Electronic Supplementary Material (ESI) for RSC Advances.

Selective release of a potent anticancer agent from supramolecular hydrogel using green light.

Johannes Karcher[a], Susanne Kirchner[a], Anna-Lena Leistner[a], Christian Hald[a], Philipp Geng[a], Tobias Bantle[a], Peter Gödtel[a], Juliane Pfeifer[b] and Zbigniew L. Pianowski*[a,c]

[a] Institut für Organische Chemie,
Karlsruher Institut für Technologie,
Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany
pianowski@kit.edu
[b] Institut für Funktionelle Grenzflächen IFG
Karlsruher Institut für Technologie, Campus Nord,
Hermann-von-Helmholtz Platz 1,
76344 Eggenstein-Leopoldshafen, Germany
[c] Institute of Biological and Chemical Systems – FMS
Karlsruher Institut für Technologie, Campus Nord,
Hermann-von-Helmholtz Platz 1,
76344 Eggenstein-Leopoldshafen, Germany

Table of Contents

1. General information

All reagents and starting materials are commercially available (SIGMA-ALDRICH, FLUOROCHEM, CHEMPUR, ALFA AESAR or BEPHARM) and were used as supplied unless otherwise indicated. All experiments were conducted in air and in deionized water (MILLIPORE) unless otherwise noted.

All experiments with molecules that can photoisomerize with visible light frequencies (>400 nm), namely the azobenzene derivatives containing fluorine atoms, were performed in absence of sunlight (brown glassware, or colorless glassware wrapped with aluminium foil, working in a room with dimmed light in a fume hood equipped with red light).

All reactions containing **air- and moisture-sensitive compounds** were performed under argon using oven-dried glassware applying common Schlenk-techniques. Liquids were added via steel cannulas and solids were added directly in powdered shape.

Column chromatography was performed on Silica gel 60 Å (40-63 µm particle size) (SIGMA).

NMR spectra were recorded using the following device: ^{1}H NMR: BRUKER 300 (300 MHz), BRUKER AVANCE 400 (400 MHz), BRUKER ASCEND 500 (500 MHz), ^{13}C NMR: BRUKER 300 (75 MHz), AVANCE 400 (101 MHz), ASCEND 500 (126 MHz), ^{19}F NMR: AVANCE 400 (377 MHz) or ASCEND 500 (471 MHz). The following solvents from EURISOTOP were used: chloroform-d_1, acetic acid-d_4, DMSO-d_6, and D_2O. Chemical shifts δ were expressed in parts per million (ppm) and referenced to chloroform-d_1 (^{1}H: δ=7.26 ppm, ^{13}C: δ=77.16 ppm), acetic acid-d_4 (^{1}H: δ=2.04 ppm, ^{13}C: δ=178.99 ppm), DMSO-d_6 (^{1}H: δ=2.50 ppm, ^{13}C: δ=39.52 ppm) and D_2O (^{1}H: δ=4.79 ppm). [1] ^{19}F-NMR were not referenced.

Mass spectra were recorded on a FINNIGAN MAT 95 mass spectrometer using electron ionization-mass spectrometry (EI-MS) or fast atom bombardment-mass spectroscopy (FAB-MS). For FAB measurements *m*-nitrobenzyl alcohol (3-NBA) was used as the matrix. The software of FAB and EI adds the mass of one electron. Electrospray ionization–mass spectrometry (ESI-MS) spectra were recorded on a THERMO FISHER SCIENTIFIC Q EXACTIVE mass spectrometer. Calibration was carried out using premixed calibration solutions (THERMO FISHER SCIENTIFIC). The molecular fragments are stated as ratio of mass per charge *m/z*.

UV-Vis spectra were recorded on a Lambda 750 (PERKINELMER) UV-Vis spectrophotometer at 20 °C, slit=2 nm.

IR spectra were recorded on a BRUKER IFS 88 using ATR (Attenuated total reflection). The intensities of the absolute peaks are given as follows: vs=very strong 0-9% T, s=strong 10-39% T, m=medium 40-69% T, w=weak 70-89% T, vw=very weak 90-100% T. All spectroscopy samples were taken at room temperature.

Analytic HPLC was measured with the 1200 Series from AGILENT TECHNOLOGIES with a YMC C18-column JH08S04-2546WT with 250 × 4.8 mm and 4 µm.

Preparative HPLC separation was performed with a LC-2000Plus series from JASCO with a VDSpher column with C18-M-SE, 250 × 20 mm and 10 µm from VDSOPTILAB.

Analytical thin layer chromatography was carried out using silica coated aluminium plates (silica 60, F_{254}, layer thickness: 0.25 mm) with fluorescence indicator by MERCK. Detection proceeded under UV light at λ=254 nm.

Rheological measurements were performed using ARES-G2 Rheometer (TA INSTRUMENTS) at room temperature.

Sample irradiation for photoisomerization of hydrogels and measurements of photostationary states was performed using LED diodes with emission maxima of 530 nm and 410 nm from LED Engin. For the time of irradiation, samples were maintained at constant temperature (22 ± 2 °C) using a metal cooling block.

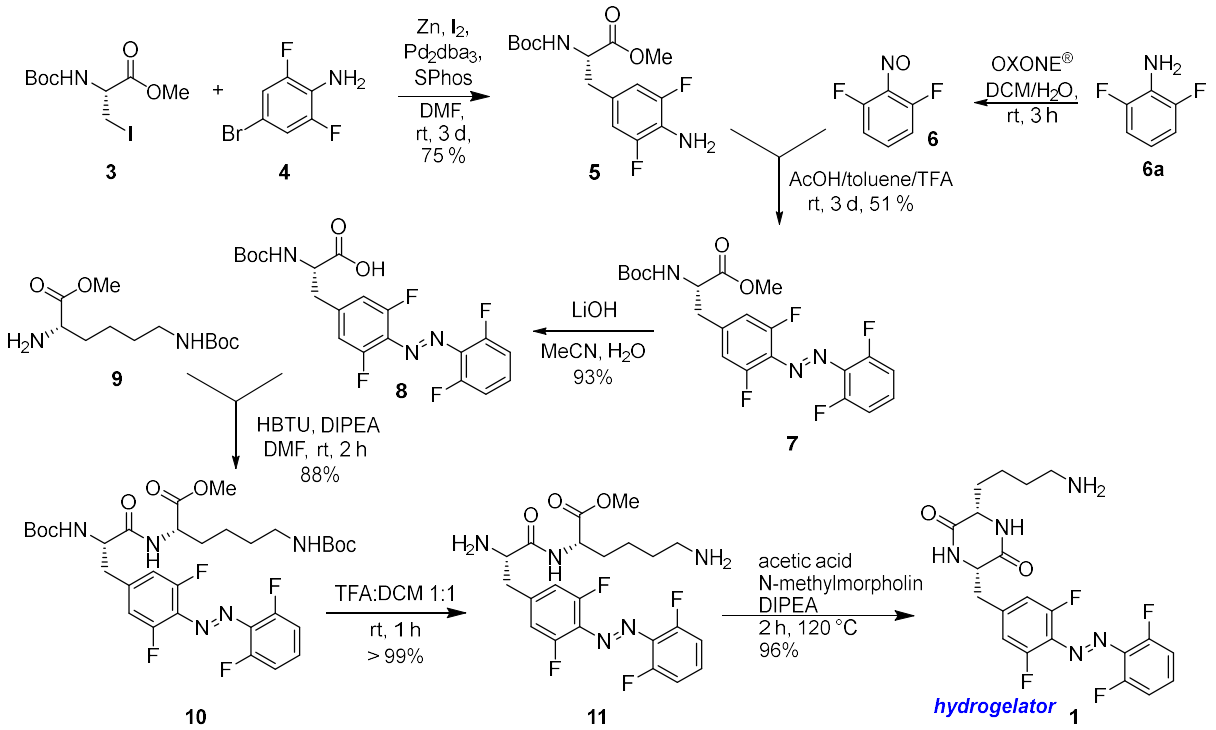
Using the PowerMax USB (type PS19Q) sensor device (Coherent®) we have measured the irradiation intensity for the particular diodes used in our experiment (5 independent measurements, the detector (diameter 19 mm) was located at the distance of 55 mm from the light source, identical as the position of irradiated samples).

Table S1. Irradiation intensity of the LED light sources used for the photoisomerization experiments.

λ_{max} of the LED diode (power input)	Light intensity (mW/cm^2)	Average power (W)	Variance (W)	SD (W)
410 nm (3 W)	9.07	$2.57*10^{-2}$	$2.72*10^{-7}$	$5.52*10^{-4}$
530 nm (3 W)	7.08	$2.01*10^{-2}$	$3.48*10^{-6}$	$1.87*10^{-3}$

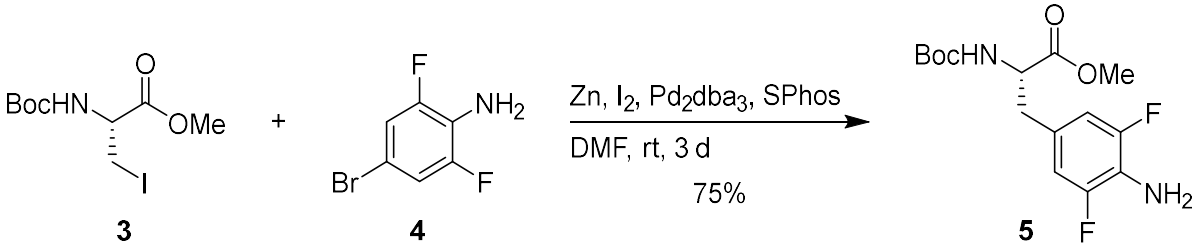
Experiments under physiological conditions were performed in Dulbecco's Phosphate-Buffered Saline (DPBS) buffer pH 7.4, (-/-): no calcium, no magnesium, Gibco™ from THERMOFISHER, cat.#: 14190136, abbreviated below and in the manuscript simply as "PBS buffer", composed of 8 g NaCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, and 0.2 g KCl in 1 L of water.

2. Synthesis:



Scheme S1. Synthesis of the hydrogelator **1**.

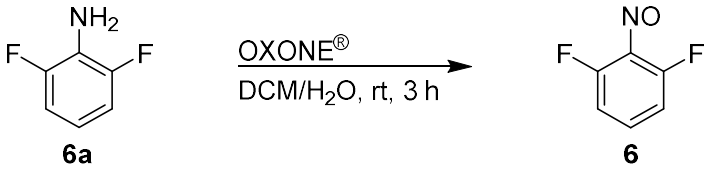
Methyl (*S*)-3-(4-amino-3,5-difluorophenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (5)



Dry DMF (30 mL) was added to zinc dust (10 Å) (2.98 g, 45.6 mmol) under argon followed by iodine (0.289 g, 2.28 mmol) and the mixture was stirred until the solution turned clear again. Methyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-iodopropanoate (**3**, Boc-Ser(I)-OMe) (5.00 g, 15.2 mmol) was added followed by iodine (0.289 g, 2.28 mmol) and the solution was stirred for 15 min until it had cooled down to room temperature again. Pd_2dba_3 (0.348 g, 0.380 mmol), SPhos (0.312 g, 0.760 mmol) and 4-bromo-2,6-difluoroaniline (**4**, 4.11 g, 19.8 mmol) were added and the reaction mixture was stirred under argon for three days at room temperature.[2] The crude product was filtrated over Celite, concentrated *in vacuo* and purified by silica gel column chromatography (cyclohexane/EtOAc 5:1 with 1% triethylamine, $R_f = 0.21$) to yield 3.01 g (11.5 mmol, 75%) of methyl (*S*)-3-(4-amino-3,5-difluorophenyl)-2-((tert-butoxycarbonyl)-amino)-propanoate (Boc-Ser(difluoro-aniline)-OMe, **5**) as a slightly brown solid. To achieve good and repeatable yields, it was critical to dry all substrates as well as the zinc dust under high vacuum overnight prior to the reaction.

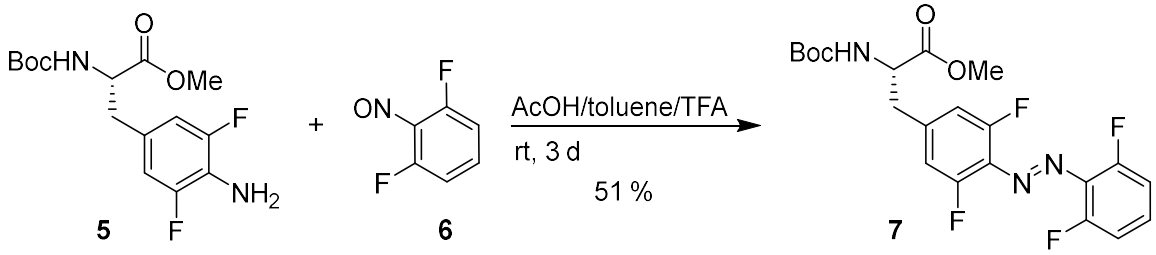
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.59 (d, *J* = 8.5 Hz, 2H), 5.01 (d, *J* = 7.8 Hz, 1H), 4.50 (dd, *J* = 13.3, 5.7 Hz, 1H), 3.72 (s, 3H), 3.59 (s, 2H), 2.95 (ddd, *J* = 34.1, 14.0, 5.5 Hz, 2H), 1.42 (s, 9H) ppm. **^{13}C NMR (101 MHz, $CDCl_3$):** δ = 172.1 (s), 155.2 (s), 152.0 (dd, *J* = 240.7, 8.4 Hz), 125.3 – 125.1 (m), 122.9 (t, *J* = 16.3 Hz), 112.0 (dd, *J* = 14.8, 7.2 Hz), 80.2 (s), 54.5 (s), 52.5 (s), 37.5 (s), 28.4 (s) ppm. **^{19}F NMR (377 MHz, $CDCl_3$):** δ = −136.56 (s) ppm. **IR (ATR):** $\tilde{\nu}$ = 3456 (vw), 3360 (m), 2978 (vw), 2954 (vw), 1731 (m), 1688 (m), 1676 (m), 1591 (w), 1519 (m), 1438 (m), 1281 (m), 1248 (m), 1156 (m), 1051 (w), 1020 (m), 991 (w), 962 (m), 841 (m), 761 (w), 593 (m), 411 (vw) cm^{-1}. **HRMS (EI+):** *m/*z = 330.1392 [M]. Calculated for $C_{15}H_{20}F_2N_2O_4$: 330.1391.

1,3-Difluoro-2-nitrosobenzene (6)



OXONE® (12.3 g, 40.0 mmol) in diH_2O (120 mL) was added to 2,6-difluoroaniline **6a** (2.35 g, 18.2 mmol) dissolved in DCM (72 mL). The mixture was stirred vigorously at room temperature for 3 hours. The layers were separated, and the organic layer was washed with 1N HCl (50 mL), water (3×50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product **6** was used for the synthesis of compound **7** without any further purification.[3]

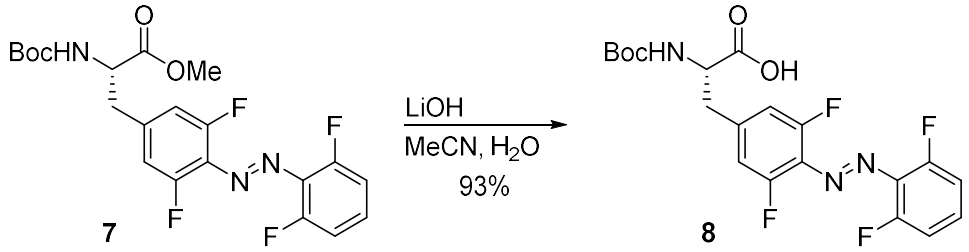
Methyl (*S*,*E*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-((2,6-difluoro-phenyl)diazenyl)-3,5-difluorophenyl)propanoate (7) "Boc-F4-PAP-OMe"



The crude product 1,3-difluoro-2-nitrosobenzene (**6**, 1.95 g, 13.6 mmol) and compound **5** (3.00 g, 9.08 mmol) were dissolved in acetic acid/toluene/TFA (6:6:1) (130 mL) and the reaction mixture was stirred at room temperature for 3 days. H_2O (60 mL) was added and the crude product was extracted with EtOAc (3×50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (cyclohexane/DCM 1:1, R_f (DCM) = 0.25) to yield 2.12 g (4.65 mmol, 51%) of compound **7** ("Boc-F4-PAP-OMe") as an orange solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (tt, *J* = 8.3, 5.9 Hz, 1H), 7.06 (t, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 9.8 Hz, 2H), 5.10 (d, *J* = 7.5 Hz, 1H), 4.62 (d, *J* = 6.9 Hz, 1H), 3.77 (s, 3H), 3.14 (ddd, *J* = 53.2, 13.8, 5.8 Hz, 2H), 1.76 (s, 1H), 1.44 (s, 9H) ppm. **^{13}C NMR (101 MHz, $CDCl_3$):** δ = 171.6 (s), 157.0 (dd, *J* = 14.9, 4.4 Hz), 155.1 (s), 154.4 (dd, *J* = 15.7, 4.4 Hz), 141.7 (t, *J* = 9.9 Hz), 132.0 (t, *J* = 9.9 Hz), 131.5 (t, *J* = 10.5 Hz), 130.6 (t, *J* = 10.0 Hz), 113.7 (dd, *J* = 21.1, 2.7 Hz), 112.8 (dd, *J* = 21.1, 2.9 Hz), 80.6 (s), 54.1 (s), 52.8 (s), 38.3 (s), 28.4 (s) ppm. **^{19}F NMR (377 MHz, $CDCl_3$):** δ = −129.81 (s), −130.40 (s) ppm. **IR (ATR):** $\tilde{\nu}$ = 3348 (w), 2980 (vw), 2954 (vw), 1733 (w), 1680 (m), 1626 (w), 1576 (w), 1519 (m), 1470 (m), 1437 (w), 1393 (w), 1349 (w), 1271 (w), 1242 (m), 1212 (m), 1158 (m), 1049 (w), 1024 (m), 845 (w), 786.4 (m), 743 (w), 605 (w), 513 (w), 471 (w), 435 (vw), 383 (vw) cm^{-1}. **HRMS (FAB+, 3-NBA):** *m/*z = 456.1547 [M+H]. Calculated for $C_{21}H_{22}F_4N_3O_4$: 456.1546. **Anal. calcd. for $C_{21}H_{21}F_4N_3O_4$ (%):** C: 55.39, H: 4.65, F: 16.69, N: 9.23, O: 14.05; found: C: 55.17, H: 4.47, N: 9.07.

