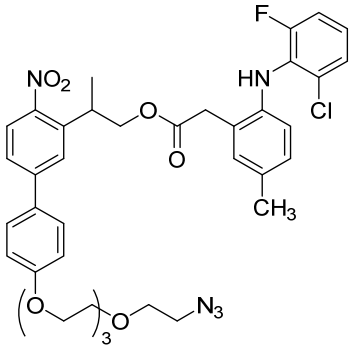
Compound 2



13 (219 mg, 403 µmol) was dissolved in 5% TFA in CH_2Cl_2 (v/v; 38 mL). The mixture was stirred under exclusion of light for 20 h. Water was added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give pure **2** as a highly viscous oil (197 mg, 99%).

IR (neat) 2921 (w), 2877 (w), 2103 (m), 1731 (m), 1604 (s), 1585 (m), 1516 (s), 1343 (s), 1249 (s), 1184 (m), 1121 (m), 943 (w), 827 (m) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) ∂ 8.06 (d, J = 8.5 Hz, 1 H), 7.62 (d, J = 1.9 Hz 1 H), 7.60–7.46 (m, 3 H), 7.06–6.99 (m, 2 H), 4.46 (q, J = 7.1 Hz, 1 H), 4.18 (m, 2 H), 3.92–3.86 (m, 2 H), 3.78–3.63 (m, 10 H), 3.40–3.34 (m, 2 H), 1.68 (d, J = 7.1 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) ∂ 176.9, 159.7, 146.8, 146.2, 135.5, 131.1, 128.6, 128.0, 126.1, 126.0, 115.3, 70.9, 70.72, 70.71, 70.68, 70.0, 69.7, 67.6, 50.7, 41.6, 17.6. HRMS m/z calcd. for $C_{23}H_{28}N_4NaO_8$ ($[M+Na]^+$) 511.17993, found 511.18034.

Caged Lumiracoxib 3

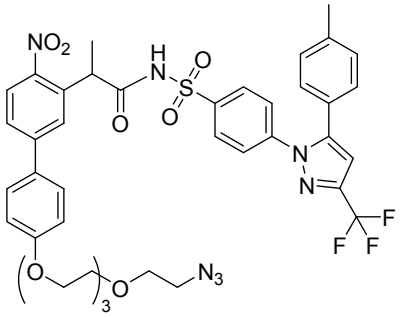


Under an atmosphere of N_2, **1** (52.4 mg, 110 µmol) was dissolved in dry CH_2Cl_2 (10 mL). At 0 °C, *N*,*N'*-dicyclohexylcarbodiimide (34.2 mg, 166 µmol), 4-dimethylaminopyridine (1.3 mg, 11 µmol), and Lumiracoxib (64.9 mg, 0.221 mmol) were added. The resulting suspension was stirred at 0 °C for 10 min and subsequently for 20 h at rt. Saturated $NaHCO_3$ solution was added and the mixture was extracted with CH_2Cl_2 (4x). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The crude residue was purified by column chromatography (hexanes/EtOAc 7:3 → 1:1). The product containing fractions were

suspended in acetonitrile and filtered with a Pasteur pipette filled with silica to give pure **3** as a slightly yellow solid (66.0 mg, 80%).

R_f = 0.51 (hexanes/EtOAc 3:7). IR (neat) 2883 (w), 2103 (m), 1730 (s), 1604 (m), 1584 (m), 1514 (s), 1479 (m), 1348 (m), 1291 (m), 1245 (s), 1137 (m), 1037 (w), 1046 (w), 906 (m), 827 (m), 764 (m), 715 (m) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) ∂ 7.83 (d, J = 8.5 Hz, 1 H), 7.52 (d, J = 1.9 Hz, 1 H), 7.48–7.41 (m, 3 H), 7.18 (dt, J = 8.1 Hz, 1.3 Hz, 1 H), 7.02–6.93 (m, 3 H), 6.91–6.85 (m, 3 H), 6.63 (bs, 1 H), 6.56 (dd, J = 8.0 Hz, 3.2 Hz, 1 H), 4.41–4.31 (m, 2 H), 4.20–4.13 (m, 2 H), 3.95–3.85 (m, 3 H), 3.74–3.63 (m, 12 H), 3.38 (t, J = 5.0 Hz, 2 H), 2.21 (s, 3 H), 1.38 (d, J = 7.0 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): ∂ 172.2, 159.4, 155.3 (d, J = 248 Hz), 148.5, 145.4, 139.7, 137.8, 131.6, 131.4, 131.2, 129.6 (d, J = 13.1 Hz), 128.42, 128.39, 127.2 (d, J = 4.36 Hz), 125.9, 125.38 (d, J = 3.43 Hz), 125.35, 125.0, 123.9, 121.9 (d, J = 8.66 Hz), 118.3 (d, J = 3.05 Hz), 115.2, 114.8 (d, J = 20.2 Hz), 70.9, 70.71, 70.69, 70.66, 70.0, 69.6, 68.6, 67.6, 50.7, 38.2, 33.2, 20.5, 17.8. ^{19}F NMR (375 MHz, $CDCl_3$): ∂ -118.9. HRMS m/z calcd. for $C_{38}H_{41}ClFN_5NaO_8$ ($[M+Na]^+$) 772.25199, found 772.25115. HPLC (1 mL/min, acetonitrile : H_2O+0.1%TFA = 0 min 10:90, 27 min 100:0, 31 min 100:0, 36 min 1:99, 38 min 1:99, 40 min 10:90, 41 min 10:90): t_R = 22.6 min. ϕ (λ=355 nm) = 0.094 ±0.02. $\delta_a\Phi_u$ (λ=800 nm) = 0.37 ±0.04 GM.

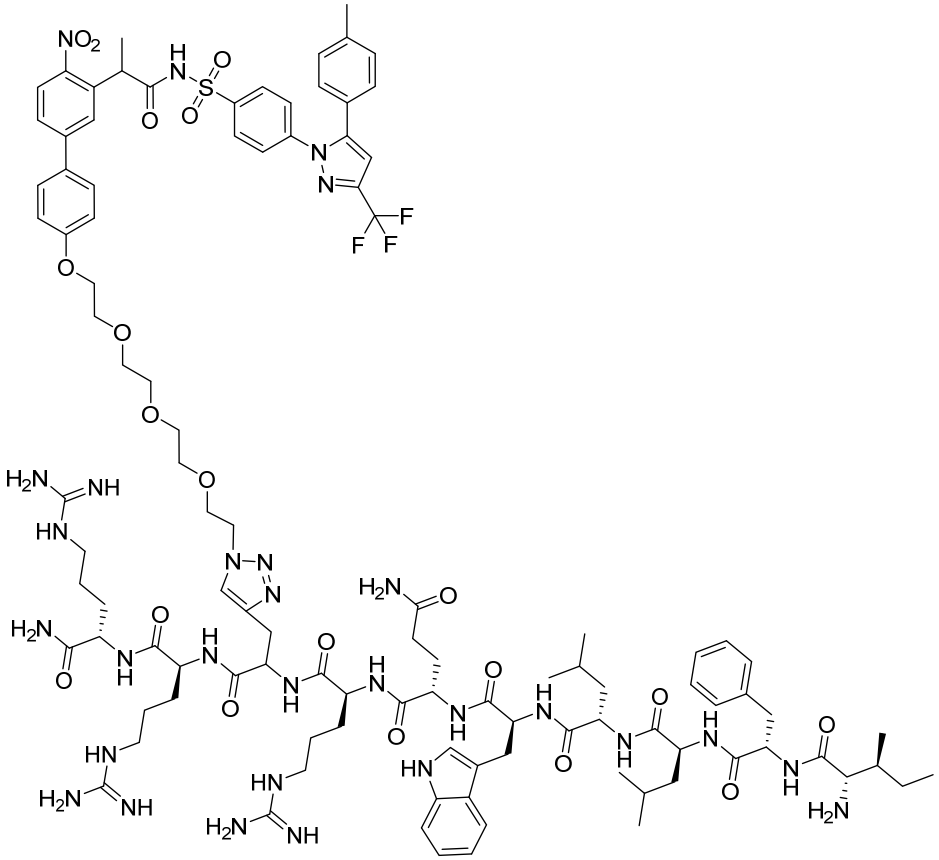
Caged Celecoxib 4



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 15.5 mg, 81.1 µmol) was added to a stirred solution of **2** (19.8 mg, 40.5 µmol), 4-dimethylaminopyridine (DMAP, 0.5 mg, 4 µmol) and Celecoxib (16.5 mg, 43.3 µmol) in CH_2Cl_2 (1.5 mL) and the resulting reaction mixture was stirred at rt under exclusion of light for 2 h. Saturated NH_4Cl solution and EtOAc were added. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The crude residue was adsorbed to silica and purified by column chromatography (hexanes/EtOAc 5:1 → 1:2) to yield pure **4** (18.5 mg, 54%) as a yellowish solid.

R_f = 0.39 (CH_2Cl_2/MeOH 95:5). IR (neat) 2923 (w), 2103 (s), 1719 (m), 1603 (m), 1516 (m), 1451 (m), 1344 (s), 1237 (s), 1185 (m), 1159 (s), 1127 (s), 1094 (s), 1024 (m), 947 (m), 821 (s) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) ∂ 8.72 (bs, 1 H), 7.99–7.91 (m, 3 H), 7.59 (dd, *J* = 8.4 Hz, 1.9 Hz, 1 H), 7.49–7.35 (m, 5 H), 7.16 (d, *J* = 8.0 Hz, 2 H), 7.06 (d, *J* = 8.0 Hz, 2 H), 6.96 (d, *J* = 8.8 Hz, 2 H), 6.73 (s, 1 H), 4.24 (q, *J* = 6.9 Hz, 1 H), 4.15 (t, *J* = 4.8 Hz, 2 H), 3.89 (t, *J* = 4.8 Hz, 2 H), 3.78–3.62 (m, 10 H), 3.38 (t, *J* = 5.0 Hz, 2 H), 2.38 (s, 3 H), 1.51 (d, *J* = 6.9 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): 169.8, 159.9, 147.0, 146.8, 145.3, 144.3, 144.0, 143.4, 139.9, 137.4, 134.0, 130.3, 129.8, 129.4, 128.7, 128.5, 127.1, 126.7, 125.9, 125.6, 125.0, 115.3, 106.5, 70.9, 70.8, 70.73, 70.70, 70.1, 69.6, 67.6, 50.7, 41.6, 21.3, 17.3. HRMS m/z calcd. for $C_{40}H_{40}F_3N_7NaO_9S$ ($[M+Na]^+$) 874.24525, found 874.24481. HPLC (1 mL/min, acetonitrile : H_2O+0.1%TFA = 0 min 10:90, 27 min 100:0, 31 min 100:0, 36 min 1:99, 38 min 1:99, 40 min 10:90, 41 min 10:90): t_R = 21.0 min. ϕ (λ=355 nm) = 0.013 ±0.001. $\delta_a\Phi_u$ (λ=800 nm) = 0.063 ±0.008 GM.

Caged Targeted Celecoxib 5

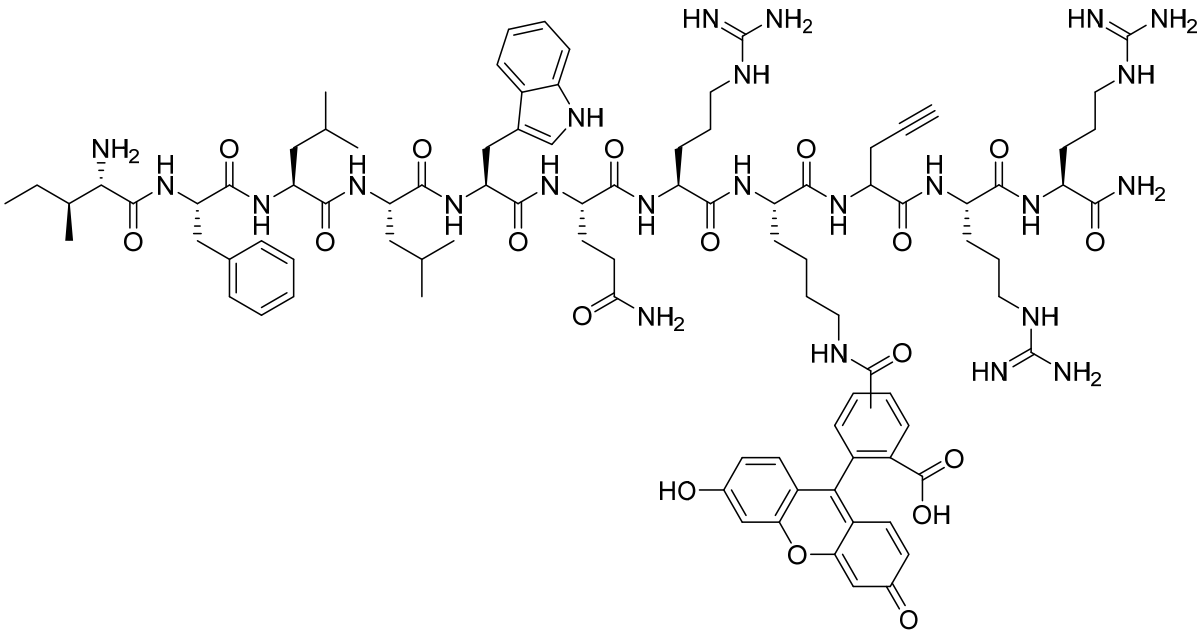


$CuSO_4$ (0.1 M in H_2O, 23.1 µL, 2.31 µmol) and sodium ascorbate (0.1 M in H_2O, 46.3 µL, 4.63 µmol) were mixed and shaken for 10 min. The red slurry was added to a solution of **4** (10.9 mg, 12.7 µmol) and targeting peptide IFLLQR(Pra)RR **14** (16 mg, 11.6 µmol) in a

mixture of THF/H_2O (2:1 v/v; 9 mL). The solution was stirred at rt for 23 h. To drive the reaction to completion, $CuSO_4$ (0.1 M in H_2O, 23.1 µL, 2.31 µmol) and sodium ascorbate (0.1 M in H_2O, 46.3 µL, 4.63 µmol) were added, the temperature increased to 60 °C, and stirring continued for 7 h. The solvents were removed by evaporation. The residue was dissolved in acetonitrile and H_2O with 0.1% TFA (1:2) and purified by preparative HPLC (20 mL/min, H_2O+0.1% TFA : acetonitrile = 0 min 95:5, 31 min 0:100, 33 min 0:100, 36 min 95:5, 37 min 95:5).

MALDI-MS calcd. for $C_{106}H_{145}F_3N_{29}O_{20}S$ ($[M+H]^+$) 2233.1, found 2233.5. HPLC (1 mL/min, acetonitrile : H_2O+0.1%TFA = 0 min 10:90, 12 min 53:47, 18 min 59:41, 27 min 100:0, 31 min 100:0, 36 min 1:99, 38 min 1:99, 40 min 10:90, 41 min 10:90): t_R = 14.9 min. ϕ (λ=355 nm) = 0.0047 ±0.0004. $\delta_a\Phi_u$ (λ=800 nm) = 0.053 ±0.008 GM.

Fluorescent Targeting Peptide IFLLQRK(fluorescein)-Pra-RR 15

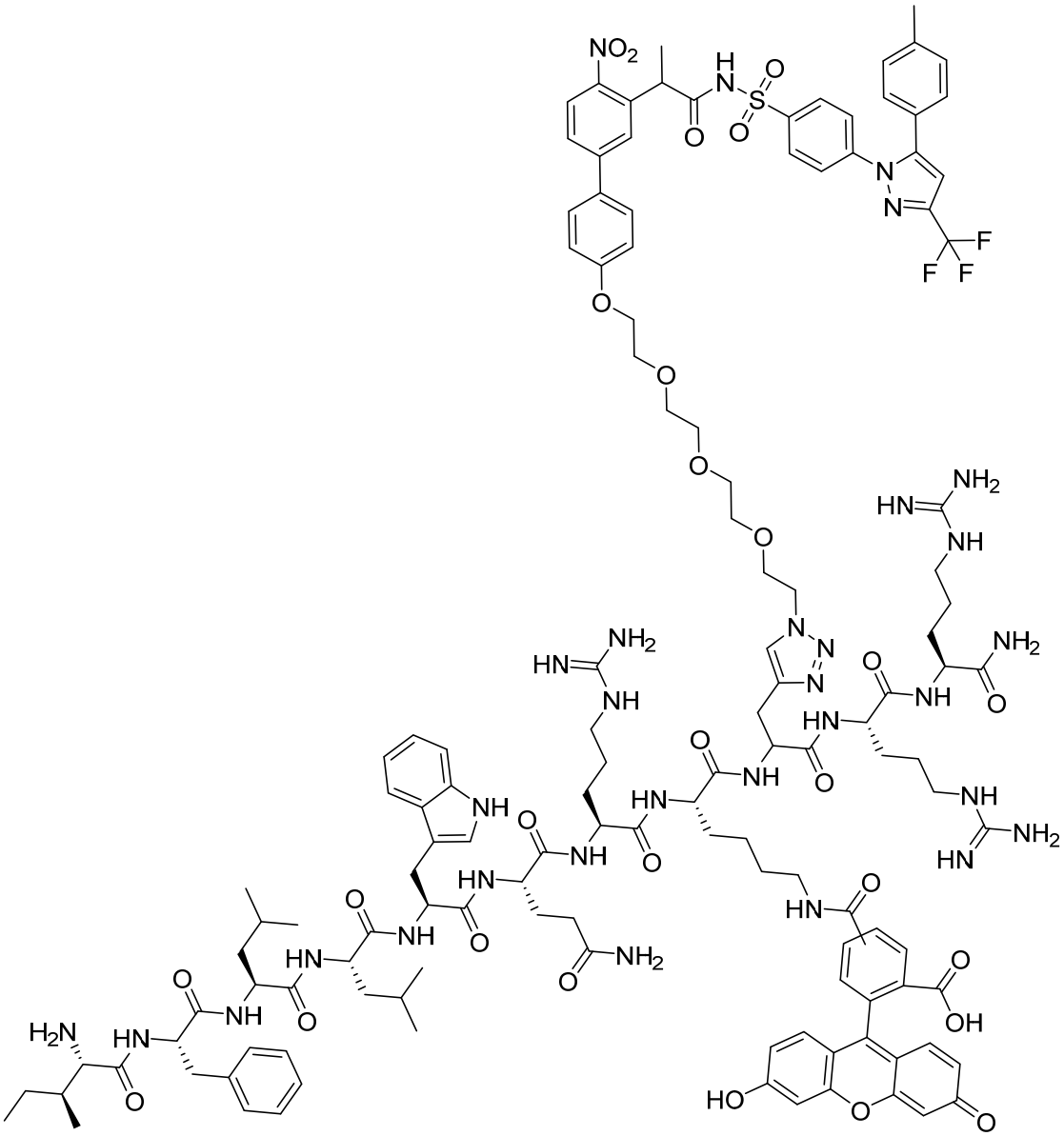


The peptide synthesis was performed as described above, with the following modifications. 4-Methyltrityl (Mtt) side chain protected lysine was additionally introduced into the sequence. After completion of the last amino acid coupling step, Kiton Red S (10 equiv.) in DMF were added to the resin and shaken together for 2 h. The reason for this additional step is that a non-covalent saturation of the resin with dye leads to improved yields for the dye-peptide coupling.[12,13] The resin was washed with DMF (5x) and CH_2Cl_2 (5x). The resin was treated with CH_2Cl_2/TFA/triisopropylsilane 94:1:5 (5x2 min) to remove the Mtt protecting group. The resin was washed (5x DMF, 5x CH_2Cl_2, 5x DMF) and shaken again for 1 h with Kiton Red S (10 equiv.) in DMF. The resin was washed with DMF (5x). NHS-fluorescein (2 equiv.)

dissolved in DMF was added and the mixture shaken for 3 h. The resin was washed with DMF (5x), CH_2Cl_2 (5x), and DMF (5x). The coupling step of the dye and the subsequent washing step was repeated. The terminal Fmoc protecting group was cleaved as described above, and the procedure continued with cleaving and purifying the peptide as described for the non-labeled variant. A mixture of isomers (resulting from the position 5 and 6 substituted fluorescein) was obtained in fair purity (90%) and used like this in the synthesis of **16**.

MALDI-MS calcd. for $C_{93}H_{127}N_{24}O_{18}$ ($[M+H]^+$) 1868.0, found 1868.0. HPLC (0.75 mL/min, acetonitrile : H_2O+0.1%TFA = 0 min 10:90, 12 min 53:47, 18 min 59:41, 27 min 100:0, 31 min 100:0, 36 min 1:99, 38 min 1:99, 40 min 10:90, 41 min 10:90): t_R = 13.4 and 13.7 min.

Caged Targeted Fluorescently Labeled Celecoxib 16



$CuSO_4$ (0.1 M in H_2O, 29 µL, 2.9 µmol) and sodium ascorbate (0.1 M in H_2O, 58 µL, 5.8 µmol) were mixed and shaken for 10 min. The red slurry was added to a solution of **4** (2.0 mg, 2.4 µmol) and fluorescently labeled targeting peptide IFLLQR(fluorescein)-Pra-RR (4.0 mg, 2.1 µmol) in a mixture of THF/H_2O (2:1 v/v; 2 mL). The solution was stirred at 60 °C for 8 h. The solvents were removed by evaporation. The residue was dissolved in acetonitrile and H_2O with 0.1% TFA (1:2) and purified by preparative HPLC (20 mL/min, H_2O+0.1% TFA : acetonitrile = 0 min 95:5, 31 min 0:100, 33 min 0:100, 36 min 95:5, 37 min 95:5).

MALDI-MS calcd. for $C_{133}H_{167}F_3N_{31}O_{27}S$ ($[M+H]^+$) 2719.2, found 2719.1. HPLC (0.75 mL/min, acetonitrile : H_2O+0.1%TFA = 0 min 10:90, 12 min 53:47, 18 min 59:41, 27 min 100:0, 31 min 100:0, 36 min 1:99, 38 min 1:99, 40 min 10:90, 41 min 10:90): t_R = 18.5 min.

Spectra

In the following all previously unreported ^1H, ^{13}C and ^{19}F NMR spectra are shown. For the compounds analyzed by HPLC the UV traces at λ=260 nm are shown.