(*S*,*E*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-((2,6-difluorophenyl)-diazenyl)-3,5-difluorophenyl)propanoic acid (8) "Boc-F4-PAP-OH"



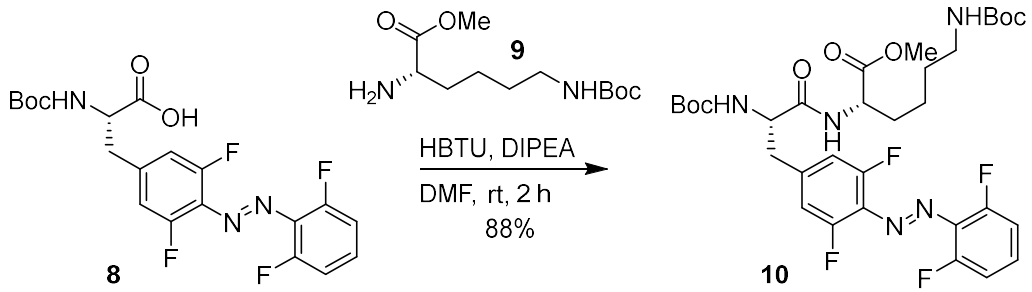
To a solution of the compound **7** (1.90 g, 4.17 mmol) in MeCN (19 mL) LiOH (1.90 g, 79.3 mmol) in H_2O (19 mL) was added and the reaction mixture was stirred at room temperature for 15 min. The reaction was quenched by adding aqueous solution of HCl (2 M, 114 mL). The mixture was extracted with EtOAc (2×100 mL) and the combined organic layers were concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (DCM/MeOH 9:1, R_f = 0.55) to yield 1.84 g (4.18 mmol, 93%) of "Boc-F4-PAP-OH" (compound **8**) as an orange solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.79 (s, 1H), 7.61 (tt, *J* = 8.5, 6.1 Hz, 1H), 7.45 – 7.14 (m, 5H), 4.29 – 4.17 (m, 1H), 3.16 (dd, *J* = 13.7, 4.5 Hz, 1H), 2.92 (dd, *J* = 13.7, 10.8 Hz, 1H), 1.31 (s, 9H). **^{13}C NMR (101 MHz, DMSO-d_6):** δ = 172.91 , 155.85 (d, *J* = 4.0 Hz), 155.63 (d, *J* = 4.6 Hz), 155.40 , 153.28 (d, *J* = 4.1 Hz), 153.05 (d, *J* = 4.8 Hz), 145.24 (t, *J* = 10.3 Hz), 132.90 (t, *J* = 10.4 Hz), 130.81 (t, *J* = 10.1 Hz),

128.98 (t, *J* = 9.9 Hz), 114.17 – 113.93 (m), 113.95 – 113.72 (m), 113.30 (d, *J* = 3.8 Hz), 113.10 (d, *J* = 3.2 Hz), 78.24 (s), 54.14 (s) , 36.30 (s), 28.06 (s).

^{19}F NMR (376 MHz, DMSO): δ = -126.59, -126.61. **IR (ATR):** $\tilde{\nu}$ = 3340 (vw), 2981 (vw), 2932 (vw), 1682 (w), 1626 (w), 1577 (w), 1519 (w), 1471 (w), 1446 (w), 1368 (w), 1242 (w), 1160 (w), 1062 (w), 1024 (m), 845 (w), 786 (w), 744 (w), 626 (w), 512 (vw), 472 (vw) cm^{-1}. **HRMS (FAB+):** *m/*z = 442.1388 [M+H]. Calculated for $C_{20}H_{20}F_4N_3O_4$: 442.1390.

Methyl N^6-(*tert*-butoxycarbonyl)-N^2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-((*E*)-(2,6-difluorophenyl)diazenyl)-3,5-difluorophenyl)propanoyl)-*L*-lysinat, „Boc-F4-PAP-Lys(Boc)-OMe" (10)

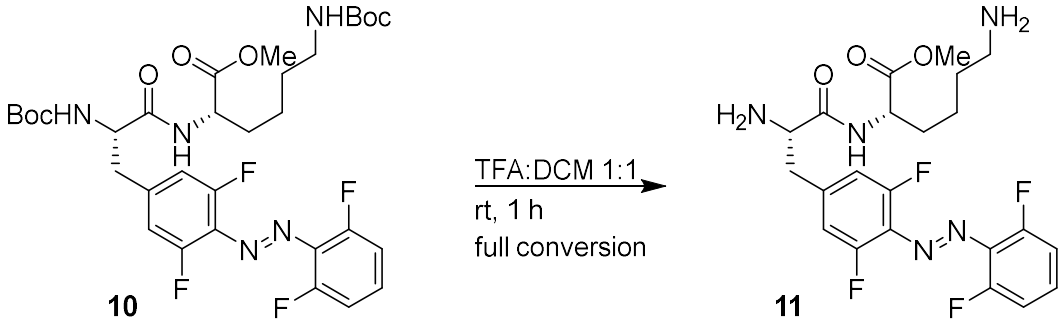


Boc-F4-PAP-OH **8** (3.09 g, 6.99 mmol, 1.0 eq.), HBTU (2.65 g, 6.99 mmol, 1.0 eq.) and DIPEA (3.0 mL, 17.5 mmol, 2.5 eq.) were dissolved in anhydrous DMF (23.3 mL) and stirred for 10 min at room temperature under argon. The same amount of DIPEA (3.0 mL, 17.5 mmol, 2.5 eq.) was added together with *ω*-*N*-Boc-lysine methyl ester hydrochloride (**9**, *H*-Lys(Boc)-OMe·HCl) (2.10 g, 7.06 mmol, 1.01 eq.) dissolved in DMF (23.3 mL). The reaction mixture was stirred at room temperature and the reaction progress was followed by TLC. After 2 hours, full conversion of the starting material was observed. The reaction mixture was quenched with sat. aq. NH_4Cl solution (200 mL) and extracted once with EtOAc (200 mL). The organic layer was washed with sat. aq. NH_4Cl solution (3×200 mL), brine (1×200 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography. The column was started with 40% EtOAc in cyclohexane to wash out colorless non-polar impurities (R_f=0.13). Then the product was eluted with EtOAc:cyclohexane 1:1 (R_f=0.38). Evaporation of combined fractions and drying *in vacuo* resulted in 4.22 g (6.17 mmol, 88% yield) of the linear dipeptide **10** as orange solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.33 (d, *J* = 7.5 Hz, 1H), 7.62 (tt, *J* = 8.5, 6.1 Hz, 1H), 7.35 (t, *J* = 8.9 Hz, 2H), 7.28 (d, *J* = 11.1 Hz, 2H), 7.04 (d, *J* = 8.8 Hz, 1H), 6.77 (t, *J* = 5.8 Hz, 1H), 4.27 (dtd, *J* = 27.4, 9.3, 8.3, 4.9 Hz, 2H), 3.62 (s, 3H), 3.06 (dd, *J* = 13.5, 4.3 Hz, 1H), 2.90 (q, *J* = 6.6 Hz, 2H), 2.83 (dd, *J* = 13.5, 10.4 Hz, 1H), 1.80 – 1.67 (m, 1H), 1.62 (dtd, *J* = 13.6, 9.1, 5.1 Hz, 1H), 1.33 (d, *J* = 31.8 Hz, 22H). **^{13}C NMR (126 MHz, DMSO-d_6):** δ = 172.4 , 171.2 , 155.6 (d, *J* = 4.5 Hz), 155.6 , 155.4 (d, *J* = 4.8 Hz), 155.2 , 153.5 (d, *J* = 4.1 Hz), 153.3 (d, *J* = 4.9 Hz), 145.3 (t, *J* = 10.2 Hz), 132.87 (t, *J* = 10.6 Hz), 130.8 (t, *J* = 10.0 Hz), 129.0 (t, *J* = 9.7 Hz), 114.0 (dd, *J* = 19.9, 3.2 Hz), 113.2 (dd, *J* = 19.6, 3.7 Hz), 78.2 , 77.3 , 54.8 , 52.0 , 51.9 , 37.4 , 30.7 , 29.1 , 28.3 , 28.0 , 22.6. **^{19}F NMR (471 MHz, DMSO-d_6):** δ = -122.26 (d, *J* = 11.5 Hz), -122.32 (dd, *J* = 10.3, 6.3 Hz). **^{1}H NMR (400 MHz, Acetic Acid-d_4):** δ = 7.46 (ddd, *J* = 14.3, 8.5, 5.8 Hz, 1H), 7.13 (t, *J* = 9.0 Hz, 2H), 7.07 (d, *J* = 10.6 Hz, 2H), 4.73 – 4.62 (m, 1H), 4.57 (dd, *J* = 8.9, 5.0 Hz, 1H), 3.72 (s, 3H), 3.20 (dd, *J* = 13.8, 6.4 Hz, 1H), 3.14 – 2.96 (m, 3H), 1.87 (dq, *J* = 13.5, 7.2 Hz, 1H), 1.71 (dq, *J* = 14.4, 7.9 Hz, 1H), 1.51 – 1.35 (m, 22H). **^{13}C NMR (101 MHz, Acetic Acid-d_4):** δ = 174.5, 174.4, 159.0, 158.6 (d, *J* = 4.3 Hz), 158.5 (d, *J* = 4.4 Hz), 158.1, 156.0 (d, *J* = 4.0 Hz), 155.9 (d, *J* = 4.6 Hz), 144.6, 133.5 (t, *J* = 9.7 Hz), 133.4, 131.9, 115.7 – 115.3 (m), 114.4 (dd, *J* = 21.0, 2.9 Hz), 82.4 , 81.4 , 56.9 , 54.3 , 53.9 , 41.7 , 39.6 , 32.8 , 30.8 , 29.4 , 29.3 , 24.3 . **^{19}F NMR (376 MHz, Acetic Acid-d_4):** δ = -126.35, -126.92. **IR (ATR):** $\tilde{\nu}$ = 3323 (vw), 2936 (vw), 1747 (w), 1680 (w), 1654 (w), 1626 (w), 1577 (w), 1520 (w), 1472 (w), 1439 (w), 1366 (w), 1324 (vw), 1242 (w), 1162 (w), 1051)w), 1024 (w), 851 (w), 786 (w), 744 (w),

643 (w), 513 (vw), 472 (vw) cm^{-1}. **HRMS (FAB+):** *m/*z = 684.3023 [M+H]. Calculated for $C_{32}H_{42}O_7N_5F_4$: 684.3020. **UV-Vis (MeCN):** λ_{max} = 314, 229 and 454 nm.

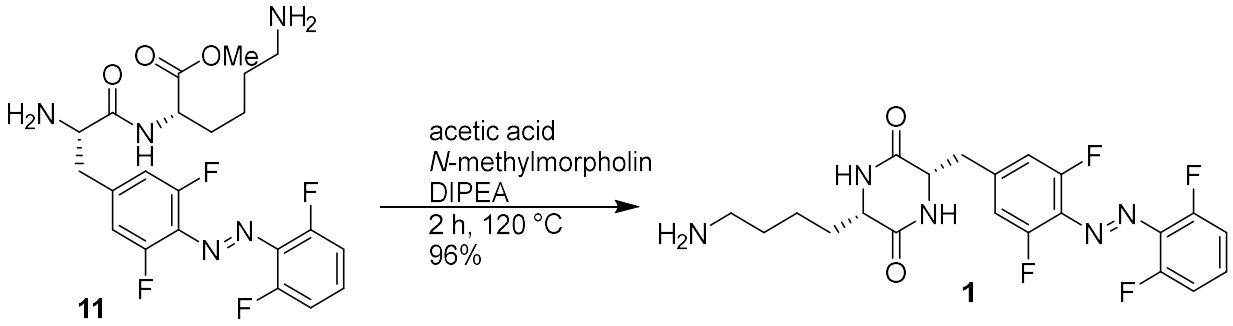
Methyl ((*S*)-2-amino-3-(4-((*E*)-(2,6-difluorophenyl)diazenyl)-3,5-difluorophenyl)propanoyl)-*L*-lysinate (11) "H-F4-PAP-Lys-OMe"



Methyl N^6-(*tert*-butoxycarbonyl)-N^2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-((*E*)-(2,6-difluorophenyl)diazenyl)-3,5-difluorophenyl)propanoyl)-*L*-lysinate **10** (4.10 g, 6.17 mmol, 1.0 eq.) was dissolved in dichloromethane (61 mL) and trifluoroacetic acid (TFA, 61 mL) and 1 vol% triisopropylsilane (1.2 mL, 6.17 mmol, 1.0 eq.) was added at room temperature. The mixture was stirred for 1 h at room temperature. Then the reaction mixture was diluted with 100 mL toluene and evaporated under reduced pressure. After drying under high vacuum, the crude deprotected linear dipeptide **11** was obtained as TFA salt (4.86 g). For the analytics, 50 mg of the product were dissolved in 2 mL DMSO and purified by preparative HPLC with the following settings: 10 mL/min, 40 min gradient 5-95% MeCN in diH_2O with 0.1% TFA, detection at 330 nm, C18-colum, retention time 22.0 min.

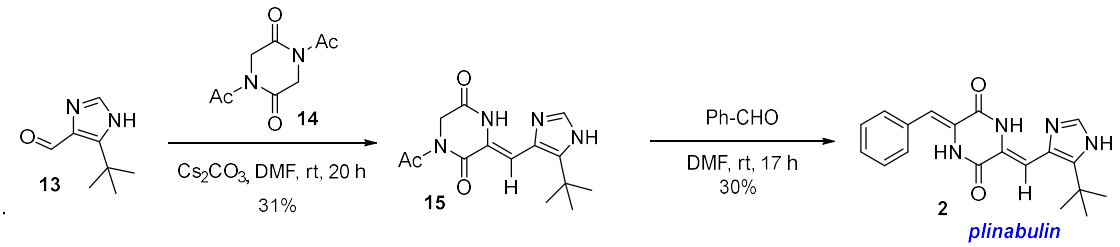
^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.03 (d, *J* = 7.4 Hz, 1H), 8.39 (s, 3H), 7.88 (s, 3H), 7.63 (tt, *J* = 8.5, 6.1 Hz, 1H), 7.36 (t, *J* = 8.9 Hz, 2H), 7.27 (d, *J* = 10.4 Hz, 2H), 4.32 (ddd, *J* = 9.0, 7.4, 5.2 Hz, 1H), 4.22 (s, 1H), 3.64 (s, 3H), 3.17 (ddd, *J* = 58.1, 14.0, 6.7 Hz, 2H), 2.77 (dq, *J* = 12.7, 5.6 Hz, 2H), 1.84 – 1.70 (m, 1H), 1.65 (dtd, *J* = 14.0, 9.3, 5.3 Hz, 1H), 1.55 (p, *J* = 7.6 Hz, 2H), 1.38 (td, *J* = 13.0, 9.5, 5.6 Hz, 2H). **^{13}C NMR (126 MHz, DMSO-d_6):** δ = 171.8 , 167.9 , 158.5 (q, *J* = 32.1 Hz), 155.6 (t, *J* = 4.3 Hz), 153.6 (t, *J* = 4.6 Hz), 141.3 (t, *J* = 10.3 Hz), 133.1 (t, *J* = 10.6 Hz), 130.8 (t, *J* = 10.1 Hz), 129.7 (t, *J* = 9.9 Hz), 117.0 (q, *J* = 298.2 Hz), 114.5 (dd, *J* = 20.4, 3.2 Hz), 113.3 (dd, *J* = 19.7, 3.6 Hz), 52.8 , 52.1 , 52.0 , 38.5 , 36.5 , 30.4 , 26.6 , 22.2. **^{19}F NMR (471 MHz, DMSO-d_6):** δ = -121.86 (d, *J* = 11.2 Hz), -122.33 (dd, *J* = 9.9, 6.2 Hz). **^{1}H NMR (400 MHz, Acetic Acid-d_4):** δ = 7.50 (ddd, *J* = 14.3, 8.5, 5.8 Hz, 1H), 7.17 (t, *J* = 9.5 Hz, 4H), 4.73 (t, *J* = 6.8 Hz, 1H), 4.61 (dd, *J* = 9.0, 4.8 Hz, 1H), 3.77 (s, 3H), 3.39 (d, *J* = 6.9 Hz, 2H), 3.10 (t, *J* = 7.5 Hz, 2H), 1.92 (ddd, *J* = 13.8, 8.5, 5.0 Hz, 1H), 1.76 (tq, *J* = 14.1, 6.5 Hz, 3H), 1.50 (q, *J* = 8.2, 7.6 Hz, 2H), 1.09 (d, *J* = 4.4 Hz, 1H). **^{19}F NMR (376 MHz, Acetic Acid-d_4):** δ = -125.86, -126.89. **IR (ATR):** $\tilde{\nu}$ = 2954 (w), 1666 (m), 1627 (w), 1579 (w), 1472 (w), 1438 (w), 1243 (w), 1181 (m), 1131 (m), 1050 (w), 1023 (w), 837 (w), 798 (w), 743 (vw), 722 (m), 624 (vw), 597 (vw), 515 (vw), 481 (vw), 410 (vw) cm^{-1}. **HRMS (FAB+):** *m/*z = 484.1971 [M+H]. Calculated for $C_{22}H_{26}O_3N_5F_4$: 484.1972. **UV-Vis (MeCN):** λ_{max} = 311 and 229 nm.

(3*S*,6*S*)-3-(4-Aminobutyl)-6-(4-((*E*)-(2,6-difluorophenyl)diazenyl)-3,5-difluorobenzyl)piperazine-2,5-dione (1), "F4-PAP-DKP-Lys"



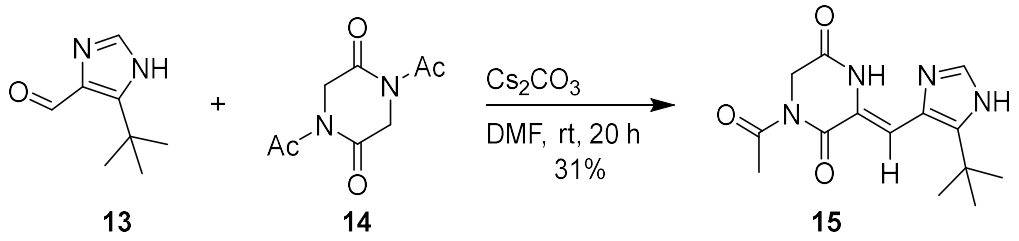
The crude (*S*)-2-amino-3-(4-((*E*)-(2,6-difluorophenyl)diazenyl)-3,5-difluorophenyl)propanoyl)-*L*-lysinate **11**, (4.87 g, 6.17 mmol, 1.0 eq.) was dissolved in 2-butanol (260 mL). It was mixed with glacial acetic acid (1302 µL, 22.8 mmol, 3.69 eq.), *N*-methylmorpholine (865 µL, 7.87 mmol, 1.28 eq.) and *N,N*-diisopropyl-*N*-ethylamine (DIPEA, 1562 µL, 8.97 mmol, 1.45 eq.). The resulting mixture was refluxed for 2 h (oil bath, external heating 120°C), then cooled down. Subsequently, approximately 80% of the solvent (c.a. 60 mL) was removed under reduced pressure. Cooling down to room temperature resulted in orange gel-like precipitation. The precipitate was filtered off, washed with a small amount of cold MeCN (3×20 mL) on the filter and dried *in vacuo* overnight resulting in the "F4-PAP-DKP-Lys" (3S,6*S*)-3-(4-aminobutyl)-6-(4-((*E*)-(2,6-difluorophenyl)diazenyl)-3,5-difluorobenzyl) piperazine-2,5-dione (**1**) (2.67 g, 5.91 mmol, 96% yield) as orange solid. If necessary, additional purification can be performed with preparative HPLC under following conditions: 10 mL/min, 40 min gradient 20-40% MeCN in diH_2O with 0.1% TFA, detection at 330 nm, C18-colum, retention time 22.5 min, the crude compound injected as solution in DMSO.

^{1}H NMR (400 MHz, Acetic Acid-*d*$_4$): δ = 7.49 (ddd, *J* = 14.3, 8.5, 5.9 Hz, 1H), 7.15 (t, *J* = 9.3 Hz, 2H), 7.09 (d, *J* = 10.4 Hz, 2H), 4.61 (t, *J* = 5.5 Hz, 1H), 4.14 (d, *J* = 6.4 Hz, 1H), 3.38 – 3.17 (m, 2H), 3.03 (t, *J* = 7.6 Hz, 2H), 1.82 – 1.53 (m, 3H), 1.46 – 1.17 (m, 3H). (amine and amid not visible) **^{13}C NMR (101 MHz, Acetic Acid-*d*$_4$):** δ = 190.6 , 189.3 , 182.3 (d, *J* = 37.1 Hz), 177.4 (d, *J* = 4.0 Hz), 177.2 (d, *J* = 4.8 Hz), 174.8 (d, *J* = 4.0 Hz), 174.6 (d, *J* = 4.7 Hz), 161.6 (t, *J* = 9.6 Hz), 152.7 (t, *J* = 10.4 Hz), 152.3 – 151.6 (m), 151.4 – 150.9 (m), 136.7 (d, *J* = 290.6 Hz), 135.0 (d, *J* = 23.4 Hz), 133.2 (dd, *J* = 20.6, 3.3 Hz), 75.9 , 74.6 , 60.1 , 59.6 , 53.7 , 46.8 , 41.5. **^{19}F NMR (376 MHz, Acetic Acid-*d*$_4$):** δ = -126.28, -126.82 ppm. **^{1}H NMR (300 MHz, Deuterium Oxide, 137 mM NaCl):** δ = 7.63 – 7.41 (m, 1H), 7.17 (t, *J* = 9.8 Hz, 2H), 7.04 (d, *J* = 11.3 Hz, 2H), 4.56 (s, 1H), 4.01 (s, 1H), 3.35 (d, *J* = 14.1 Hz, 1H), 3.20 – 2.97 (m, 1H), 2.78 (t, *J* = 7.7 Hz, 2H), 1.55 – 1.20 (m, 3H), 1.11 – 0.72 (m, 3H). **IR (ATR):** $\tilde{\nu}$ = 2952 (vw), 1664 (m), 1627 (w), 1578 (w), 1450 (w), 1332 (w), 1242 (vw), 1199 (w), 1174.4 (w), 1129 (w), 1052 (w), 1024 (w), 833 (w), 786 (w), 741 (w), 720 (w), 621 (vw), 480 (vw), 436 (vw), 382 (vw) cm^{-1}. **HRMS (FAB+):** *m/z* = 452.1708 [M+H]. Calculated for $C_{21}H_{22}O_2N_5F_4$: 452.1710. **UV-Vis (MeCN):** λ_{max} = 312, 228 and 456 nm.



Scheme S2. The modular synthesis of the low-nM antimitotic agent plinabulin **2** from the aldehyde **13** and the acetylated glycine anhydride **14** according to references [S4] and [S5].

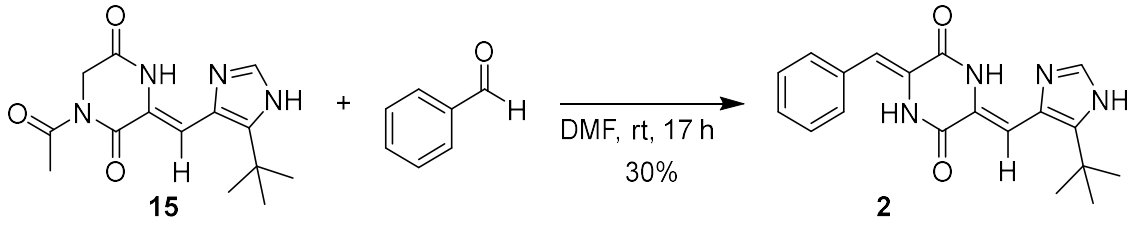
(*Z*)-1-Acetyl-3-((5-(*tert*-butyl)-1*H*-imidazol-4-yl)methylene)piperazine-2,5-dione (15)



A mixture of (5-(*tert*-butyl)-1*H*-imidazol-4-carbaldehyde (**13**) (1.19 g, 7.79 mmol, 1.00 eq), 1,4-diacetyl-piperazine-2,5-dione (**14**) (3.09 g, 15.6 mmol, 2.00 eq) and Cs_2CO_3 (3.81 g, 11.7 mmol, 1.50 eq) in 10 mL of dry DMF was stirred under argon at room temperature for 20 h. The reaction mixture was poured on ice water (20 mL) and the precipitate was filtered off and dried to yield 703 mg (2.42 mmol, 31%) of compound **15** as a yellow solid.[5]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.15 (br s, 1H), 9.26 (br s, 1H), 7.57 (s, 1H), 7.18 (s, 1H), 4.47 (s, 2H), 2.65 (s, 3H), 1.46 (s, 9H). **^{13}C NMR (101 MHz, $CDCl_3$):** δ = 173.0 (s), 162.6 (s), 160.7 (s), 141.0 (s), 132.5 (s), 131.7 (s), 124.0 (s), 109.0 (s), 77.4 (s), 46.6 (s), 32.0 (s), 30.9 (s), 27.5 (s). **IR (ATR):** $\tilde{\nu}$ = 2962 (w), 1671 (m), 1617 (w), 1506 (w), 1419 (m), 1366 (m), 1229 (m), 1190 (m), 1103 (w), 1051 (w), 1022 (w), 999 (w), 948 (w), 872 (w), 818 (w), 777 (w), 746 (w), 729 (w), 692 (w), 661 (w), 577 (w), 535 (w), 508 (vw), 471 (w), 437 (w) cm^{-1}. **HRMS (EI+):** *m/z* = 290.1378 [M]. Calculated for $C_{14}H_{18}N_4O_3$: 290.1379.

(3*Z*,6*Z*)-3-Benzylidene-6-((5-(*tert*-butyl)-1*H*-imidazol-4-yl)methylene)piperazine-2,5-dione (2), *Plinabulin*

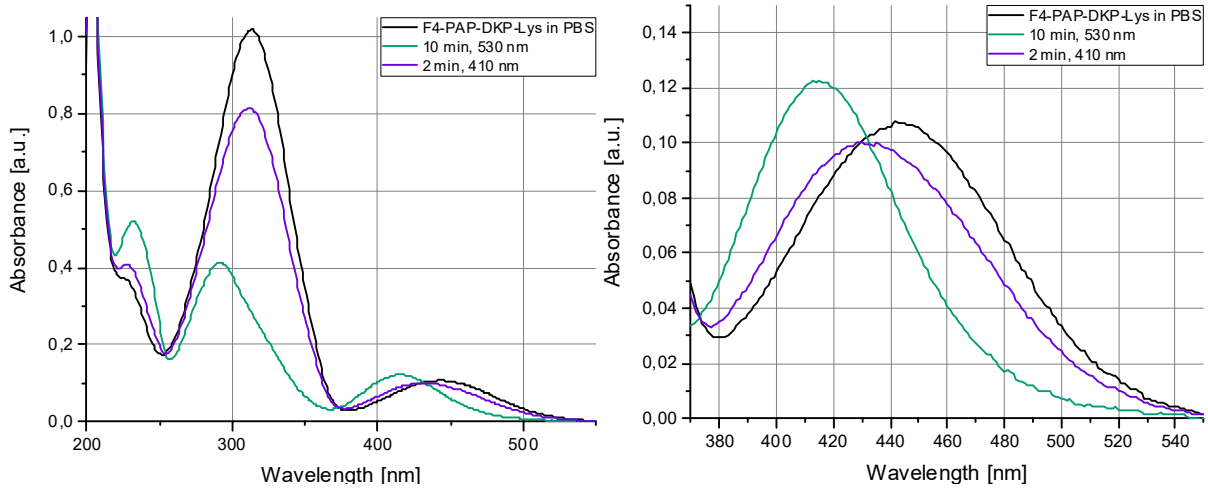


A mixture of *(Z)*-1-acetyl-3-((5-(*tert*-butyl)-1*H*-imidazol-4-yl)methylene)piperazine-2,5-dione (**15**) (200 mg, 0.689 mmol, 1.00 eq), benzaldehyde (110 mg, 1.03 mmol, 1.50 eq) and Cs_2CO_3 (336 mg, 1.03 mmol, 1.50 eq) in 2.1 mL of dry DMF was stirred at room temperature under argon for 17 h. The reaction mixture was poured on ice water (20 mL) and the precipitate was filtered off. The crude product was purified by HPLC with 41 min gradient from 20-95% MeCN in diH_2O with 0.1% TFA, retention time = 21.7 min to yield 70 mg (0.208 mmol, 30%) of compound **2** as a pale-yellow solid.[5]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.11 (bs, 1H), 11.33 (bs, 2H), 7.79 (s, 1H), 7.64 (d, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.4 Hz, 1H), 6.80 (s, 1H), 6.65 (s, 1H), 1.38 (s, 9H). **^{13}C NMR (101 MHz, DMSO-d_6):** δ = 158.0 (s), 156.7 (s), 140.2 (s), 134.3 (s), 133.8 (s), 130.9 (s), 129.3 (s), 128.6 (s), 128.0 (s), 127.6 (s), 124.2 (s), 113.3 (s), 104.5 (s), 31.9 (s), 30.7 (s). **IR (ATR):** $\tilde{\nu}$ = 2977 (vw), 1684 (w), 1644 (w), 1400 (w), 1353 (w), 1186 (w), 1144 (w), 953 (vw), 801 (w), 765 (w), 719 (w), 689 (w), 645 (vw), 520 (vw), 459 (vw), 442 (vw) cm^{-1}. **HRMS (FAB+):** *m/*z = 337.1665 [M]. Calculated for $C_{19}H_{20}N_4O_2$: 337.1665.

3. Photophysical properties of the gelator 1

a)



b)

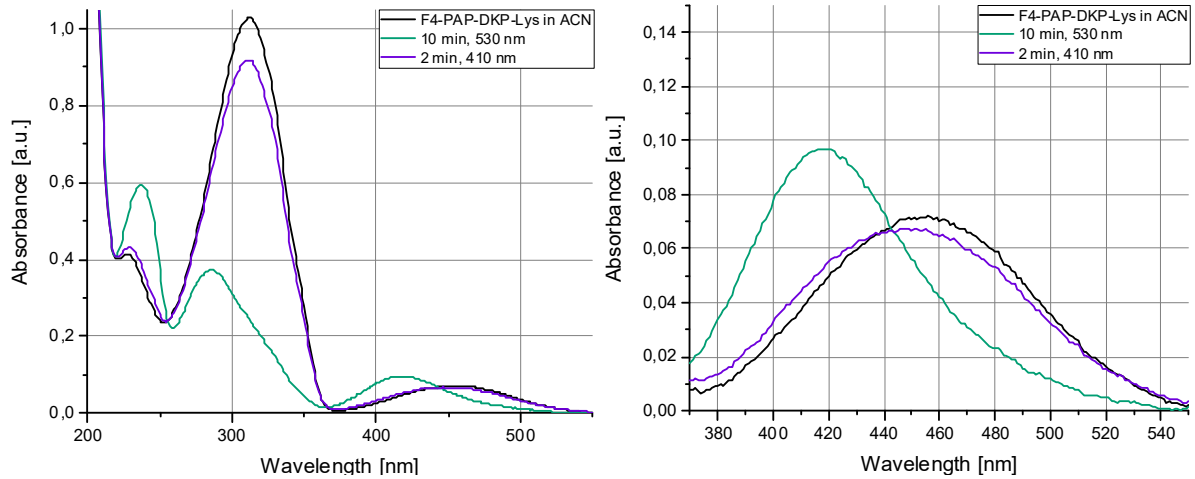


Figure S1. UV-Vis absorbance of the compound **1** ("F4-PAP-DKP-Lys"). **a)** 55 µM of **1** in aqueous PBS buffer pH 7.4 in absence of light or upon irradiation with green (530 nm) or violet (410 nm) light diodes (right side – magnification) **b)** 55 µM of **1** in MeCN, under identical conditions.