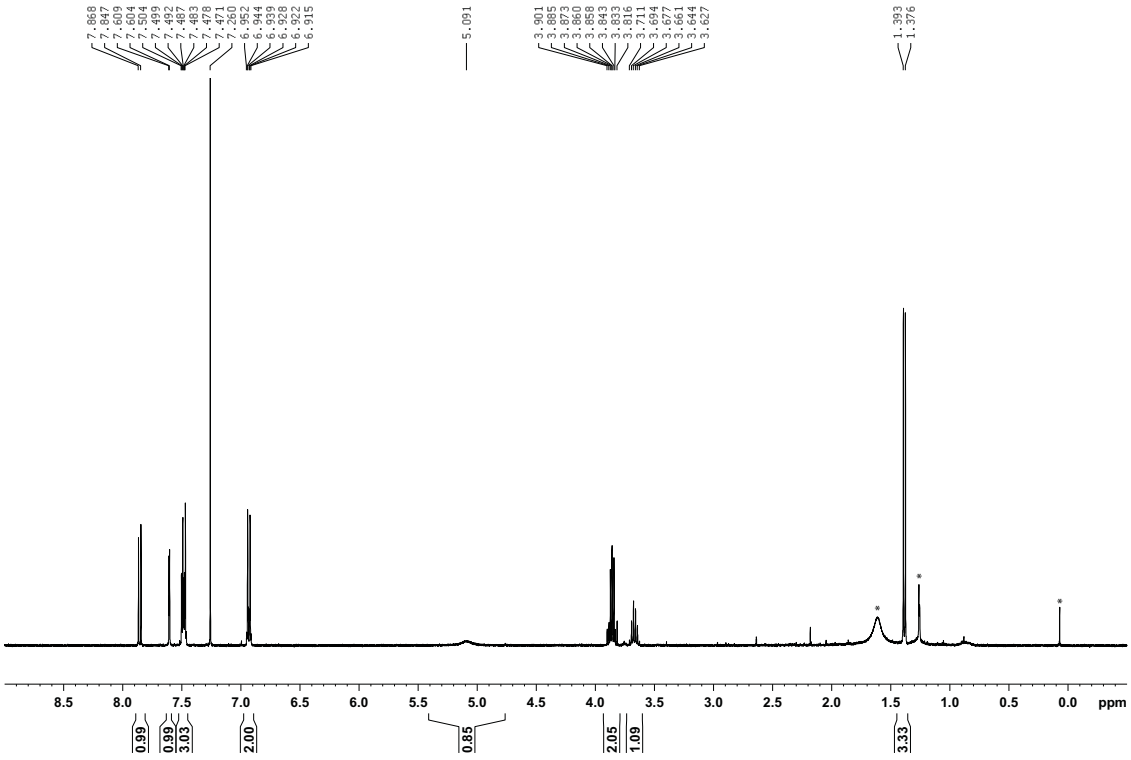


Figure S12. ^{1}H-NMR spectrum of **8** in $CDCl_3$.

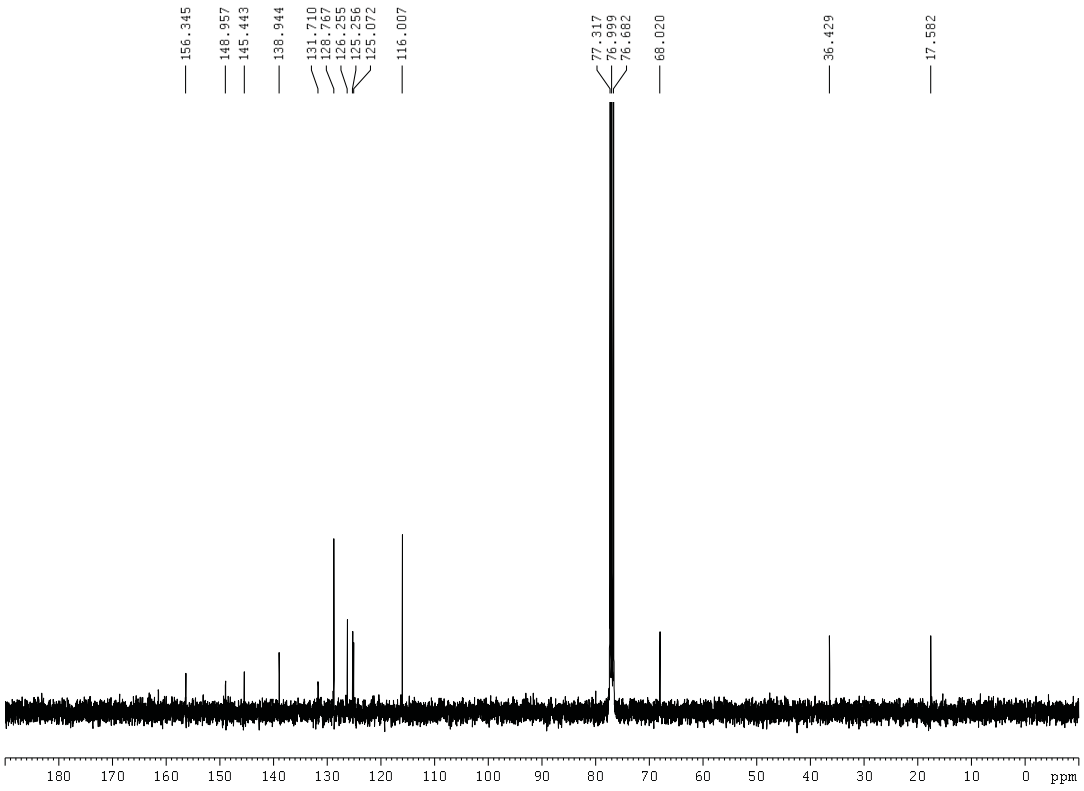


Figure S13. ^{13}C-NMR spectrum of **8** in $CDCl_3$.

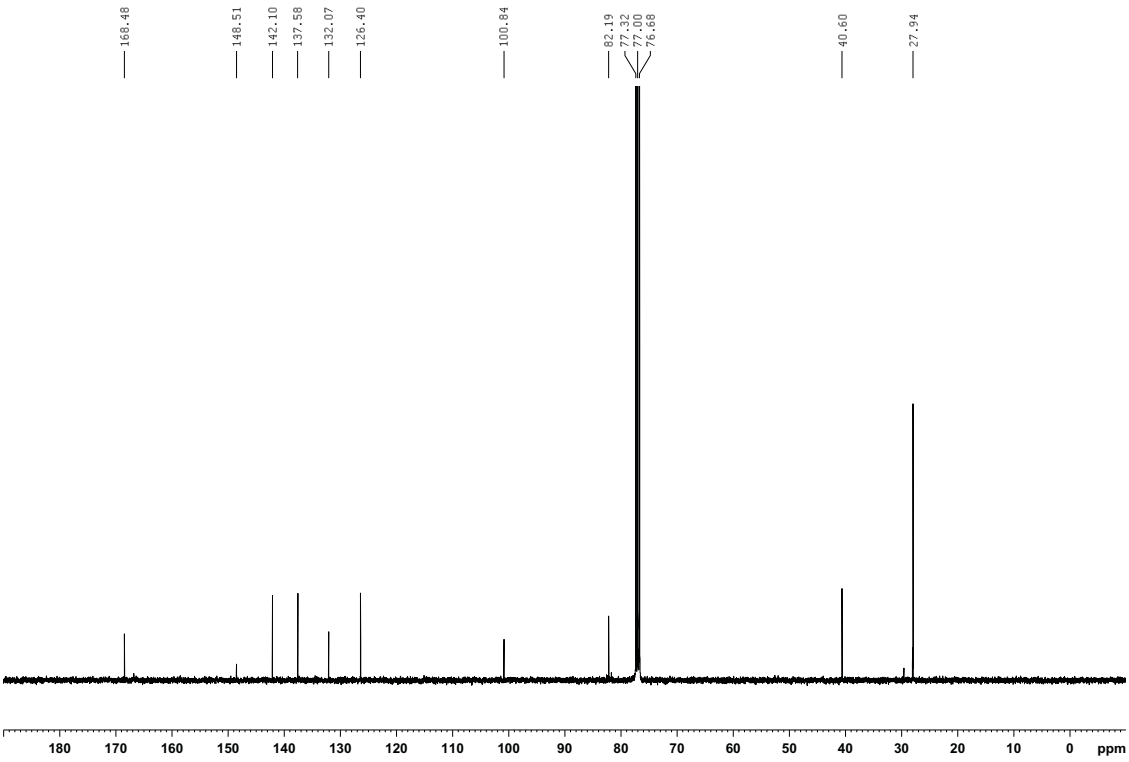


Figure S14. ^{13}C--NMR spectrum of **9** in $CDCl_3$.

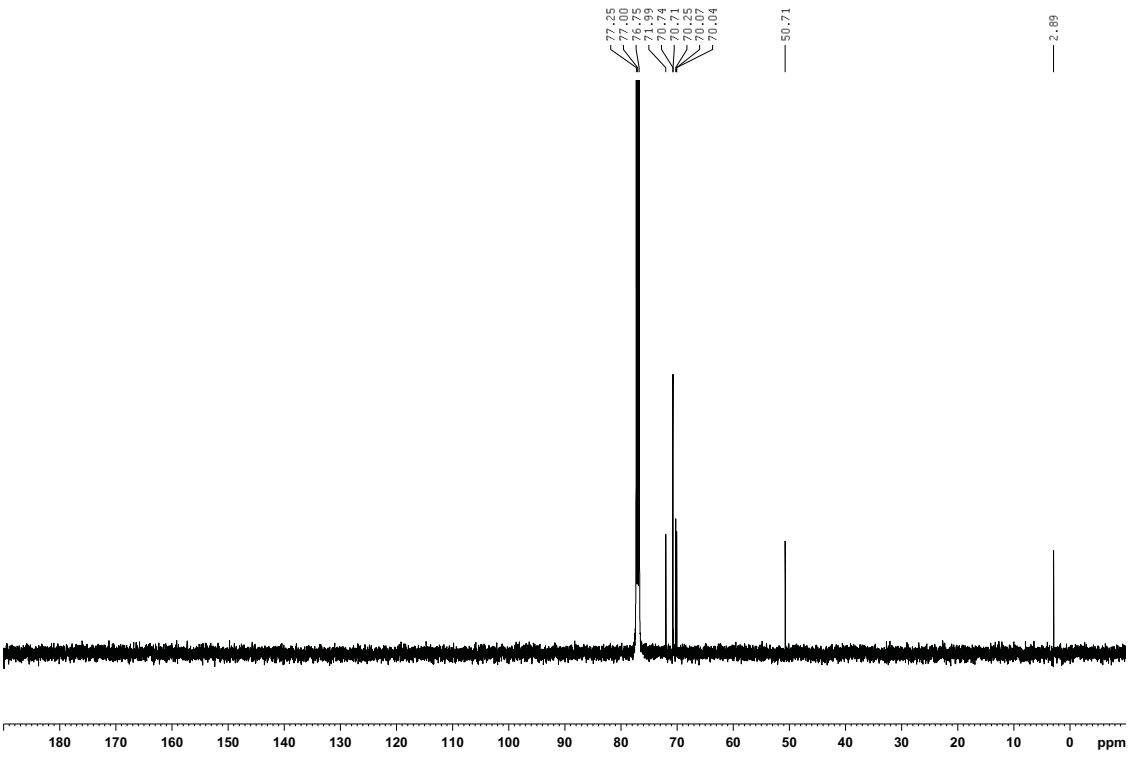


Figure S15. ^{13}C-NMR spectrum of **12** in $CDCl_3$.

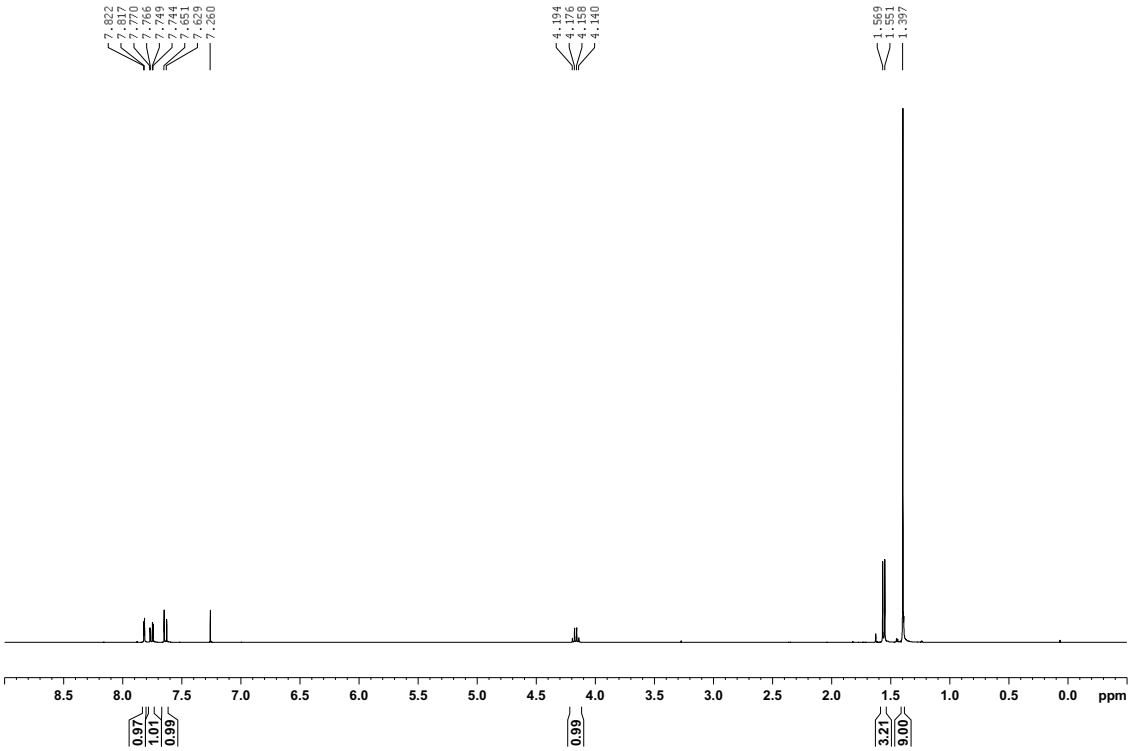


Figure S16. ^{1}H-NMR spectrum of **10** in $CDCl_3$.

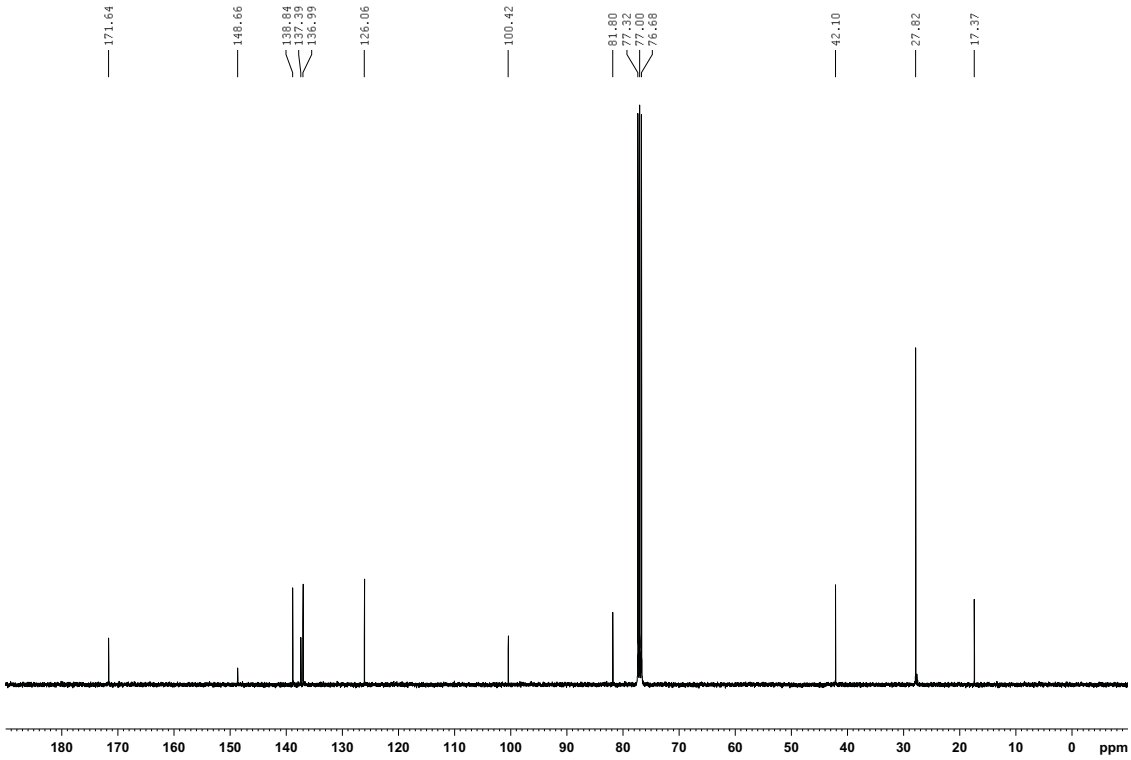


Figure S17. ^{13}C-NMR spectrum of **10** in $CDCl_3$.

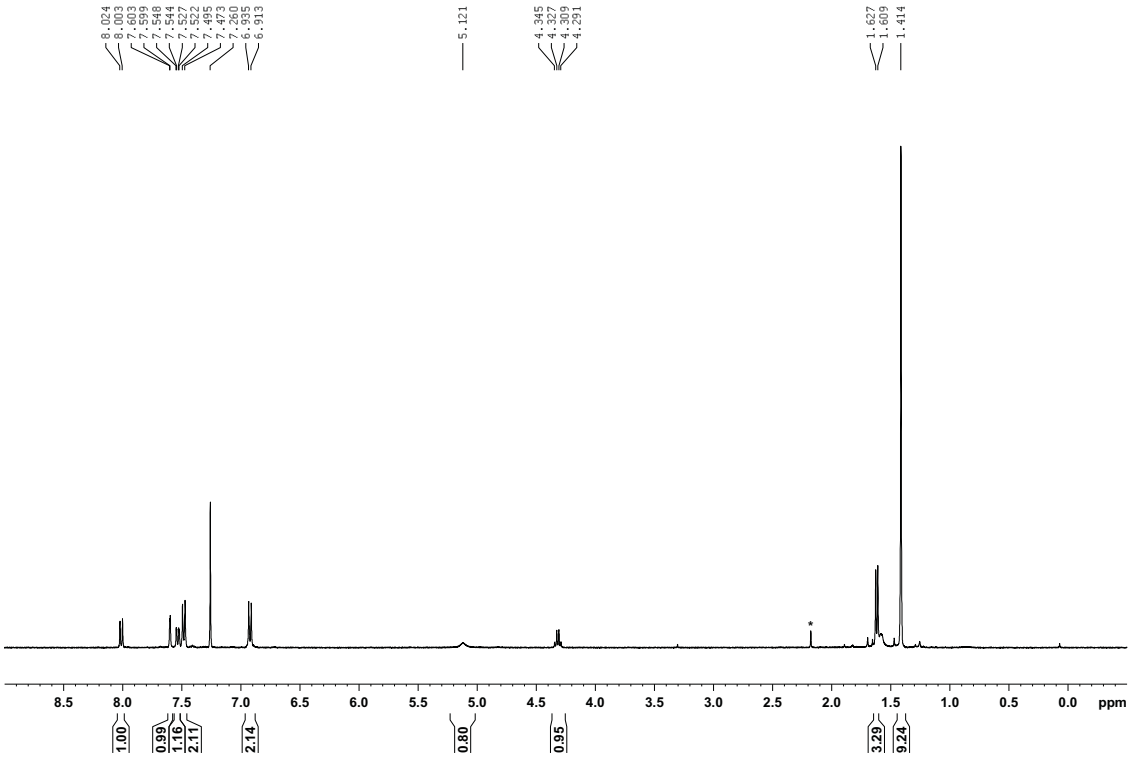


Figure S18. ^{1}H-NMR spectrum of **11** in $CDCl_3$.

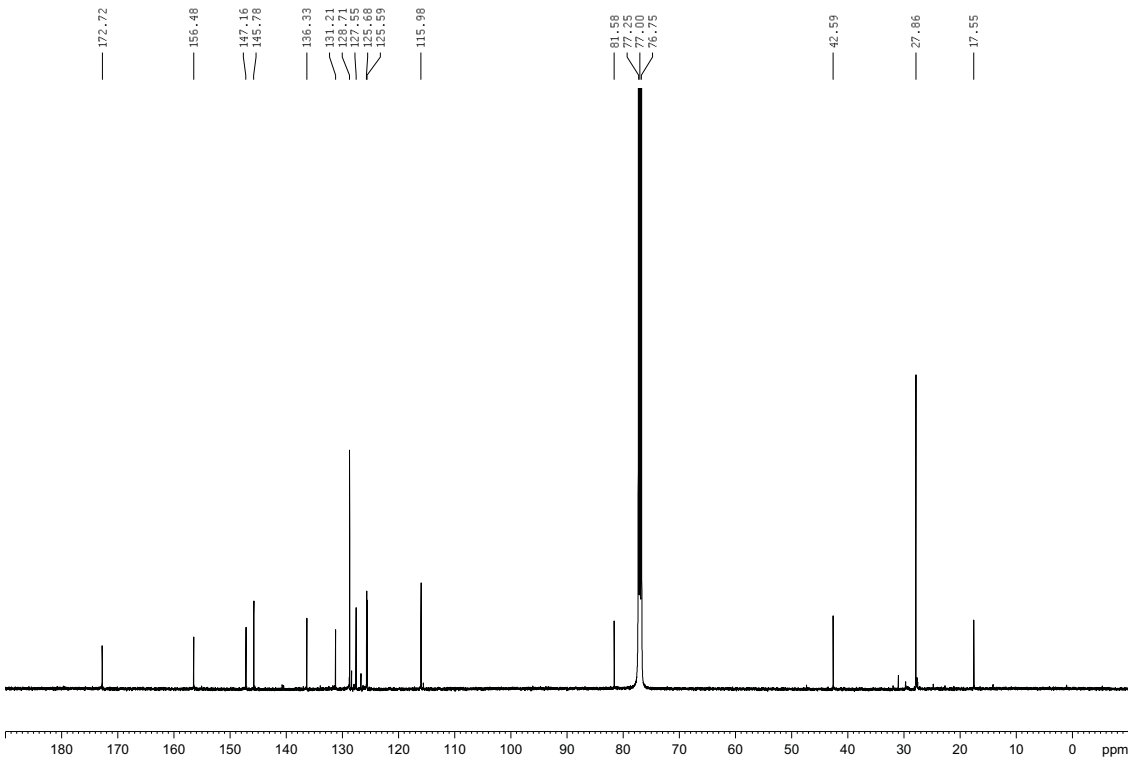


Figure S19. ^{13}C-NMR spectrum of **11** in $CDCl_3$.