The 'dark state' refers to the *trans*-isomer obtained by thermal equilibration during the purification procedure (crystallization from 2-butanol/MeCN) during synthesis of the compound **1** (F4-PAP-DKP-Lys) and its further storage in absence of light. For UV-Vis measurements, the HPLC-purified F4-PAP-DKP-Lys **1** containing >99% *trans*-isomer was used. Daylight irradiation results in decrease of the *trans*-isomer in the mixture.

For the HPLC-based quantification of the *cis*-/*trans*-isomer ratio of **1**, we have chosen λ = 254 nm as the isosbestic point (were the molar absorptivity of both photoisomers is equal) with a medium absorption 0.1782 au ± 0.0012 au for the 55 µM solution of F4-PAP-DKP-Lys **1** in PBS. Due to different polarities of the azobenzene photoisomers, both isomers of compound **1** exhibit different retention times, and can be independently quantified.

A 1.0 mM solution of **1** (F4-PAP-DKP-Lys) in PBS was irradiated for 30 min with 530 nm (green LED), or 10 min with 410 nm (violet LED) to achieve the photostationary state (PSS). 25 µL of each solution, as

well as 25 µL of the non-irradiated solution were injected in the RP-HPLC under following conditions: run of 20 min, gradient 20% H_2O to 40% MeCN with 0.1% TFA (4 nm slit, wavelength 254 nm):

PSS values in aqueous PBS buffer: $PSS_{530\ nm}$ = 89% *cis*-**1**; $PSS_{410\ nm}$ = 23% *cis*-**1**.

The same experiment was performed using acetonitrile (MeCN) as a solvent instead of the aqueous PBS buffer. The PSS in 1.0 mM solution of **1** in MeCN was achieved upon 10 min of irradiation with both 530 nm and 410 nm light frequencies:

PSS values in MeCN solution: $PSS_{530\ nm}$ = 87% *cis*-**1**; $PSS_{410\ nm}$ = 18% *cis*-**1**.

Overall, the values of photostationary states for the compound **1** slightly depend on the solvent. Due to the extraordinarily long lifetime of the *cis*-**1** (**Figure S2**), both photoisomers can be then separately stored in darkness at ambient temperature without significant interconversion.
It has to be underlined, that the samples must be stored and handled in absence of daylight (at least below 600 nm) by using red lamps, brown glass, or wrapping the transparent glassware with aluminium foil to prevent prompt *trans/cis* ratio changes.

Measurement of the lifetime of the cis-1

The *cis*-isomers of azobenzenes are thermally less stable than the respective *trans*-isomers and undergo spontaneous thermal equilibration. However, the rate of equilibration depends on the substitution pattern on both aromatic rings of the azobenzene system and can span from microseconds to years. For the *ortho*-fluorinated azobenzenes, lifetime of the *cis*-isomer is enhanced in comparison to the non-substituted azobenzene.
Our measurements of thermal isomerization parameters for the *cis*-**1** have been compared with the existing literature data.[3,9] For measurements, the thermally equilibrated **1** (almost exclusively the *trans*-**1**) was purified by preparative RP-HPLC, lyophilised, dissolved in MeCN (1.0 mM) and irradiated for 10 min with 530 nm LED diode to achieve the photostationary state (87% *cis*-**1**, see above). Thermal decay of the *cis*-**1** to its *trans*-isomer was measured by analytical RP-HPLC (by using the same conditions like for quantification of the *cis/trans* ratio). We determined the half-life of *cis*-**1** to be 70.9 ± 0.6 h at 60 ± 2 °C in MeCN (**Figure S2** *left*), which is comparable to values reported previously by Bleger *et al.* for the tetra-*ortho*-fluoroazobenzene and other *ortho*-fluoroazobenzenes.[9]
It comes in agreement with our observation that, upon light-induced dissipation of hydrogels composed of *trans*-**1** with green light to the respective fluid (sol), the fluid (containing now mostly the *cis*-**1**) cannot solidify again to the hydrogel after incubation of the sample in darkness at room temperature (hydrogel with 2% of **1**, n=5), at least within 7 days. On the other hand, during last step of the synthesis of **1** (*vide supra*), the reaction mixture that is warmed up to 120 °C for 2 h – under these conditions *cis*-**1** accidentally formed, e.g. upon irradiation with daylight, will thermally back-isomerize, providing the product **1** almost exclusively in form of the *trans*-isomer.
The gelator **1** does not show any measurable degradation upon 50 h incubation at 60 °C. However, warmed up to the boiling point in aqueous buffer (PBS pH 7.4), **2** exhibits degradation to multiple products with the $\tau_{1/2}$ = 2.7 h ± 0.2 h at 100 ± 2 °C. Therefore heating over 60 °C in aqueous solutions should be limited to the necessary minimum. This problem has not been observed for non-aqueous solutions, e.g. during the synthesis of **1** from **11** upon heating to 120 °C in 2-butanol.

Stability of F4-PAP-DKP-Lys against glutathione

We investigated the stability of **1** in the reducing environment, emulating the intracellular redox potential values. A solution of 0.50 mM (1.0 eq.) of **1** and 5.0 mM reduced glutathione (10 eq.) was prepared in PBS and incubated in darkness at room temperature. After 3 days, 94% of **1** remained

unaffected, as evidenced by RP-HPLC (218 nm) (**Figure S2** *right*). After 6 days, 92% of the initial concentration of **1** was still detected. Then the experiment was stopped.

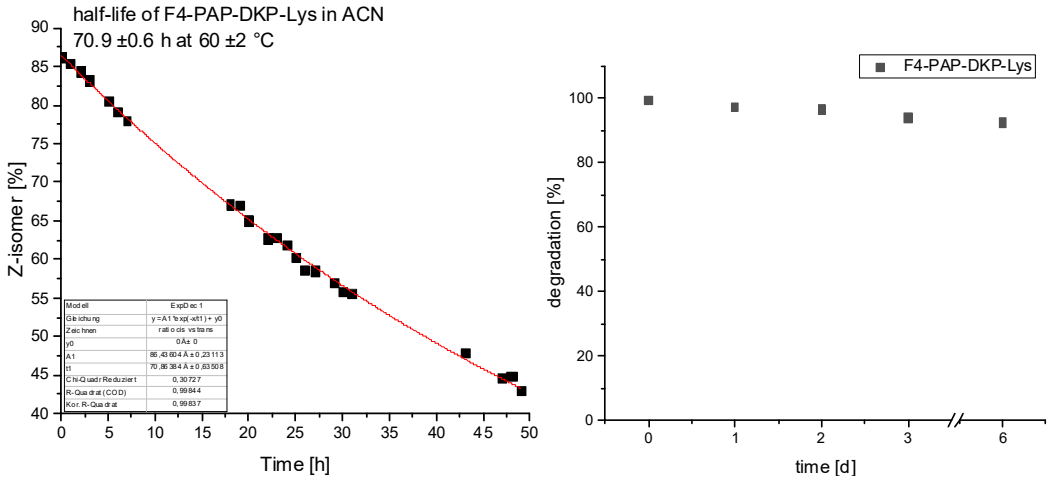


Figure S2. *Left*: 70.9 ± 0.6 h half-life of *cis*-**1** (F4-PAP-DKP-Lys) at 60 ± 2 °C in acetonitrile (MeCN); *Right:* Stability of the gelator **1** (0.50 mM of **1**) against 5.0 mM reduced glutathione solution.

4. Gelation properties of the compound 1.

Measuring the melting temperature of the hydrogels.

The gel-to-sol transition temperature is characteristic for the particular gel composition, and can be used to estimate its relative stability in comparison to the other gel samples. The value of that transition, referred to as "the melting temperature" of a gel, was measured according to the following protocol: in a 1.5 mL glass vial (crimp top, 12×32 mm), the photochromic gelator **1** was added to PBS buffer pH 7.4 (500 µL) (mass of the gelator and its approximate final percentage in the gel are listed in the **Table S2**). The crimped vial was warmed up vertical in a water bath at 80 °C for 5 minutes. The suspension was dissolved in the crimped vial after heating it up to the boiling point (<1 min) with a heat gun. **Warning:** prolonged boiling of the hydrogel under these conditions should be avoided, since decomposition of **1** to unidentified products (with a half-life of **1** being 2.7 h ± 0.2 h at 100 ± 2 °C in the PBS buffer) has been observed.

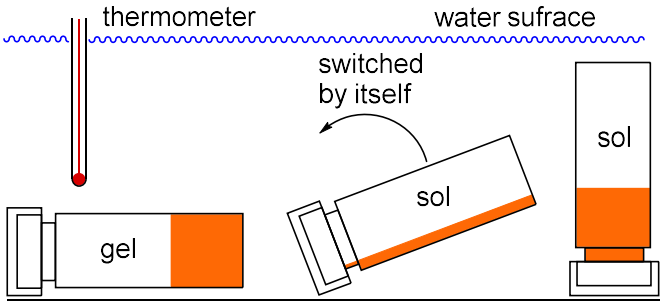
The hot fluid turned to an orange solution and this fluid was gelated upon cooling to r.t. (typically within 1 h). Before a measurement, the hydrogel (unless indicated otherwise) was kept overnight in darkness at room temperature in order to allow equilibration of the components, which in turn minimized statistical deviation of the measured behaviour.

To measure the melting temperature, a sample of the hydrogel prepared above was swimming horizontally on the surface of a slowly stirred (60 rpm) water bath at 25 °C. The bath was then warmed up with the heating rate of ca. 2 °C/min. The hydrogel starts melting slowly before the resulting sol (fluid) will abruptly flow down at the given gel-to-sol transition temperature. The measurement was done with 5 (n=5) identical samples and the average transition temperature was reported as "the melting temperature" T_m.

Gels prepared from **1** in PBS buffer pH 7.4 were stable and homogenous in the range between 15 g/L and 50 g/L (1.5% - 5% of **1**), also at ambient daylight. At the lower concentration of ≤12.0 g/L (1.2%) gelation was slow (several days), the resulting viscous gel had low mechanical stability (almost immediately turned into liquid upon slight shaking of the vial) and therefore the respective melting points have not been determined (**Table S2**).

Table S2. Gel to sol transition temperatures of hydrogels comprising the gelator **1** in PBS buffer

*Composition of the gel (x mg of **1** + 500 µL PBS)*	Approx. Concentration	T_m (^{0}C)	*Gelation time at room temperature*
5.0	"1%" (10 g/L)	-	n.d.
6.3	"1.2%" (12 g/L)	-	n.d.
7.5	"1.5%" (15 g/L)	46 ± 8	1 h-16 h
8.8	"1.7%"(17 g/L)	**57 ± 3**	c.a. 1 h
10	"2%" (20 g/L)	82 ± 1	< 1 h



To examine the applicability of our hydrogelator at pH values deviated from the physiological standard of pH 7.4, we have prepared samples containing 17 g/L of the gelator **1** in aqueous buffers with pH values of 4, 6, 8 and 10 (the exact composition and measured pH values summarized below) according to the same protocol, as stated above for the hydrogels formed at pH 7.4 (PBS buffer). The solid **1** did not dissolve in the buffer at the pH 10 up to the boiling point, and upon cooling no gelation was observed in that sample. The gelator dissolved fully in buffers at the pH range 4-8 above c.a. 60 °C. Within 1 h of cooling at room temperature, all these samples yielded homogenous hydrogels. The hydrogel formed at pH 4 was mechanically unstable and dissipated upon slight shaking or vial inversion. Hydrogels formed at pH 6 and pH 8 were stable under these conditions, and mechanically similar to the hydrogel formed at pH 7.4 with the same gelator concentration. Also the melting temperatures, measured according to the protocol described above, were almost identical to the temperature determined beforehand for the hydrogel prepared from 17 g/L of **1** at pH 7.4 (57± 3 °C) – 57 °C at the pH 6 and 59 °C at the pH 8. The molten hydrogel samples solidified again at room temperature within 1 hour.

pH 4 **pH 6** **pH 8** **pH 10**

Figure S3. Gelation process of **1** at various pH values: (from left to right) pH 4, pH 6, pH 8 and pH 10. Homogenous hydrogels were generated within 1 h at room temperature upon short boiling of the samples in aqueous buffers at the range of pH 4-8. The gelator **1** was insoluble at pH 10.

Composition and exact pH values of the used buffers:

pH=4

Components	MW (g/mol)	Conc. (mg/L)	mM
Citric acid	192	856	4.46
KCl	75.0	200	2.67
NaCl	58.0	8000	138
$Na_2HPO_4*7H_2O$	268	292	1.09

The final exact pH was adjusted to 3.96 by addition of diluted aqueous NaOH solution and measuring the pH on a pH meter (WTW pH 3310 with a SenTix® 41 electrode).

pH=6

Components	MW (g/mol)	Conc. (mg/L)	mM
KCl	75.0	200	2.67
NaCl	58.0	8000	138
NaH_2PO_4	120	1051	8.76
$Na_2HPO_4*7H_2O$	268	330	1.23

The final exact pH was adjusted to 5.96 by addition of diluted aqueous HCl solution and measuring the pH on a pH meter (WTW pH 3310 with a SenTix® 41 electrode).

pH=8

Components	MW (g/mol)	Conc. (mg/L)	mM
KCl	75.0	200	2.67
NaCl	58.0	8000	138
NaH_2PO_4	120	468	3.90
$Na_2HPO_4*7H_2O$	268	1635	6.10

The final exact pH was adjusted to 8.02 by addition of diluted aqueous HCl solution and measuring the pH on a pH meter (WTW pH 3310 with a SenTix® 41 electrode).

pH=10

Components	MW (g/mol)	Conc. (mg/L)	mM
KCl	75.0	200	2.67
NaCl	58.0	8000	138
Na_2CO_3	106	6360	60
$NaHCO_3$	84.0	3360	40

The final exact pH was adjusted to 10.05 by addition of diluted aqueous HCl solution and measuring the pH on a pH meter (WTW pH 3310 with a SenTix® 41 electrode).

5. Light-induced gel-sol transitions

First of all, we have to clearly indicate that the speed of light-induced gel-sol transition in our material depends on the factors like: concentration of the gelator, the presence of other components (like NaCl or TFA) in the gelation medium, the preparation protocol of the hydrogel, the ratio between height and diameter of the glass vial (and consequently, the area of light-absorbing surface of the gel), as well as the light intensity (including the respective distance of the light source from the irradiated sample). All the results reported here were measured in multiplicates and reproducible within the same experimental setup.

Samples of the hydrogels (1.5-2 wt% of **1** in PBS buffer pH 7.4, prepared according to the **Table S2**) in 1.5-mL-vials (crimp top, 12×32 mm) have been irradiated with green light (LED diode 530 nm, 7.08 mW/cm^2) at room temperature (22 °C). The vials were inverted every 5 min. After 30 min irradiation, the samples of 1.5% and 1.7% gels turned into homogenous fluids without need for any mechanical stimulation upon inversion. The gel samples at the concentration of 2.0% after 30 min of irradiation became so unstable, that they dissipated to fluid upon slight shaking. The gel samples at the concentration of 3.0% and above have been irradiated for 60 min without an effect – they remained stable gels. With the "2.0%" hydrogel we have observed reproducible (n=5) back-isomerisation (*cis*-to-*trans*) of the sol samples after irradiation with violet light (LED diode 410 nm, 9.07 mW/cm^2) for 60 min and incubation at room temperature overnight, without additional heating.

The gel composition "1.7%" prepared from 8.8 mg of **1** in 500 µL PBS buffer pH 7.4 (**Table S2** – the entry highlighted in blue) was selected as the most optimal formulation for the encapsulation and release experiments described below due to its mechanical stability, quick gelation upon cooling, and its efficient gel-to-sol transition without any mechanical stimulus.

Figure S4. Transparent hydrogel (1.7 wt% of **1** in PBS pH7.4) (**left**) turns into sol after irradiation with green light (**right**)

6. Rheology

The rheological characterization of hydrogels formed by the compound **1** in PBS buffer pH 7.4 ("1.7%") was performed on samples generated by cooling the warm solution (formed from 140 mg of **1** and 8.00 mL of buffer) directly on the rheometer plate from 95°C to room temperature. The hydrogel was covered with Parafilm and incubated overnight at room temperature in the dark. Strain sweep experiments were performed at 10 rad/s to determine the linear viscoelastic regime and the mechanical strength of the hydrogel at 20 °C. Frequency experiments were performed at low strain within the linear viscoelastic region (LVR) of the sample. For regeneration experiments, the samples were exposed to a deformation of 100% for thirty seconds to destroy the supramolecular network, afterwards the regeneration of G' was measured at low strain within the LVR.