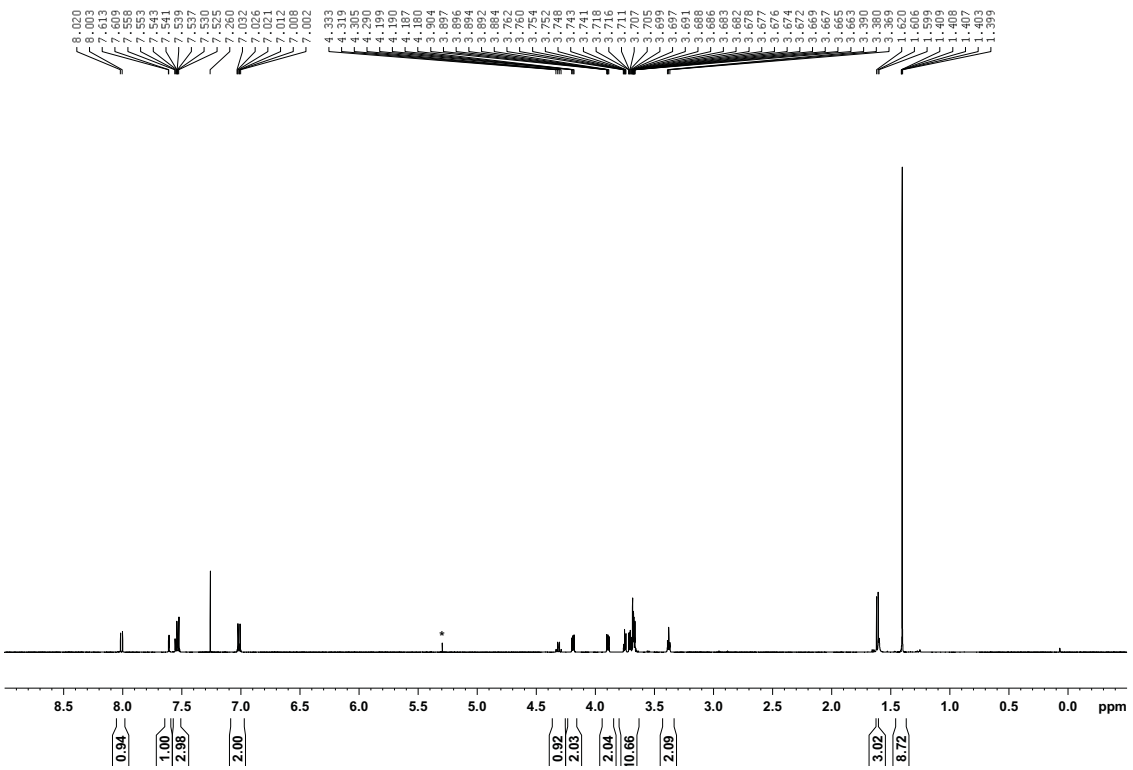


Figure S20. ^{1}H-NMR spectrum of **13** in $CDCl_3$.

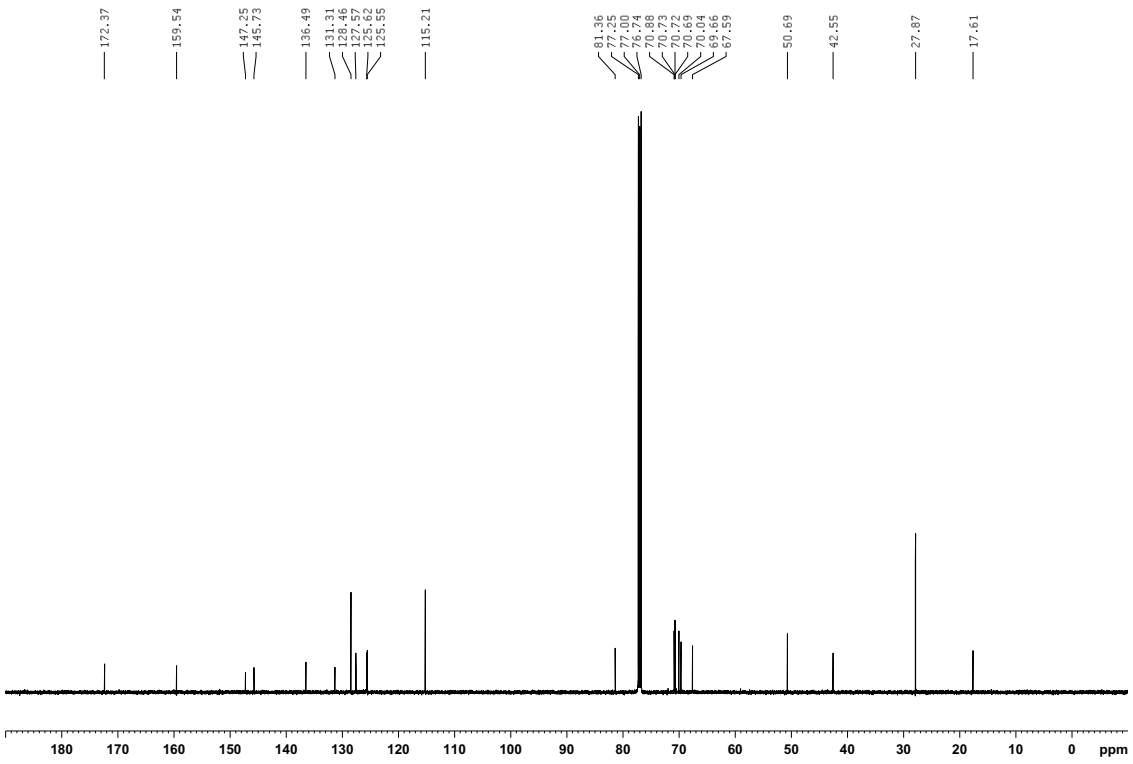


Figure S21. ^{13}C-NMR spectrum of **13** in $CDCl_3$.

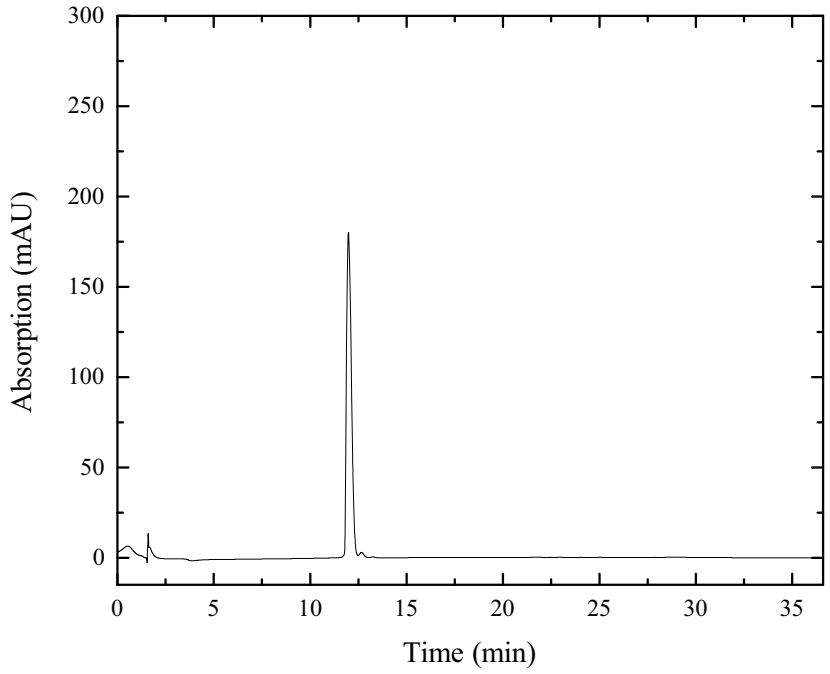


Figure S22. Analytical HPLC trace of the compound **14**.

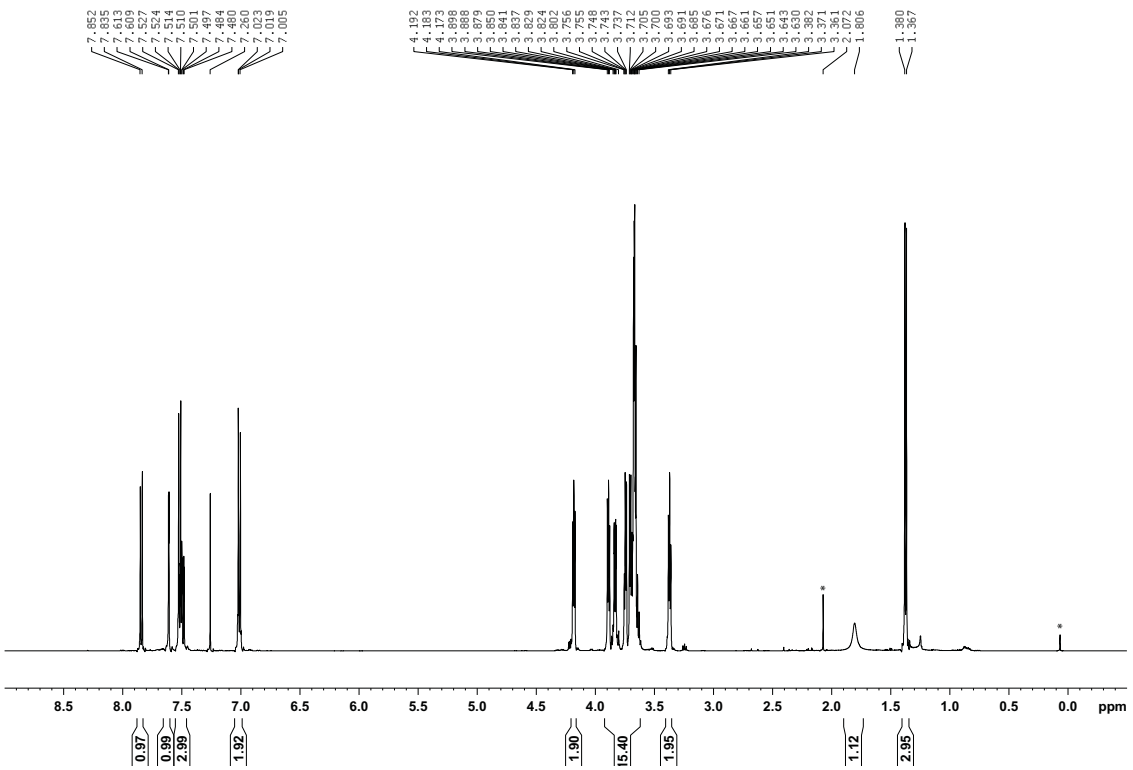


Figure S23. ^{1}H-NMR spectrum of compound **1**in $CDCl_3$.

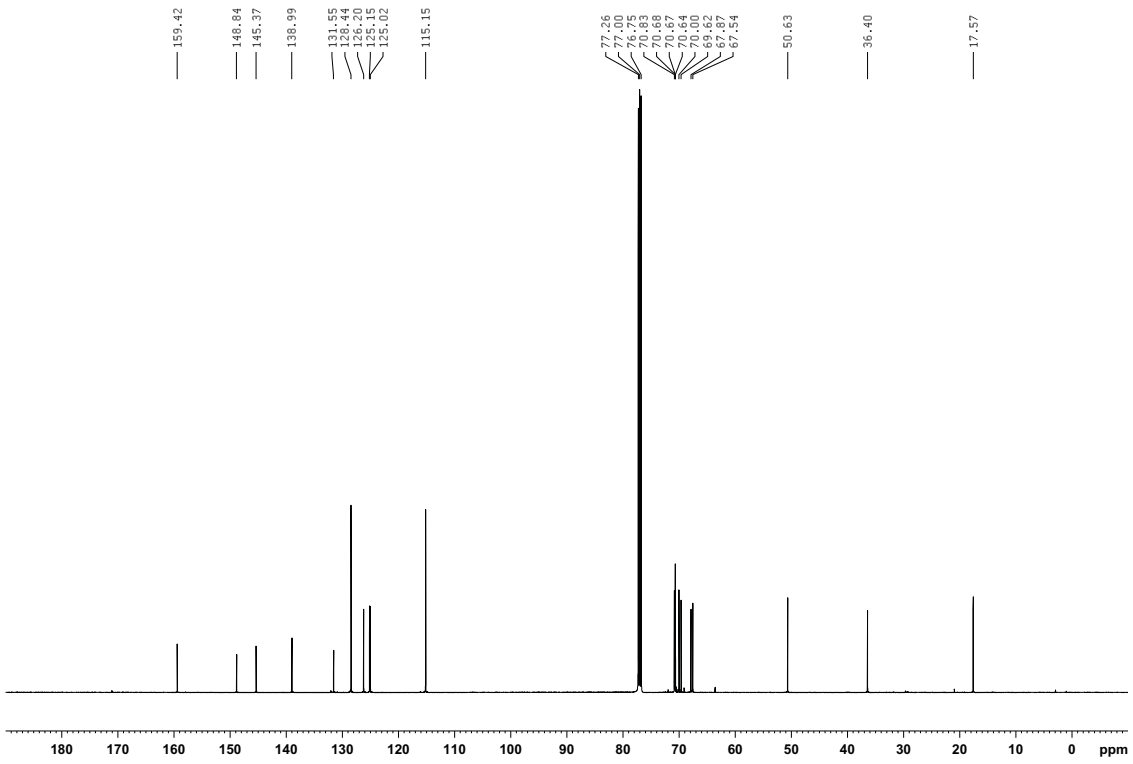


Figure S24. ^{13}C-NMR spectrum of compound **1** in $CDCl_3$.

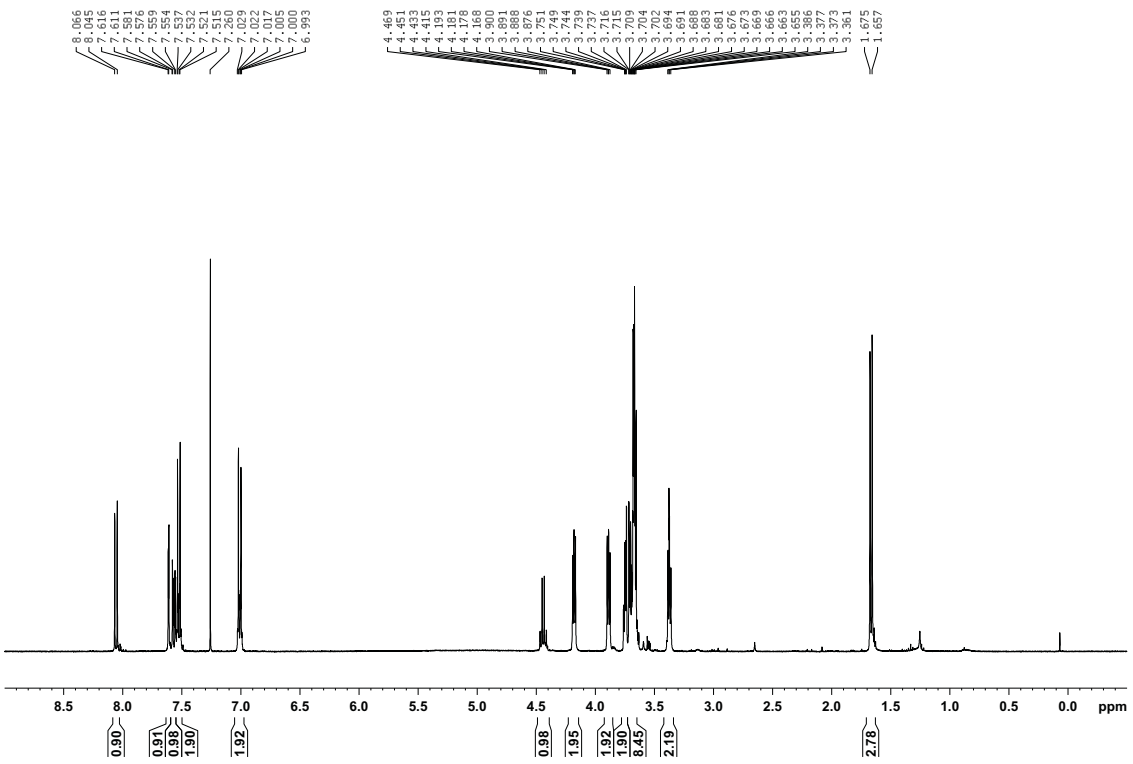


Figure S25. ^{1}H-NMR spectrum of compound **2** in $CDCl_3$.

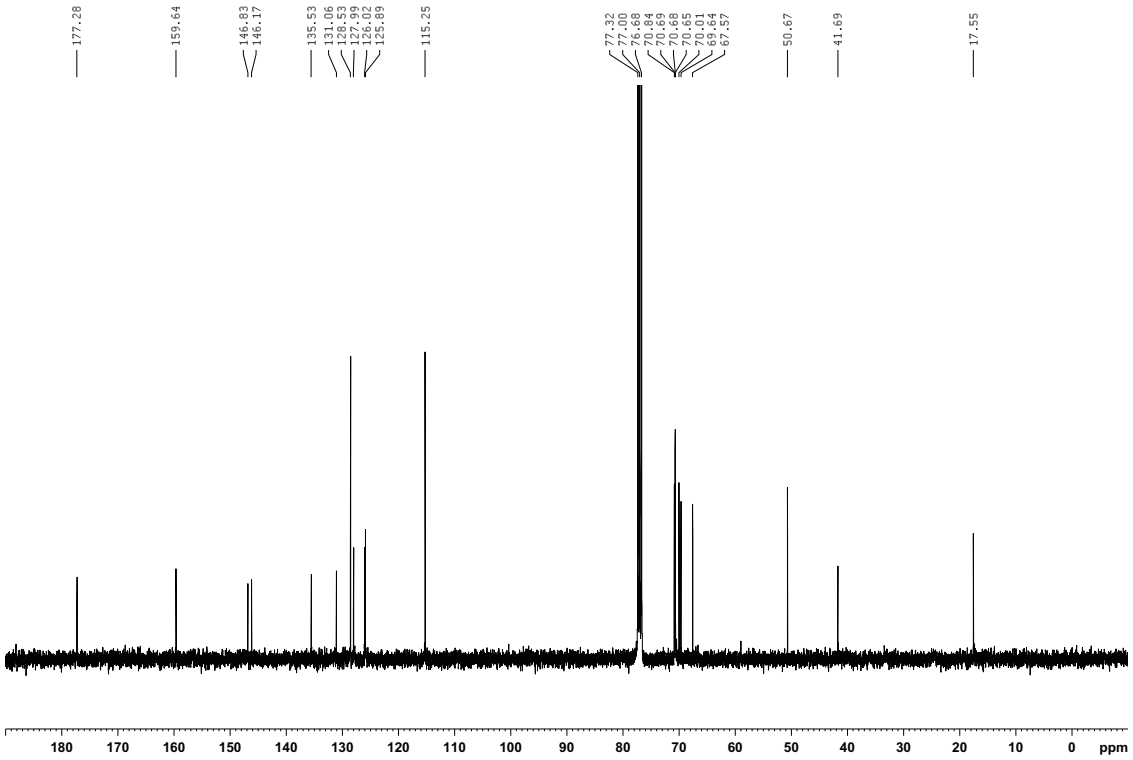


Figure S26. ^{13}C-NMR spectrum of compound **2** in $CDCl_3$.

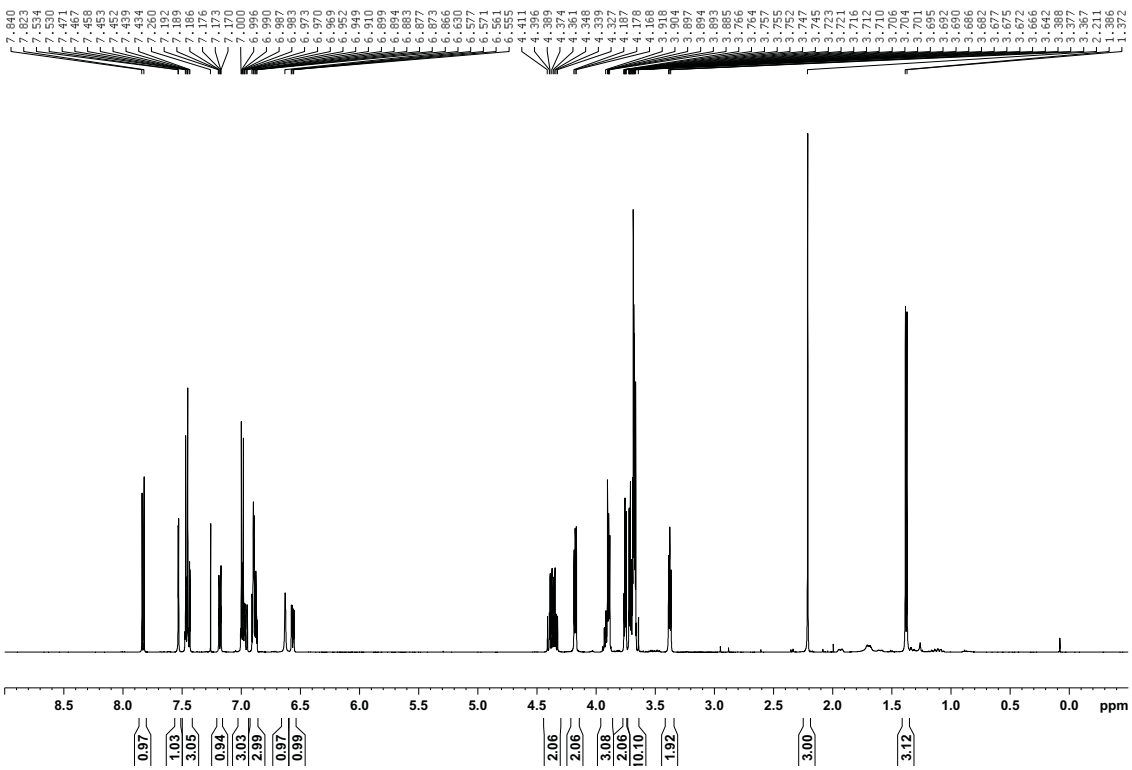


Figure S27. ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

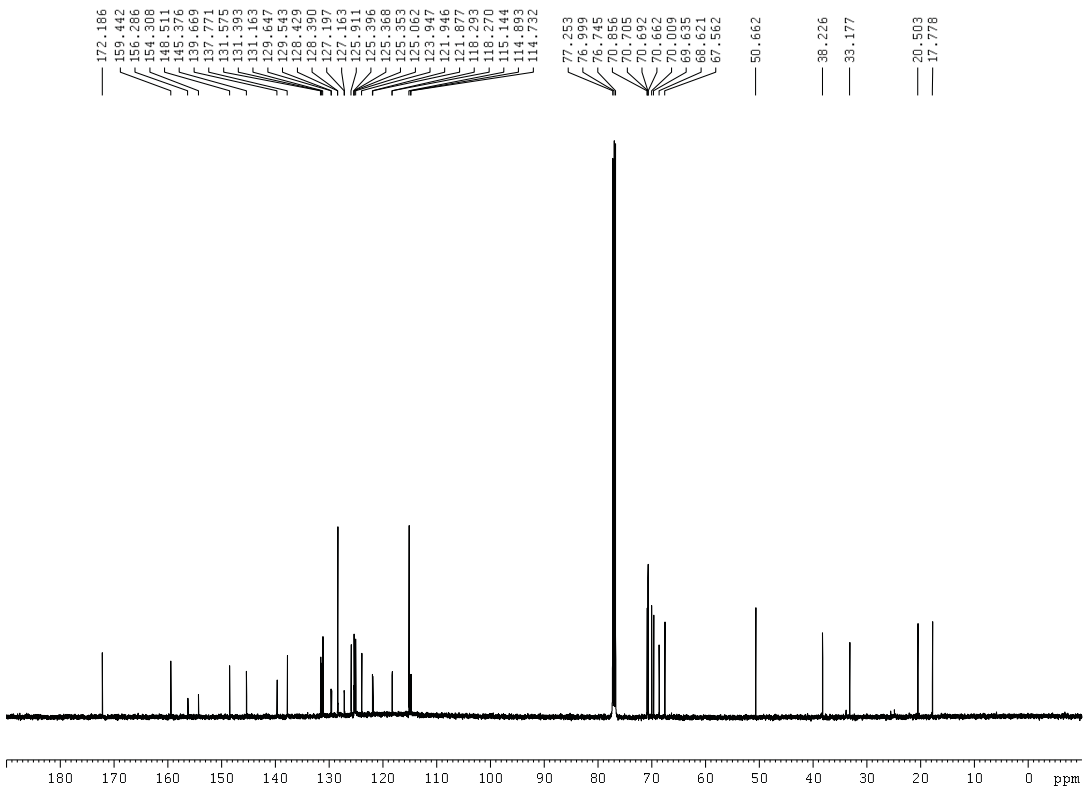


Figure S28. ^{13}C-NMR spectrum of compound **3** in $CDCl_3$.