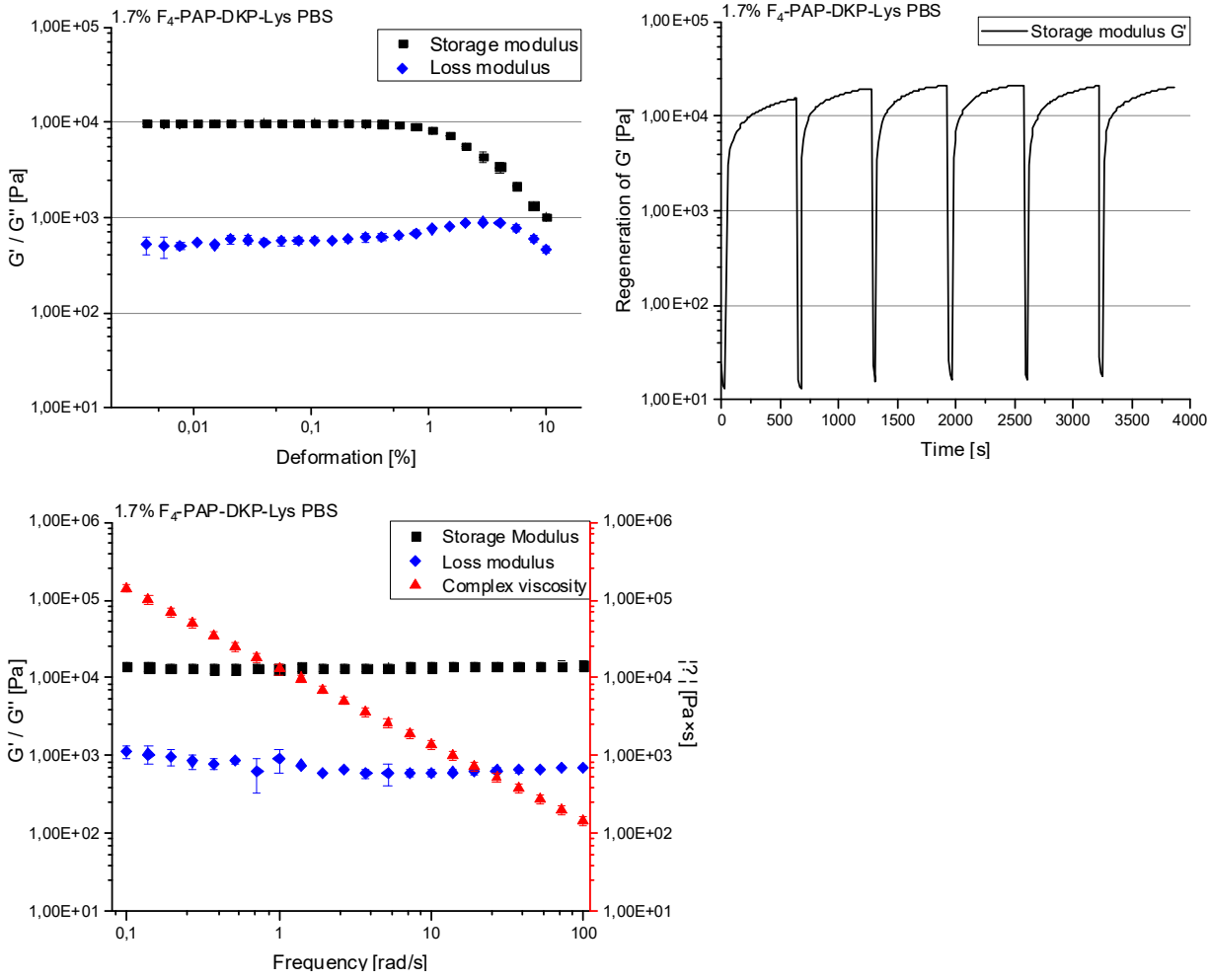
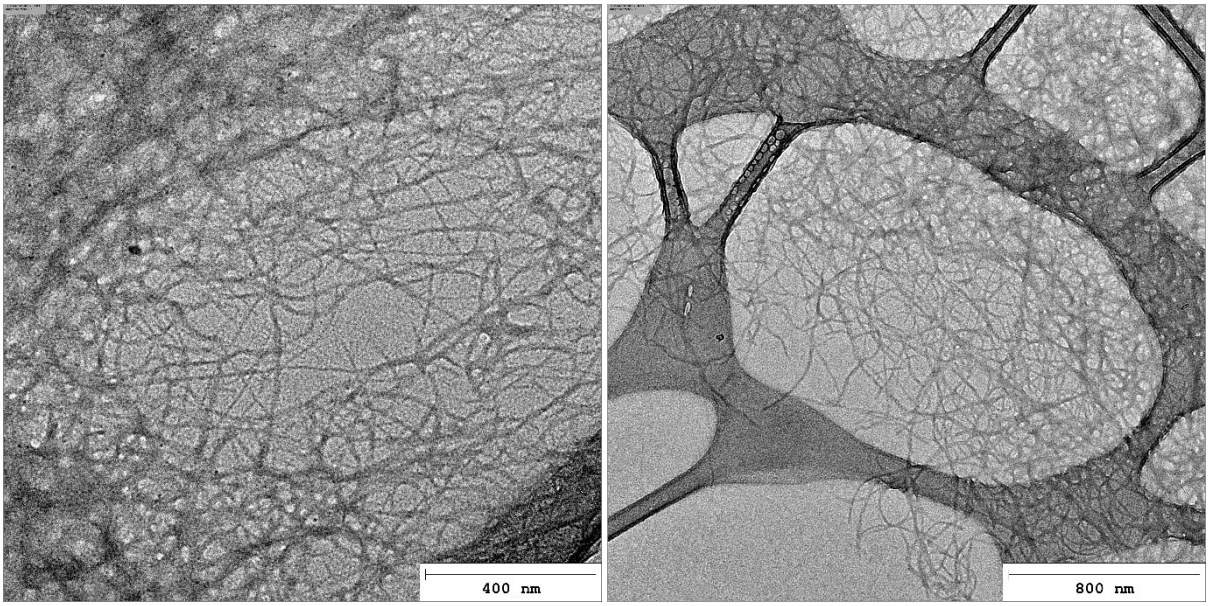


Figure S5. Rheological properties of the hydrogel (140 mg of **1** and 8.00 mL of PBS buffer pH 7.4). Strain sweep experiment (top left); frequency sweep experiment (bottom); regeneration of G' after shearing the gel for 30 seconds at 100% deformation (top right);

7. TEM characterization of the hydrogelator 1

Two diluted solutions were prepared from 17 mg F4-PAP-DKP-Lys **1** dissolved in 1 mL of diH_2O with or without 8 mg of NaI. Carbon-coated copper grids (400 Mesh) were covered with the material by short exposure on spray generated from the aforementioned solutions with ultrasounds. The grids were dried under atmospheric pressure and subsequently examined using a Philips CM200 transmission electron microscope. Under these conditions, the fine fibrous structure of our material was revealed.

Pictures registered without NaI:



Pictures registered with 50 mM NaI:

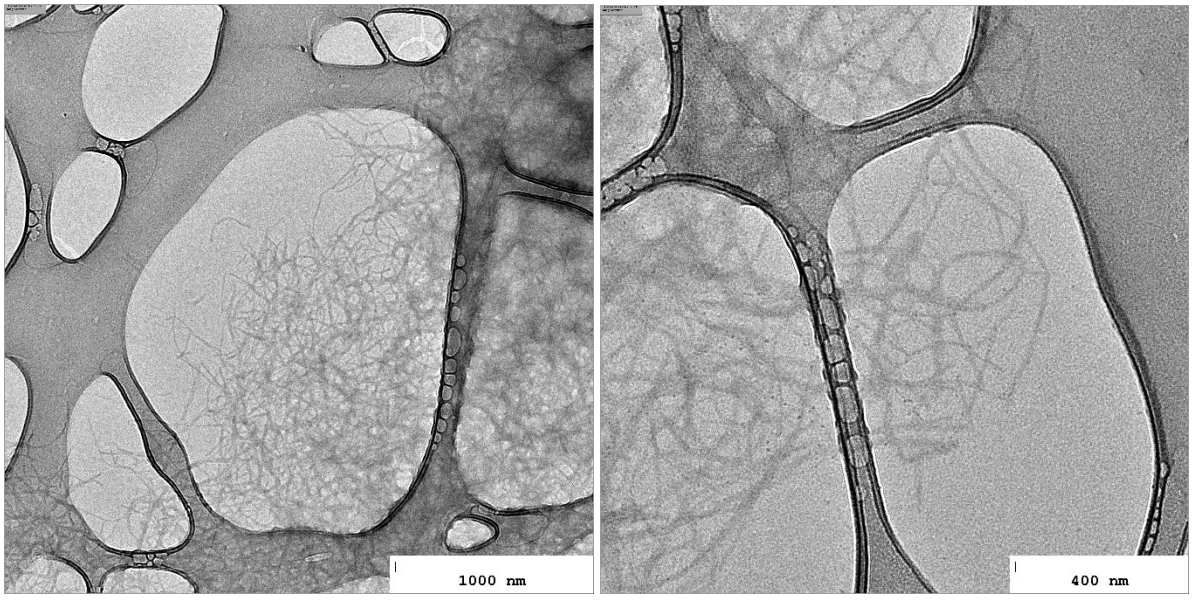


Figure S6. Transmission electron microscopy (TEM) images of the gelator **1** in diluted aqueous solutions.

8. Guest release from hydrogels using green light

Here we wanted to investigate how efficient are hydrogels based on the gelator **1** in releasing encapsulated guest molecules by means of diffusion (in darkness) or dissipation of the inner gel structure upon irradiation with green light. We have chosen the composition of 8.8 mg of the gelator **1** in 500 µL of PBS buffer pH 7.4 as the gel matrix for all experiments in this section. As described in the **section 4** of this supporting information (**Table S2**), it forms a stable gel in absence of light, and this gel is completely converted to sol upon 30 min of irradiation with green light (530 nm, 7.08 mW/cm^2) without need for mechanical stimuli.

Preparation of the hydrogel samples

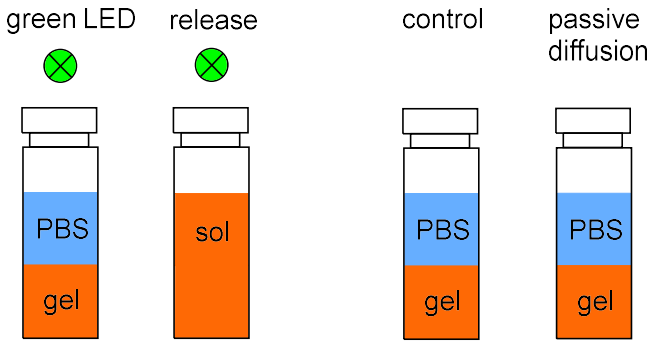
In a 1.5-mL glass vial (crimp top, 12×32 mm) we mixed the photochromic gelator **1** (8.8 mg, powder) and PBS buffer pH 7.4 (495 µL). To this opaque suspension was added a 100× stock solution (5 µL) of the particular cargo molecule dissolved in in DMSO (for plinabulin **2**) or diH_2O (ciprofloxacin). The crimped vial was warmed up vertically in a water bath at 80 °C for 5 minutes. The suspension was then homogenized inside the vial after heating it up to the boiling point (<1 min) with a heat gun. The hot mixture turned into an orange solution (homogenous), which was gelated after 1 h at room temperature. Before a release experiment, the hydrogels were kept overnight in dark at room temperature.

The measurements of the release rate of plinabuiln **2** were performed in triplicates and the average values were taken for the final conclusions and result plots. The release processes of ciprofloxacin hydrochloride were carried out as single experiments, to provide the comparison to the previously reported light-induced release system based on a gelator bearing bis-fluorinated azobenzene.[10] Concentrations of the cargo were chosen to obtain the optimal accuracy for the HPLC detection range.

Quantification of the passive diffusion – cargo "leaking" from hydrogels in darkness

500 µL of PBS buffer pH 7.4 was slowly added on top of a gel sample (on the wall of the vial) and immediately removed with micropipette, to wash away unbound or loosely bound guest molecules from the surface. Addition of fresh 500 µL of PBS buffer followed. The gel was incubated together with the buffer on the top in darkness. 500 µL of the liquid was collected after 5 minutes by gently turning the vial sideways and pipetting off the liquid from the side wall of the vial. Then, fresh 500 µL of PBS buffer was added on the side wall of the vial, incubated in darkness and removed after 5 min in the same way as described above. That process was repeated for the total duration of 30 minutes by collecting 7 subsequent volume aliquots. After that time, all gels remained visually unaffected. By measuring the remaining liquid volume after removal of the last 500 µL aliquot from the top of the gel we estimated that the total decay of the gel volume was lower than 15%.

Procedure of the light-induced release



Scheme S3. The light-triggered release of encapsulated molecules from hydrogel samples vs. passive diffusion of the cargo in the absence of light (schematic representation of the experiment).

To measure the release process upon green light irradiation, we exactly repeated the procedure described above, but after initial washing of the gel surface the sample was placed in an irradiation chamber and illuminated with one 3 W LED (530 nm, from the distance of 5 cm). Short breaks in irradiation (<30 sec.) were taken for the replacement of 500 µL aliquot with fresh 500 µL of PBS buffer every 5 minutes, but the summary irradiation time was 30 min. The irradiation time was sufficient to fully convert all the gel samples into sol. All aliquots were weighted before the HPLC measurement to calculate the released amount of the substance. The concentration of the aliquots were calculated by a previously measured calibration curve of the substances. In all aliquots no precipitate of ciprofloxacin was observed by the naked eye.

In case of ciprofloxacin (**Figure S7**), the "leaking" rate in darkness approached 25-30% of its light-induced release rate from the hydrogel formed of **1** (17 g/L **1** in PBS pH 7.4) within the 30 min. period. This selectivity was very similar to the one achieved previously with the same molecule encapsulated in the gelator containing the bis-*ortho*-fluoroazobenzene photochrome (50 g/L of the gelator in PBS pH 7.4, 180 min. release time).[10]

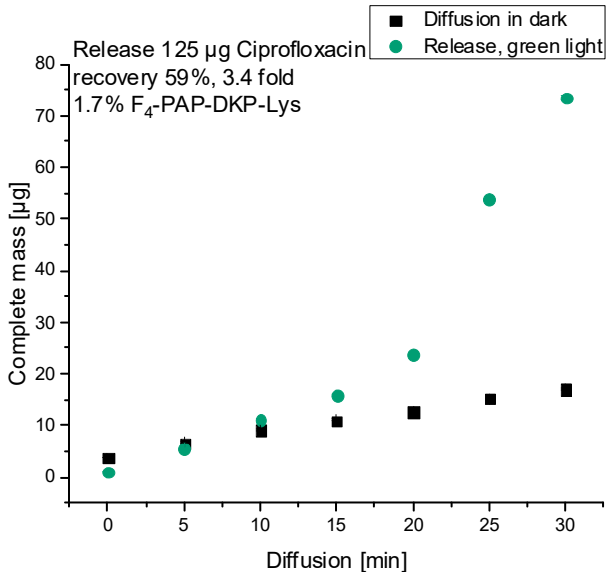


Figure S7. Light-induced release of encapsulated ciprofloxacin (125 µg) (a common antibiotic) from the hydrogel (0.5 mL) containing 17 g/L of **1** ("1.7 % gel") upon irradiation with green light (530 nm

LED diode, 7 mW/cm2) (green circles). Passive diffusion ("leaking") of cargo from the same hydrogel in darkness (black squares) occurs at the rate of at least 20% of the light-induced release rate.

Green light-induced release of plinabulin **2** encapsulated in the hydrogel (17 g/L of **1** in PBS pH 7.4) was achieved almost quantitatively within 30 min of the experiment, upon complete gel dissipation. Within the same time period in darkness, spontaneous diffusion of the cargo **2** was negligible (below 1% of the rate of the light-induced release, within the experimental error of the HPLC quantification method), as depicted on the **Figure 3** in the manuscript.

It was reported, that solubility of plinabulin in aqueous solutions is relatively low. This is one of the major obstacles for broad pharmaceutical applications of this compound. Therefore, stock solutions of plinabulin were prepared in DMSO and diluted 100-fold in aqueous solutions for the final formulations. For quantification of our release experiments we have determined the solubility of plinabulin in 1% DMSO in PBS to be 7.5 ± 1.7 µmol/L.

More concentrated solutions, prepared by 100-fold dilution of homogenous DMSO solutions in PBS, formed visible precipitation after c.a. 1h. However, hydrogel (1.7% of **1**) prepared with plinabulin at the concentrations up to 350 µmol/L was still transparent and no precipitation of the plinabulin was observed overnight (n=7). In combination with the unusually low leaking rate (below 1%) of plinabulin measured in darkness and its molecular structure similar to the gelator **1**, we hypothesize that these effects can be overall explained by plinabulin molecules being incorporated into the supramolecular structure of the hydrogel. Some sort of association between the *cis*-**1** or the residual *trans*-**1** and plinabulin molecules in solution (like the one depicted on the **Figure S8**) may also explain the fact that concentrations of plinabulin in PBS buffer (still in homogenous solution) measured upon certain light-induced release experiments have been up to 32.7 µmol/L, which is 4.4 times higher than the solubility of plinabulin in 1% DMSO in PBS alone.

Still, to prevent incorrect assignment of the plinabulin concentrations in both the diffusion- and light-induced release experiments, 50 µL of AcOH (c.a 10 vol%) were added to all aliquots after the removal of the aliquots from the gel surface. Acetic acid was chosen to dissolve aggregates or precipitations and reduce the hypothetical supramolecular interactions between plinabulin and molecules of the gelator **1** for the quantification purpose. It was not possible to reduce the concentration of plinabulin much below its solubility limits, because the detection by the HPLC would become inaccurate.

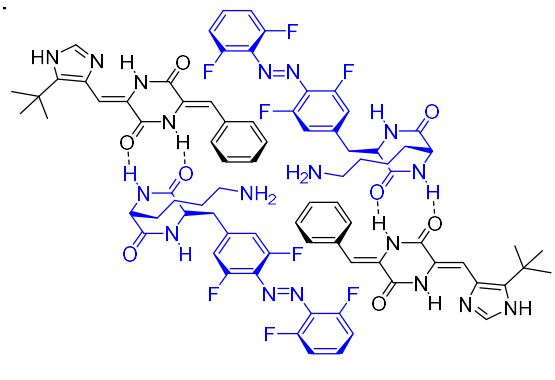


Figure S8. Hypothetical supramolecular aggregates between plinabulin **2** and the gelator **1** might be the reason of the observed enhanced solubility of **2** in aqueous solutions upon its release from the hydrogels, above the measured solubility limit for pure compound **2** in the same medium.

9. Cell viability assays of the hydrogelator 1

Toxicity of every vehicle for therapeutics delivery against human cells is an important parameter in future applications. We have assessed this parameter for our hydrogelator **1** using cell viability assays (MTT assays). We have treated one human cancerous (HeLa) and one non-cancerous (NHDF - fibroblasts) cell line with increasing concentrations of the gelator **1** in order to determine the range of IC_{50} values. Under potential therapeutic circumstances, our material can occur in two forms – either as pure *trans*-isomer (the thermally stable form) that forms hydrogels in aqueous solutions, or as a mixture obtained upon irradiation of gels composed of *trans*-**1** with green light. We have tested the effect of both forms on the HeLa and NHDF cells. The latter mixture was formed by irradiation of stock solution of *trans*-**1** with green light (530 nm), until photostationary state was achieved (30 min), and it corresponds to the dissipated hydrogel after cargo release. The experiment protocols were summarized below, and the results, summarized in **Tables S4-S7**, were plotted in **Figure S9**. Briefly, for the NHDF cells, IC_{50} values of **1** exceed 1 mM (c.a. 0.45 g/L, slightly below the CGC values) for both forms (irradiated and non-irradiated). The IC_{50} value of the irradiated mixture (containing both the *cis*-**1** and *trans*-**1**) against HeLa cells also exceeds 1 mM. When HeLa cells were exposed to the pure (non-irradiated) solution of *trans*-**1**, the IC_{50} value decreased to the range between 0.1 mM and 1 mM.

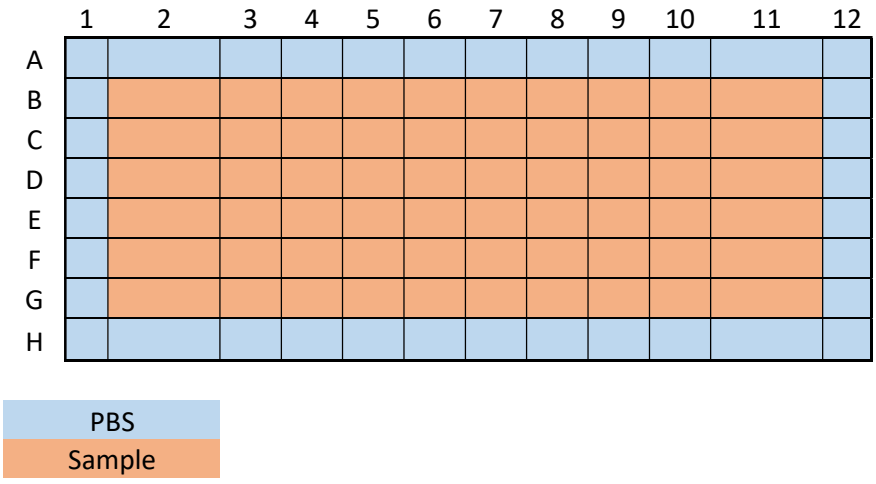
Protocol for the cell viability assay on non-cancerous NHDF cells

NHDF (normal human dermal fibroblasts) were cultured with Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10 % fetal bovine serum (FBS, Gibco) and 1 % penicillin/streptavidin (Gibco) at 37 °C and 5 % CO_2. For all cytotoxicity assays, cells were trypsinized (0.05 % Trypsin-EDTA, Gibco) and seeded in 96-well-plates (96 Well Cell Culture Cluster, Cellstar) in the required densities. Cytotoxicity of the compounds was determined with a CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega). NHDF P9 were seeded in 96-well-plates at a density of 1,5 x 10^4 cells/well in cell culture medium. After incubation at 37 °C and 5 % CO_2 for 24 h, the medium was removed and the cells were treated with different dilutions of the substances in cell culture medium and incubated at 37 °C and 5 % CO_2 for 72 h. As a negative control, the cell culture medium was exchanged with fresh DMEM. For the positive control, 1 % Triton X-100 was added before finally adding 15 µl MTT (3-(4,5-dimethylthiazol2-yl)-2,5-di-phenyltetrazolium bromide) solution to each well. Three replicates were done for each treatment group. After 3 h of incubation at 37 °C and 5 % CO_2, the cells were lysed by adding 100 µl Solubilization/Stop Solution. The cell viability was determined by measuring the absorbance of the resulting formazan at 595 nm using a multiwell plate reader (SpectraMax ID3, Molecular Devices, USA) and setting it into correlation with positive and negative controls.

Protocol for the cell viability assay on cancerous HeLa cells

Hela cells were grown in DMEM (Dulbecco`s Modified Eagle Medium) which was modified with 10% FCS (fetal calf serum) and 1% penicillin/streptomycin solution (10,000 units/mL of penicillin and 10,000 µg/mL of streptomycin) in a humid incubator at 37 °C with 5% CO_2. Cells were detached from the surfaces with Trypsin-EDTA (0.25%) from Gibco®. Cells were washed with PBS (Phosphate-Buffered Saline) from Gibco®. 96-well plates (**Table S3**) with a flat bottom were prepared by filling all wells on the outer border with 200 µL PBS and the remaining wells with 100 µL of a cell suspension (30.000 cells/mL) in DMEM. The prepared plate was incubated overnight to ensure cell attachment to the well-bottom and cell growth.

Table S3: *Scheme of the 96-well plate. The sample positions were filled as follows: row 2 with the positive control (all cells are dead), row 11 with the negative control (all cells are alive) and row 3 to 10 with one specific concentration respectively.*



For the dilution-series of each compound, a stock solution of DMEM modified with 0.25% DMSO was prepared to ensure that all cells are treated with the same conditions. Consequently, the first sample of the dilution series was prepared by dissolving the substance in DMSO and adding a specific amount of this solution to a specific amount of non-modified DMEM so that a final concentration of 0.25% DMSO is reached.

To apply the substances to the 96-well plate, the DMEM was removed without disturbing the cells grown in the plate and adding subsequently 100 µL to each well. To ensure the same treatment to the control rows, the DMEM was removed from the wells and DMSO-modified DMEM (100 µL) was added to the corresponding wells. The 96-well plate was incubated for 48 h.

The positive control was treated with 5 µL of Triton™ X-100 detergent (10% solution (w/v)) per well for at least 5 min before adding 15 µL of MTT dye-solution (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid in water / CellTiter 96®Non-Radioactive Cell Proliferation Assay from Promega) to all sample wells and incubating for 3 h in the dark. 100 µL of a fresh prepared stop solution (10% sodium dodecyl sulfate, 0.01 M HCl in water) was added after incubation to stop the reduction of MTT to formazan, therefore prevent from overreaction and solubilize the formazan crystals. After 24 h of solubilization in the incubator, the plate was read out with a plate reader (BioTek® EPOCH^2, Gen5 Data Analysis) by measuring the absorption of each well at 570 nm.

The raw data was processed as followed by first subtracting the positive control (all cells are dead) from all measured values in one row to remove background absorption. Each concentration was measured sixfold per plate therefore, the values for each concentration and the negative control (all cells alive) were averaged and the standard deviation was calculated. The cell viability was calculated as a percentage of the negative control and normalized by assuming the highest obtained viability as 100%.

Table S4: F4-PAP-DKP-Lys **1** dark adapted HeLa			
Concentration	Concentration [M]	Cell viability [%]	Stdev
1 mM	1.00E-03	20	7.67
100 µM	1.00E-04	87	7.27
10 µM	1.00E-05	90	7.99
1 µM	1.00E-06	85	4.69
100 nM	1.00E-07	86	8.24
10 nM	1.00E-08	83	2.62
1 nM	1.00E-09	92	15.57
100 pM	1.00E-10	100	6.74

Table S5: F4-PAP-DKP-Lys **1** dark adapted NHDF			
Concentration	Concentration [M]	Cell viability [%]	Stdev
1 mM	1.00E-03	76	1.13
100 µM	1.00E-04	84	0.84
10 µM	1.00E-05	97	1.31
1 µM	1.00E-06	92	3.42
100 nM	1.00E-07	99	1.43
10 nM	1.00E-08	97	1.86
1 nM	1.00E-09	100	2.87
100 pM	1.00E-10	96	0.43

Table S6: F4-PAP-DKP-Lys **1** 523 nm 30 min HeLa			
Concentration	Concentration [M]	Cell viability [%]	Stdev
1 mM	1.00E-03	81	7.25
100 µM	1.00E-04	88	4.94
10 µM	1.00E-05	94	5.81
1 µM	1.00E-06	94	8.16
100 nM	1.00E-07	96	9.64
10 nM	1.00E-08	203	8.81
1 nM	1.00E-09	100	6.19
100 pM	1.00E-10	90	2.20

Table S7: F4-PAP-DKP-Lys **1** 523 nm 30 min NHDF			
Concentration	Concentration [M]	Cell viability [%]	Stdev
1 mM	1.00E-03	79	5.09
100 µM	1.00E-04	85	2.50
10 µM	1.00E-05	86	2.71
1 µM	1.00E-06	89	1.22
100 nM	1.00E-07	95	2.33
10 nM	1.00E-08	100	1.97
1 nM	1.00E-09	99	3.22
100 pM	1.00E-10	96	3.83

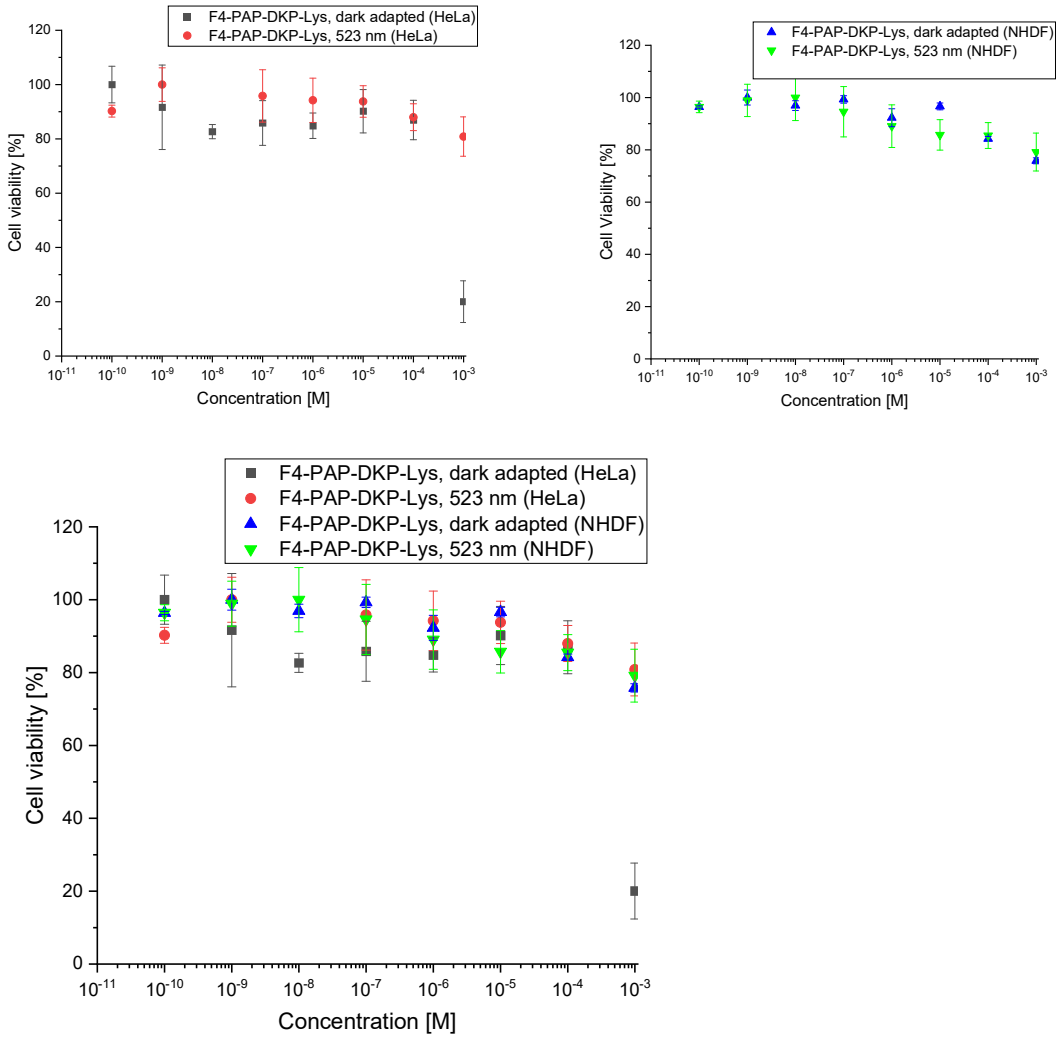


Figure S9. Cell viability assays of human cancerous HeLa cells and non-cancerous NHDF cells treated with increasing concentrations of dark adapted **1** (*trans*-**1**) and **1** irradiated with green light until the photostationary state is achieved (F4-PAP-DKP-Lys is the hydrogelator **1**)

10. Chiral HPLC analysis

The stereochemical outcome of the synthesis of amino acid **8** has been investigated with chiral RP-HPLC chromatography on the HPLC chromatograph 1100 Series from AGILENT TECHNOLOGIES with an amylose-SA column (KSA99S05-2546WT) with 250 × 4.6 mm and 5 µm. The separations were performed with a 20 min gradient from 95% H_2O to 95% MeCN with 0.1% TFA, flow rate 1 mL/min, slit = 4 nm, wavelength 256 nm or 330 nm for detection. In order to validate separation conditions for enantiopure aminoacids, we have initially analysed chromatograms of Fmoc-(*R*)-Arg(Pbf)-OH and Fmoc-(*S*)-Arg(Pbf)-OH (**Figure S10**), as well as with their racemic mixture (**Figure S11**) on our experimental setup. In the case of racemate, peaks of both enantiomers were separated by Δt=0.30 min. The substrate (Boc-iodo-Ala-OMe, **3**) exhibited >98% ee (**Figure S12**). The amino acid Boc-(*S*)-4-amino-3,5-difluoro-Phe-OMe **5** was obtained in >98% .e (**Figure S13**). The photochromic amino acid *(S)-N*-Boc-TFAB-alanine **8** was obtained in >96% ee (**Figure S14**).