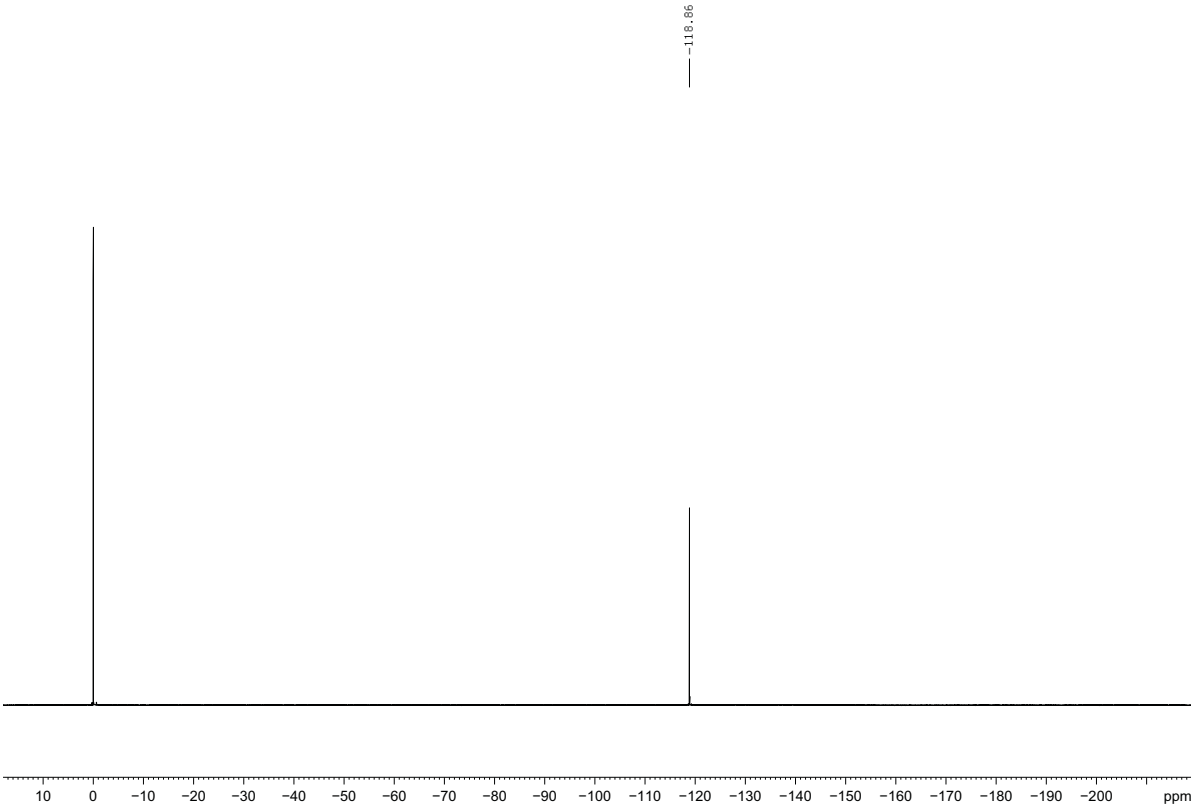


Figure S29. ^{19}F-NMR spectrum of compound **3** in $CDCl_3$ with CCl_3F as standard.

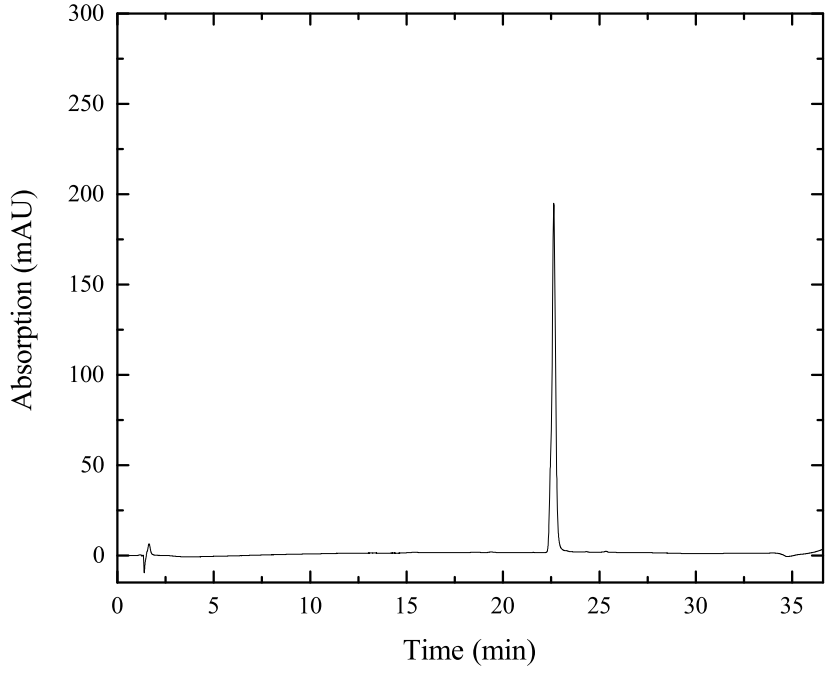


Figure S30. Analytical HPLC trace of compound **3**.

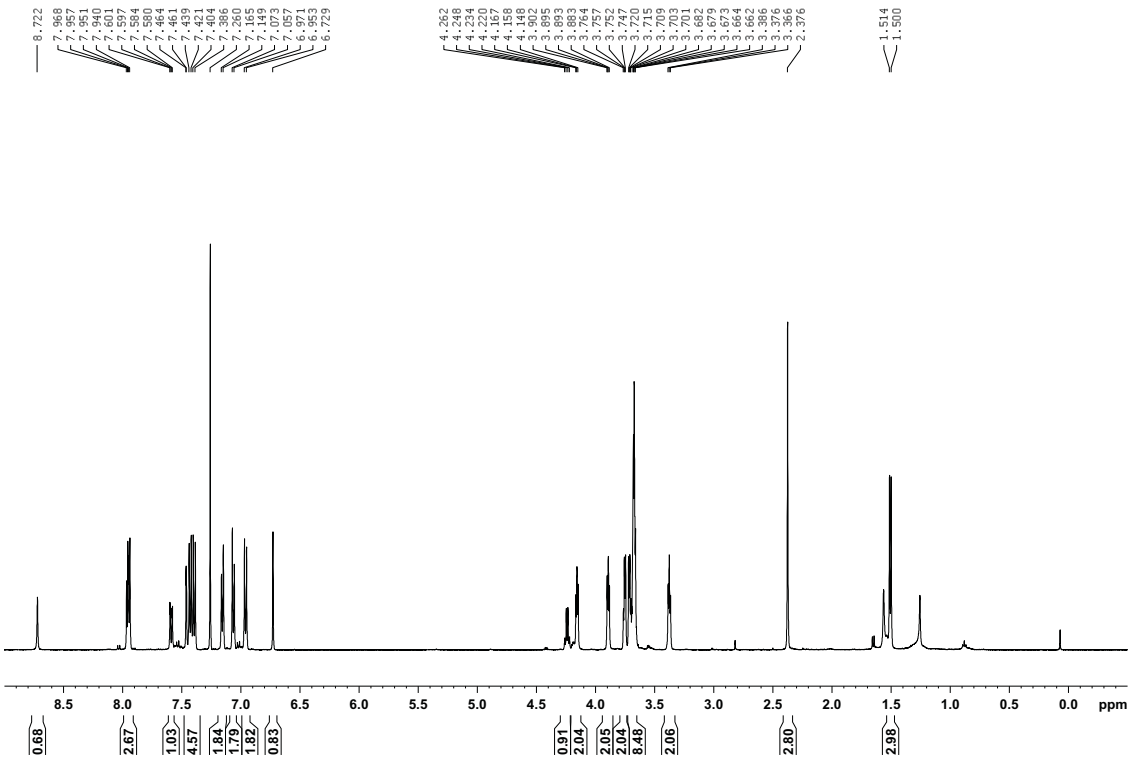


Figure S31. ^{1}H-NMR spectrum of compound **4** in $CDCl_3$.

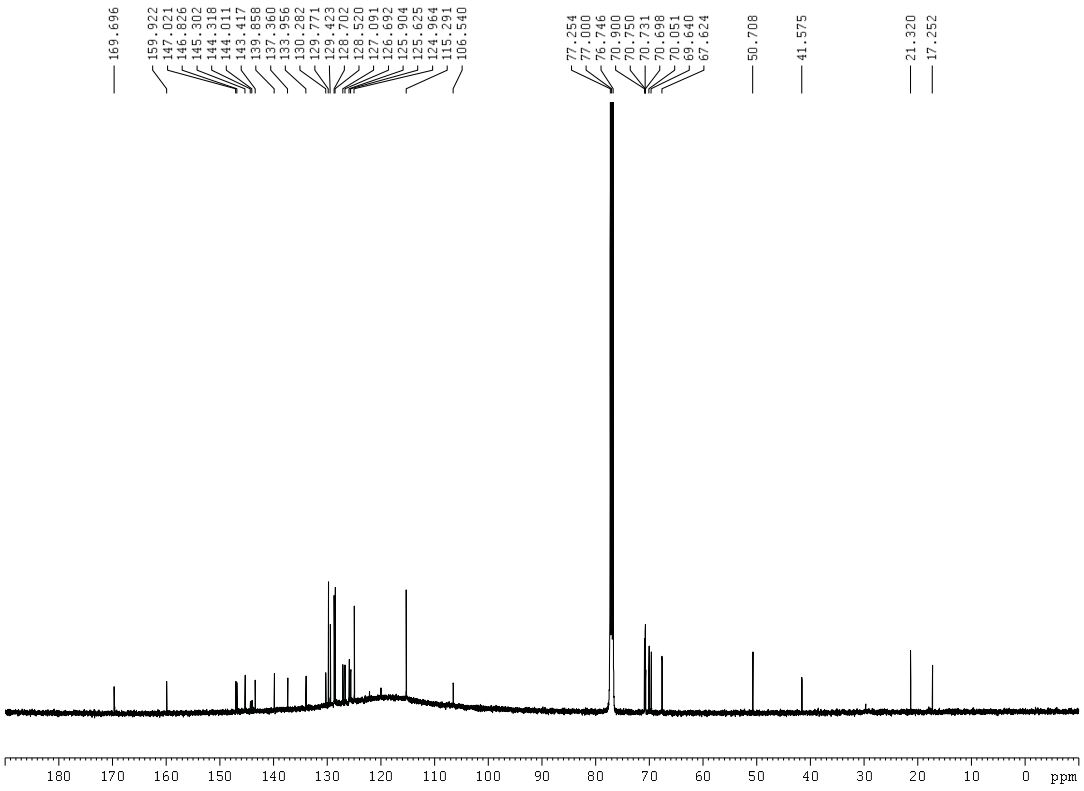


Figure S32. ^{13}C-NMR spectrum of compound **4** in $CDCl_3$.

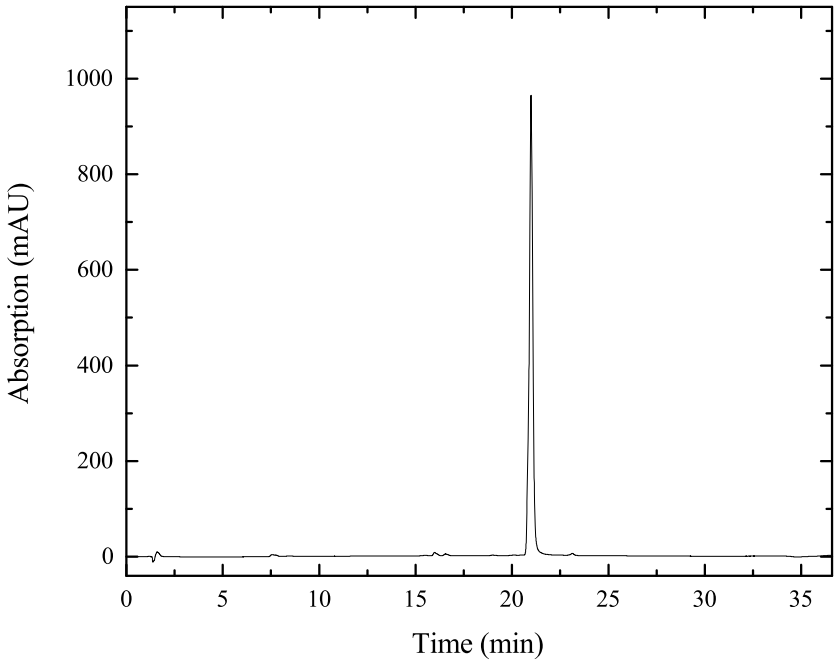


Figure S33. Analytical HPLC trace of compound **4**.

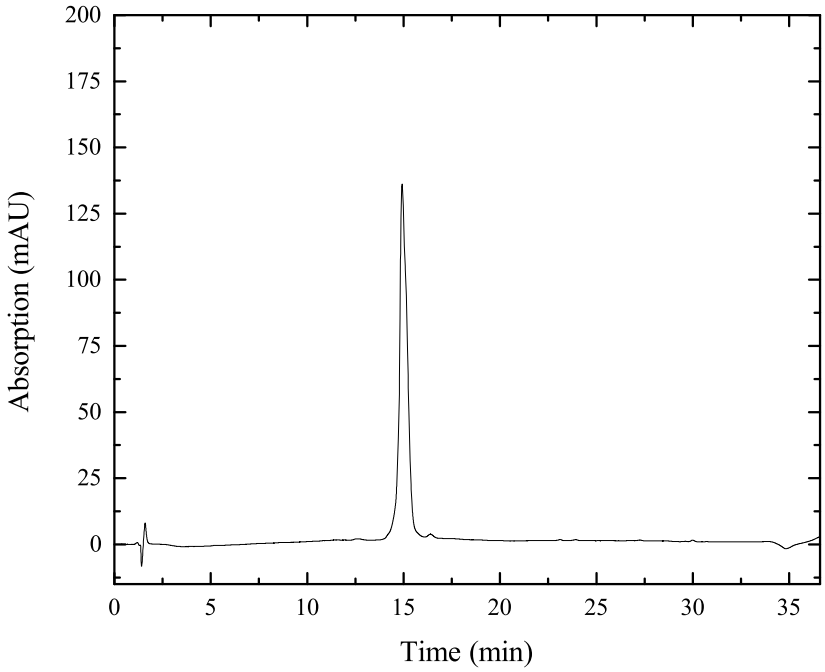


Figure S34. Analytical HPLC trace of compound **5**.

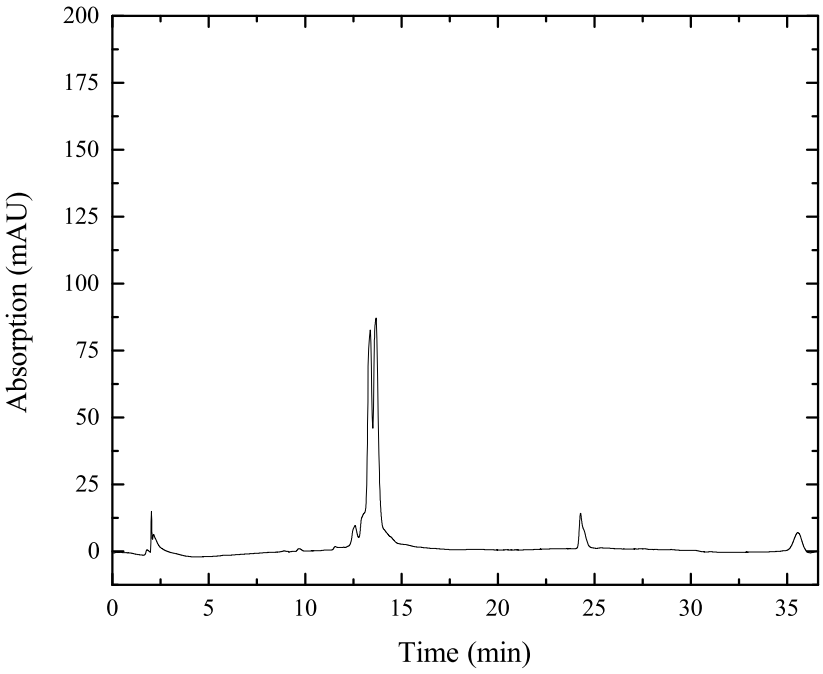


Figure S35. Analytical HPLC trace of compound **15**.

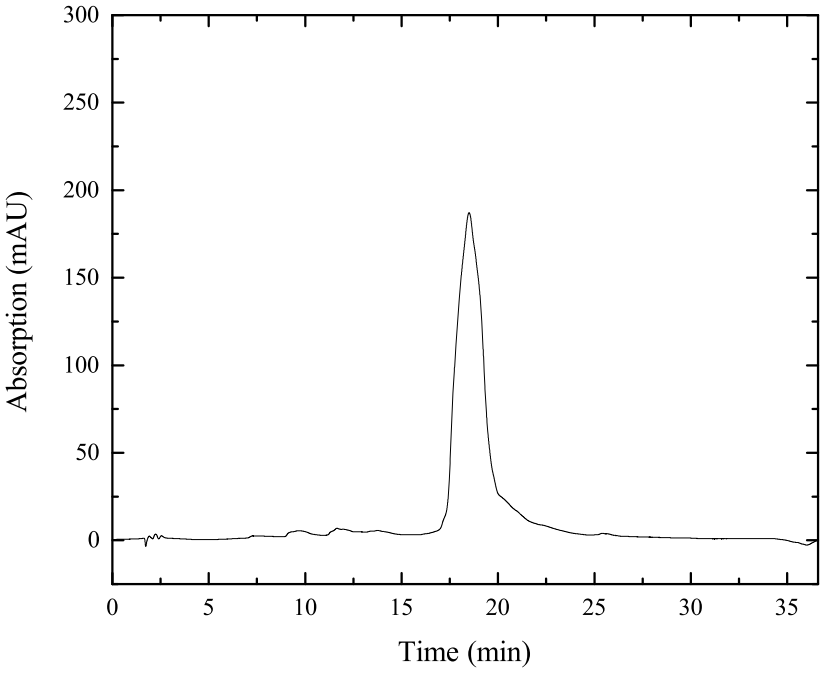


Figure S36. Analytical HPLC trace of compound **16**.

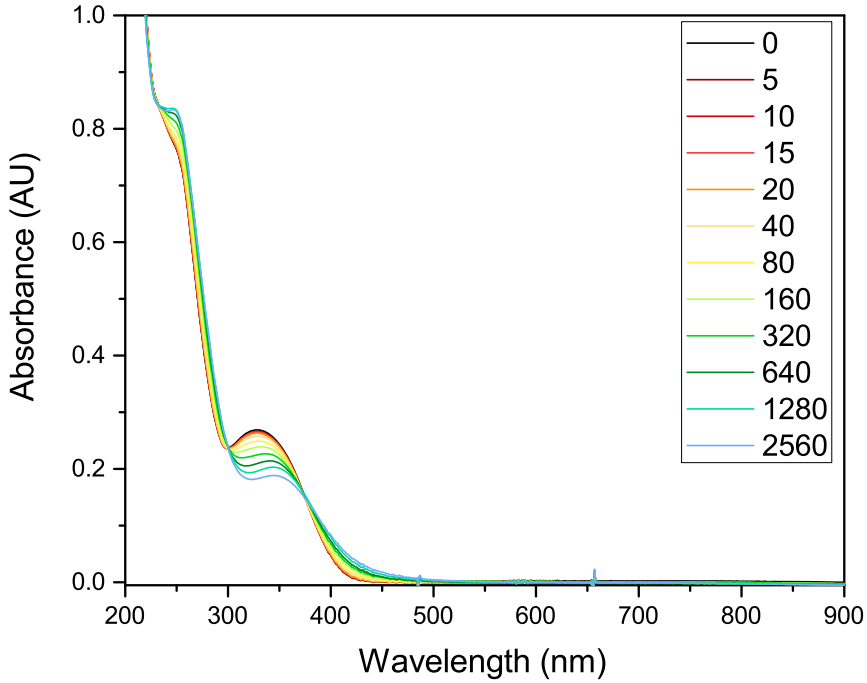


Figure S37. UV/Vis spectra of a solution containing **4** (OD(355 nm)=0.23, PBS/acetonitrile 1:1, pH=7.4) upon irradiation at 355 nm. The numbers in the legend indicate the number of laser shots ($1.9 \cdot 10^{-8}$ einsteins each).

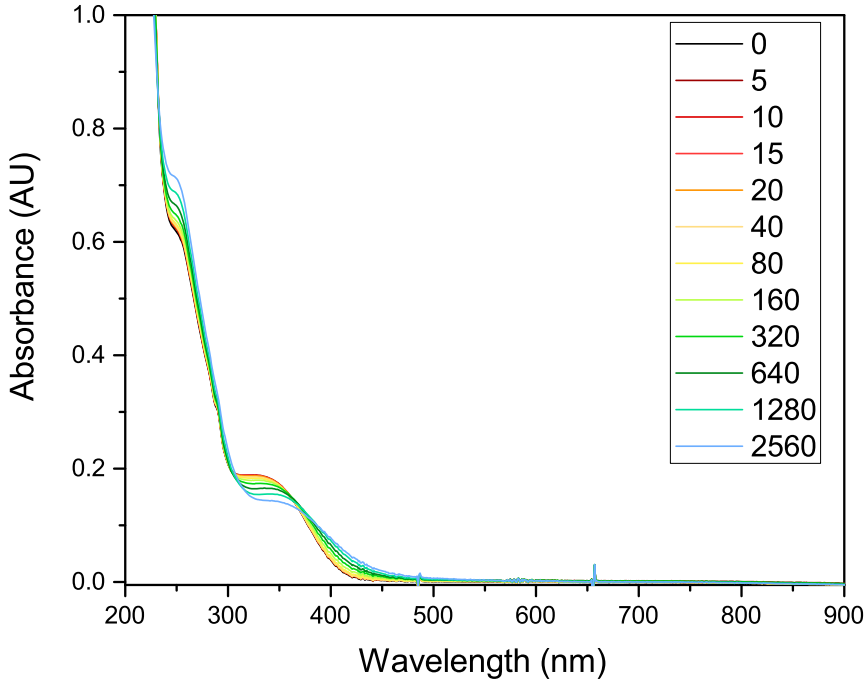


Figure S38. UV/Vis spectra of a solution containing **5** (OD(355 nm)=0.16, PBS/acetonitrile 1:1, pH=7.4) upon irradiation at 355 nm. The numbers in the legend indicate the number of laser shots ($1.9 \cdot 10^{-8}$ einsteins each).

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