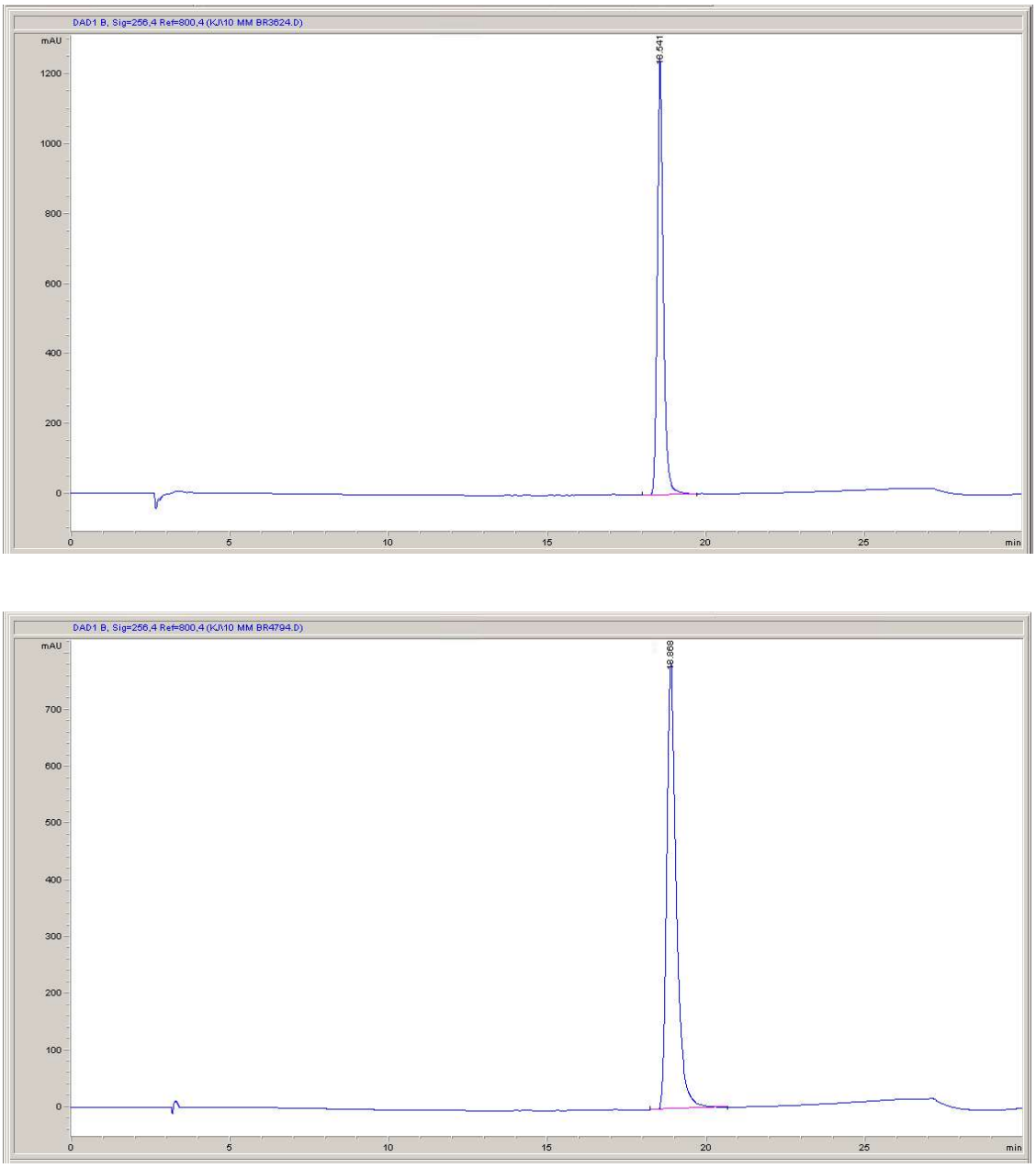


Figure S10. Chiral HPLC of Fmoc-(*R*)-Arg(Pbf)-OH (*top*) and Fmoc-(*S*)-Arg(Pbf)-OH (*bottom*), 256 nm

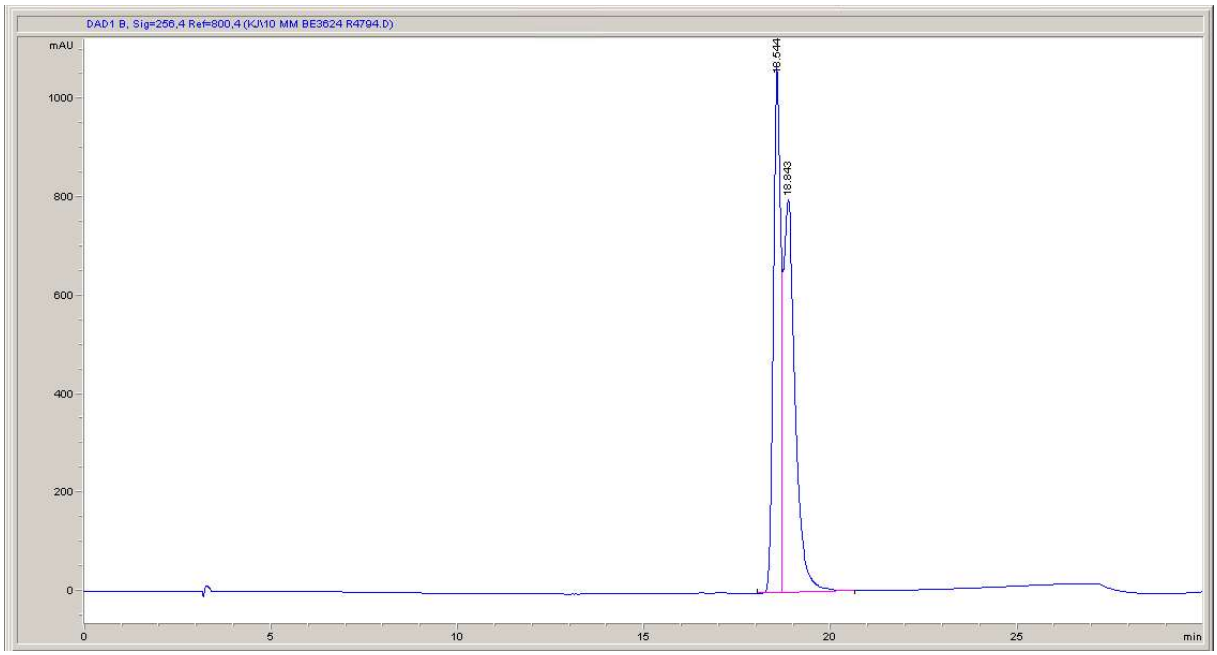


Figure S11. Chiral HPLC of the racemic mixture of Fmoc-(*R*)-Arg(Pbf)-OH and Fmoc-(*S*)-Arg(Pbf)-OH, 256 nm

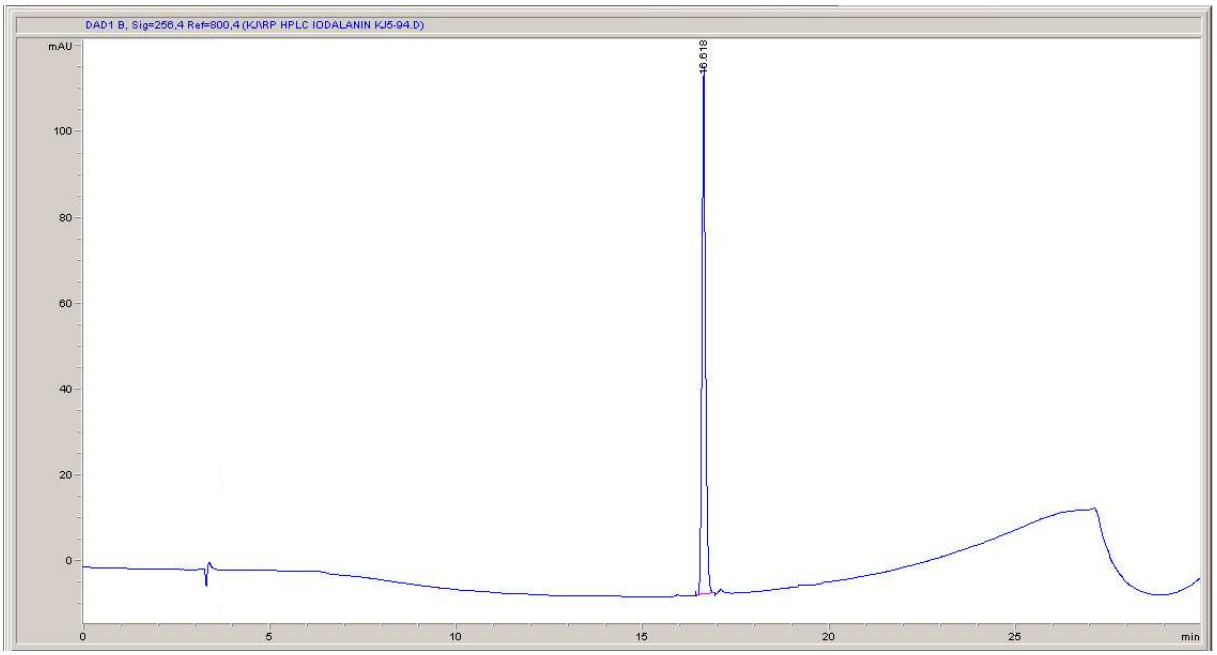


Figure S12. Chiral HPLC of methyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-iodopropanoate **3**, 256 nm

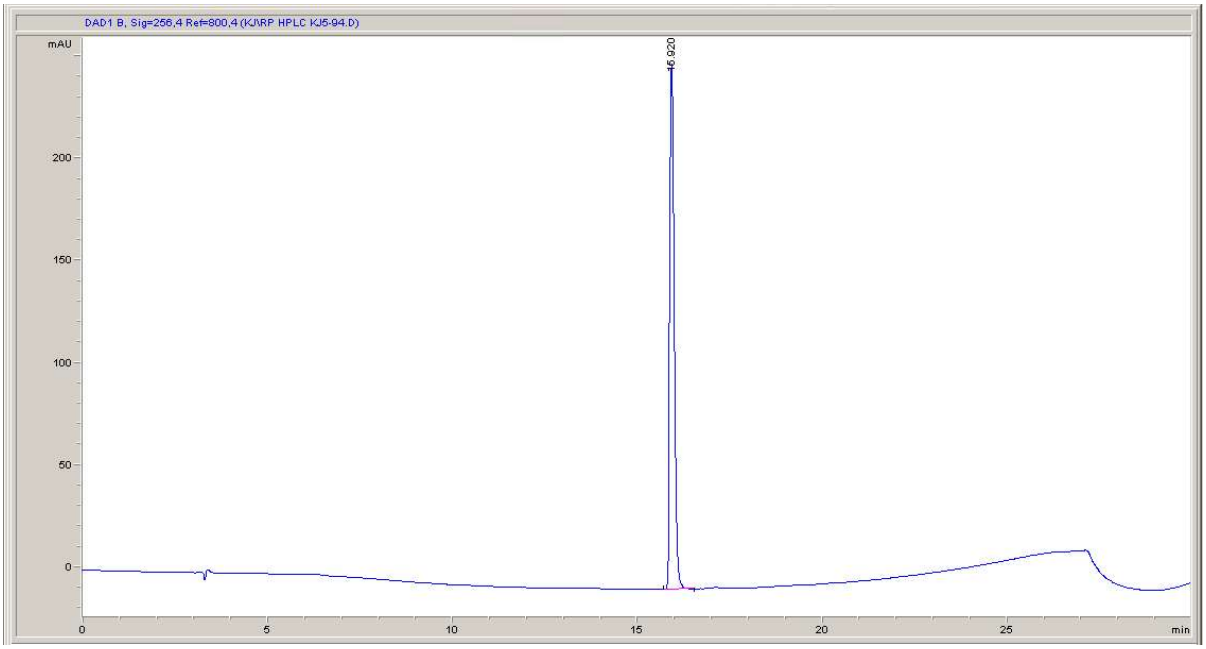


Figure S13. Chiral HPLC of Boc-(*S*)-Ser(difluoro-aniline)-OMe **5**, 256 nm

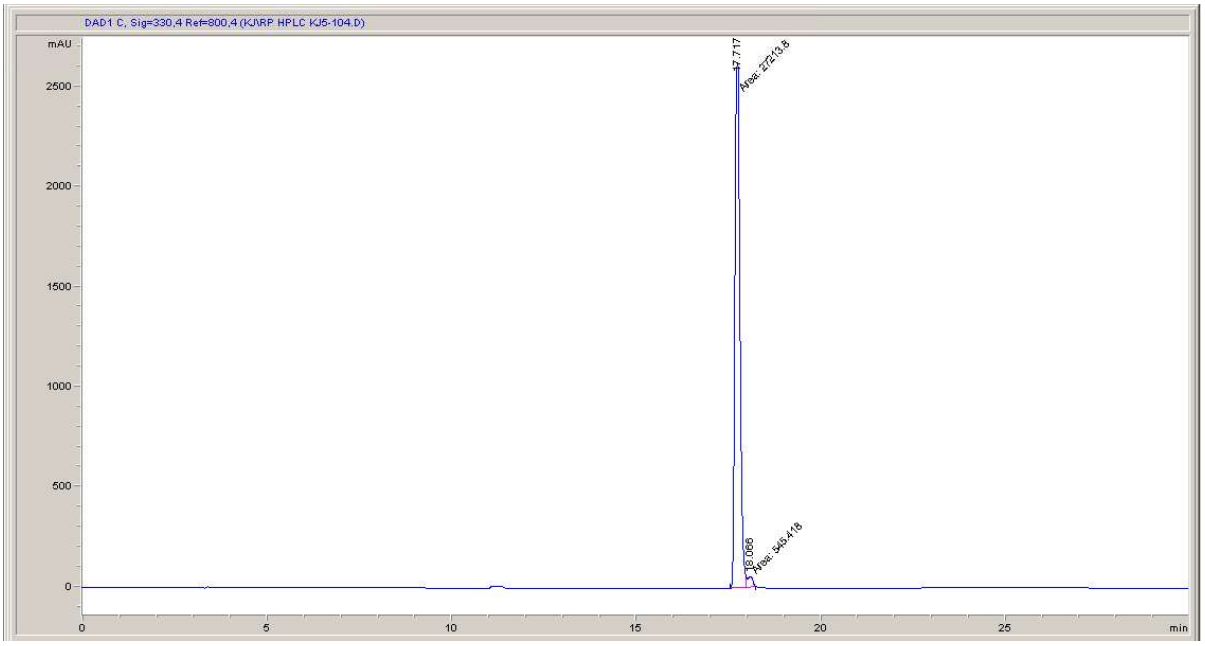


Figure S14. Chiral HPLC of*(S)-N*-Boc-TFAB-alanine (Boc-F_4-(*S*)-PAP-OH) **8**, 330 nm

11. NMR Spectra of the synthesized compounds

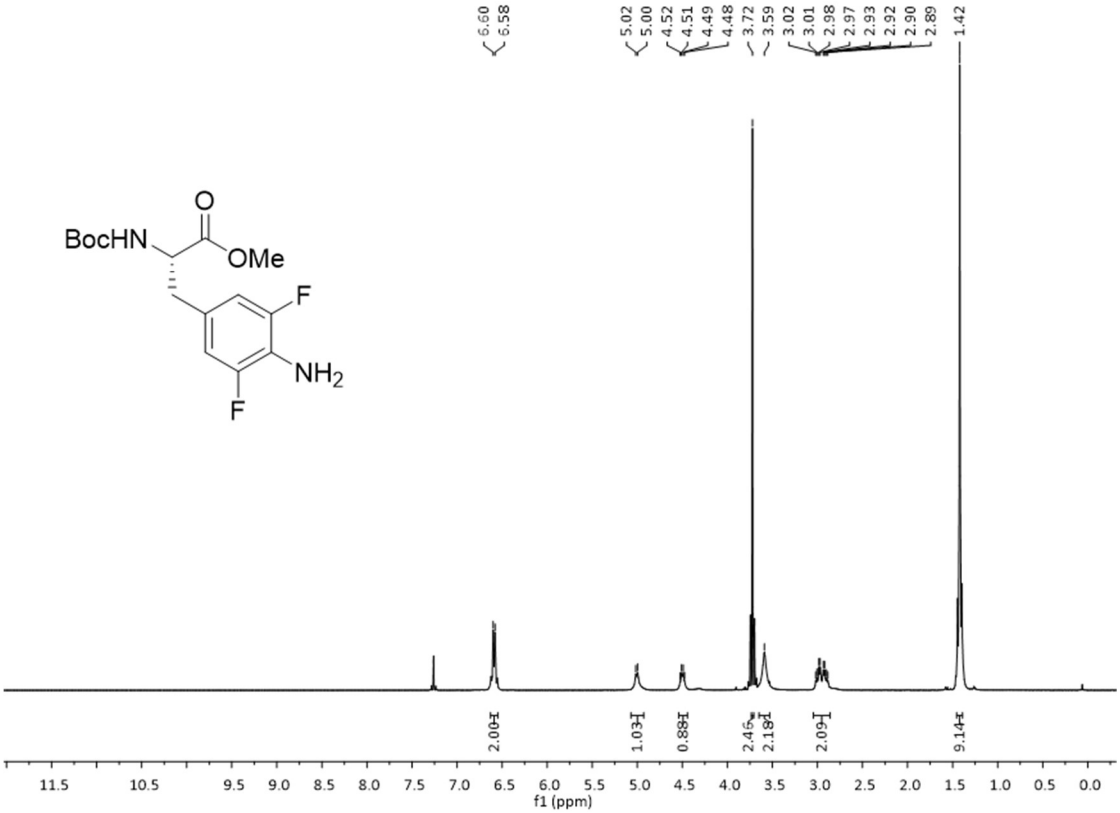


Figure S15. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **5**.

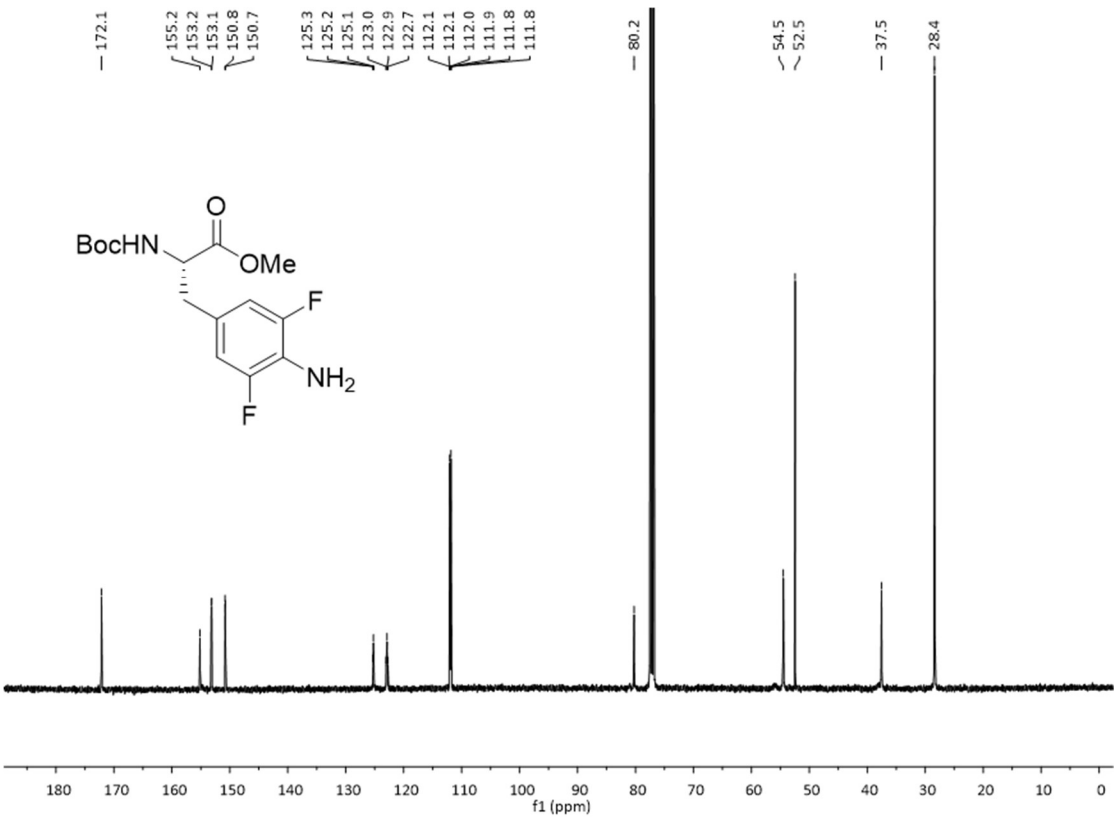


Figure S16. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of compound **5**.

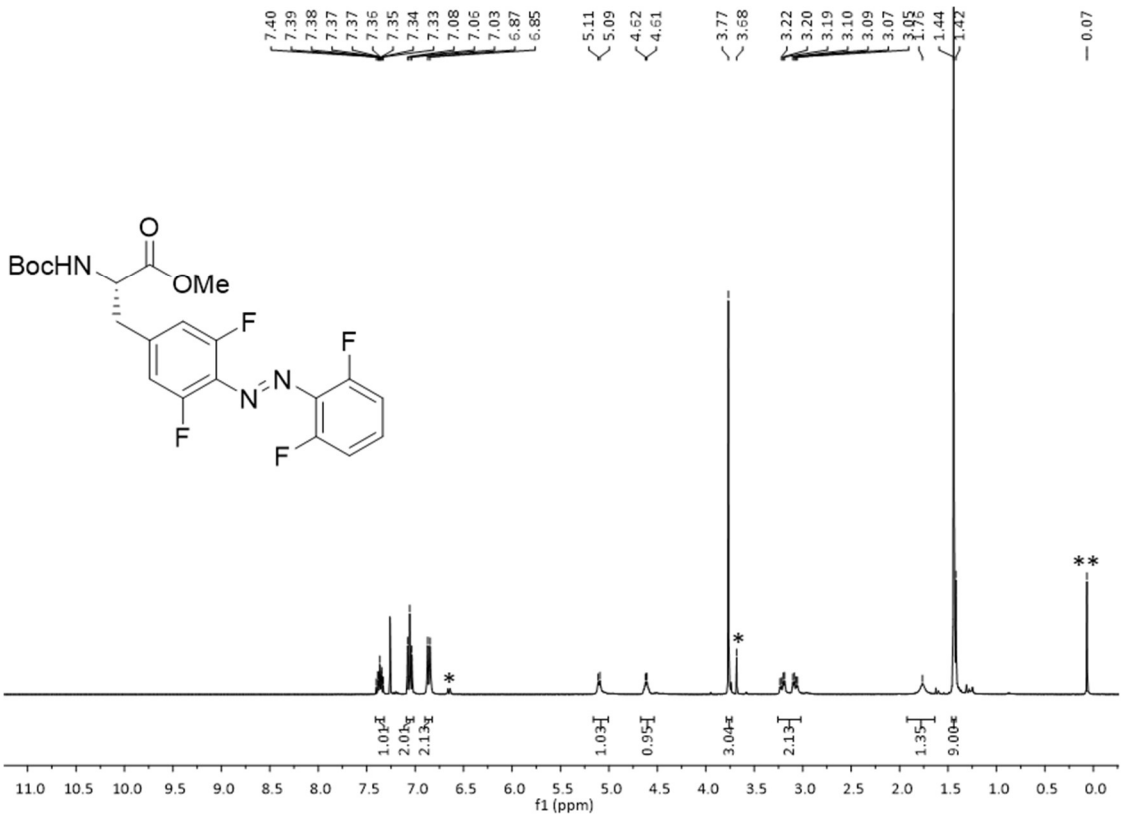


Figure S17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **7**. **cis*-isomer, ***silicon grease.

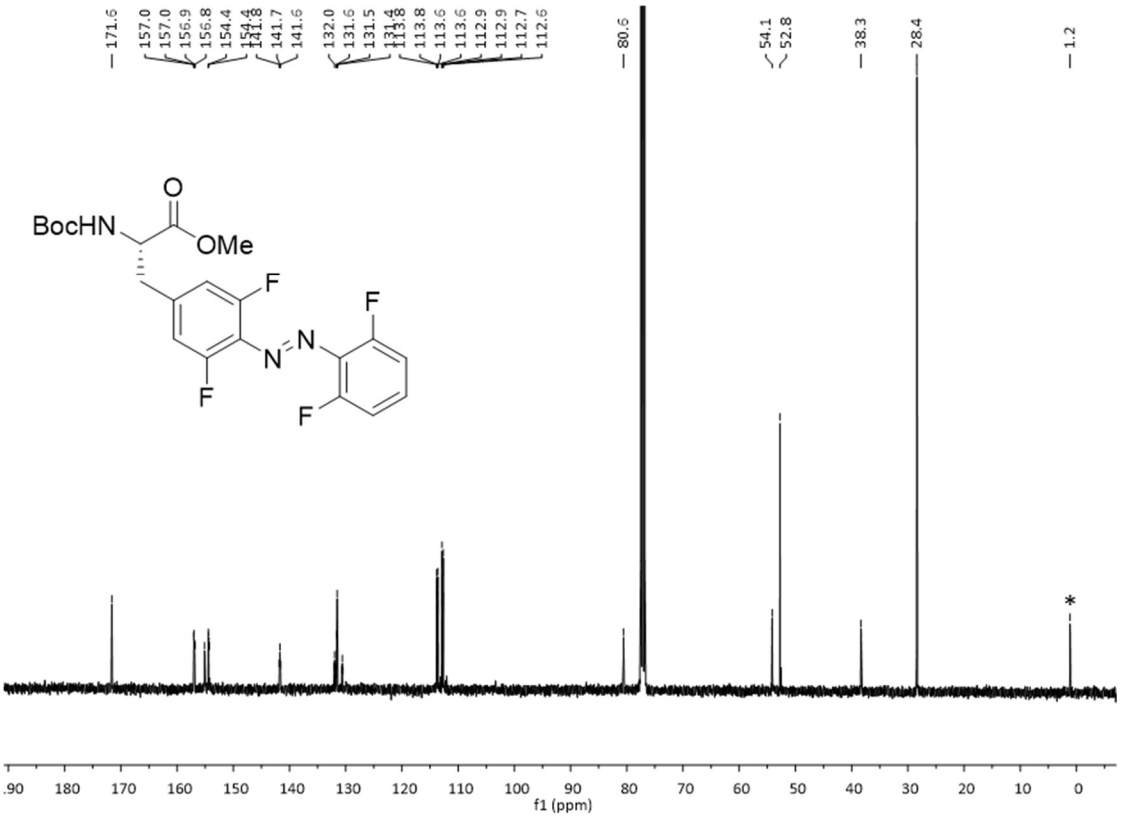


Figure S18. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of compound **7**. *silicon grease.

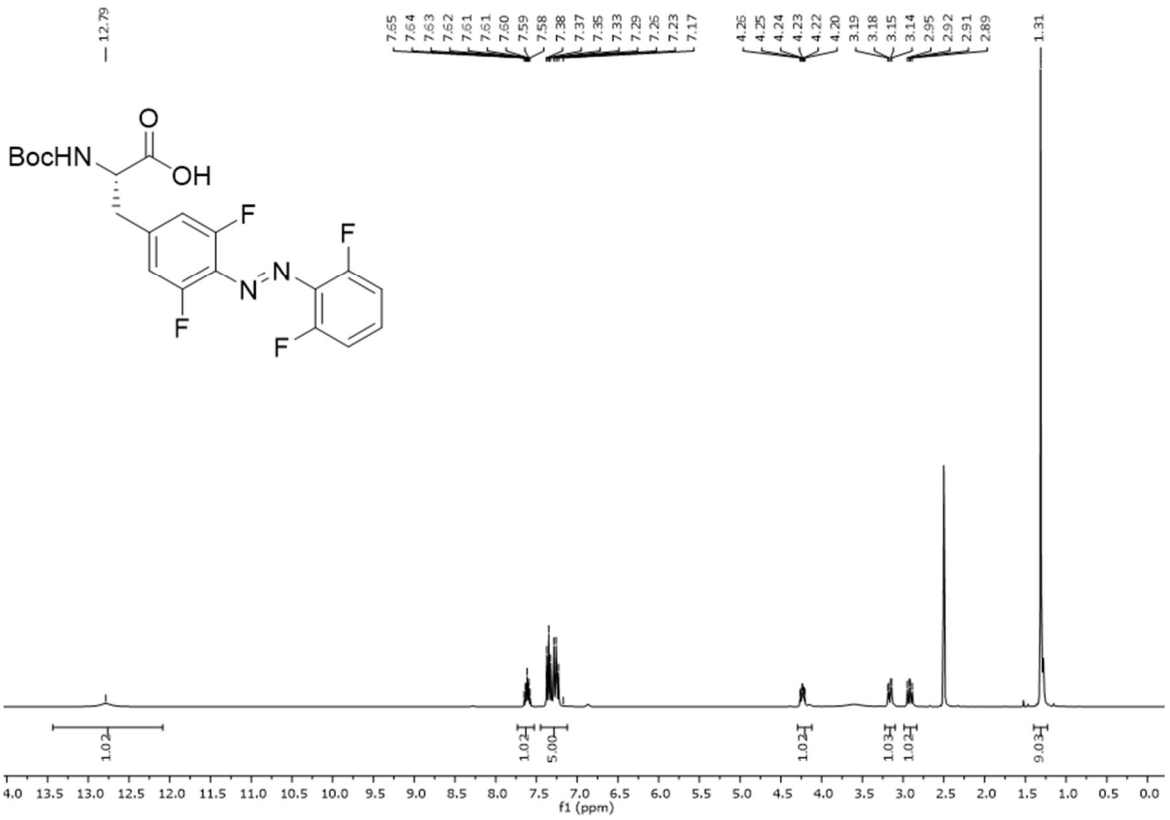


Figure S19. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **8**.

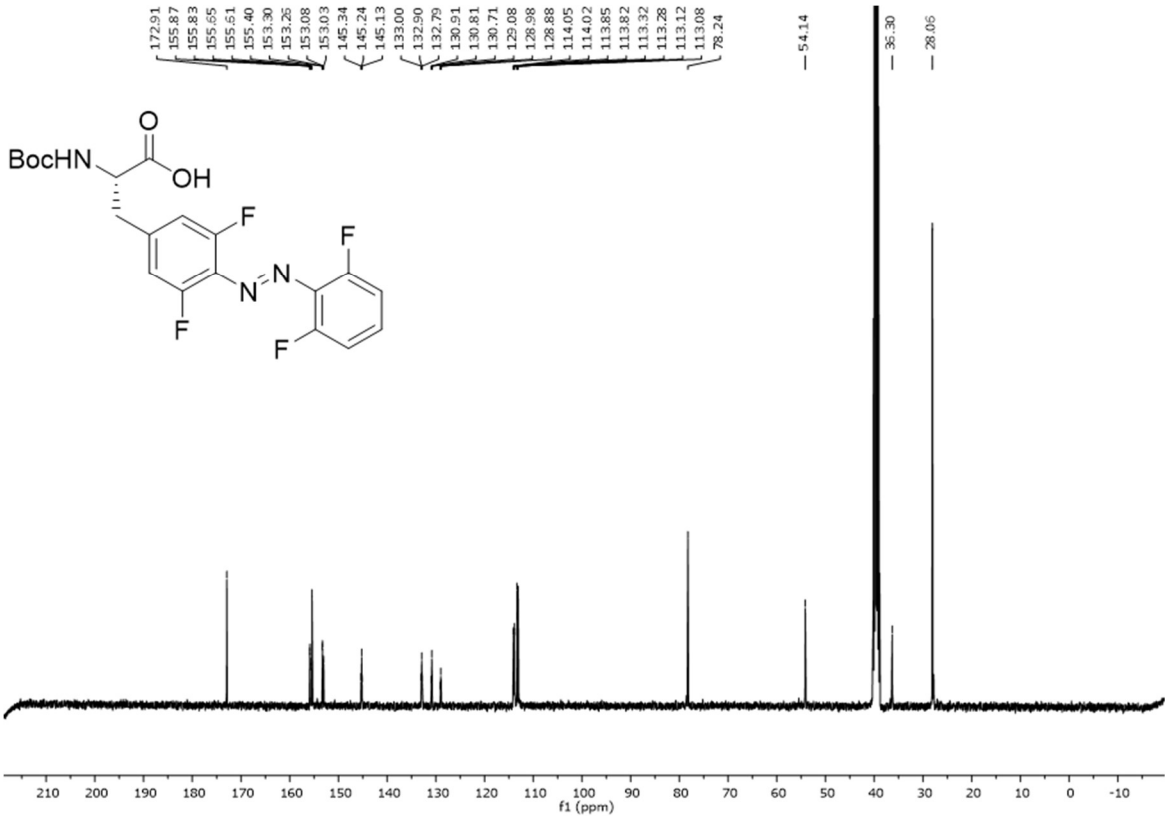


Figure S20. ^{13}C NMR spectrum (101 MHz, DMSO-d_6) of compound **8**.

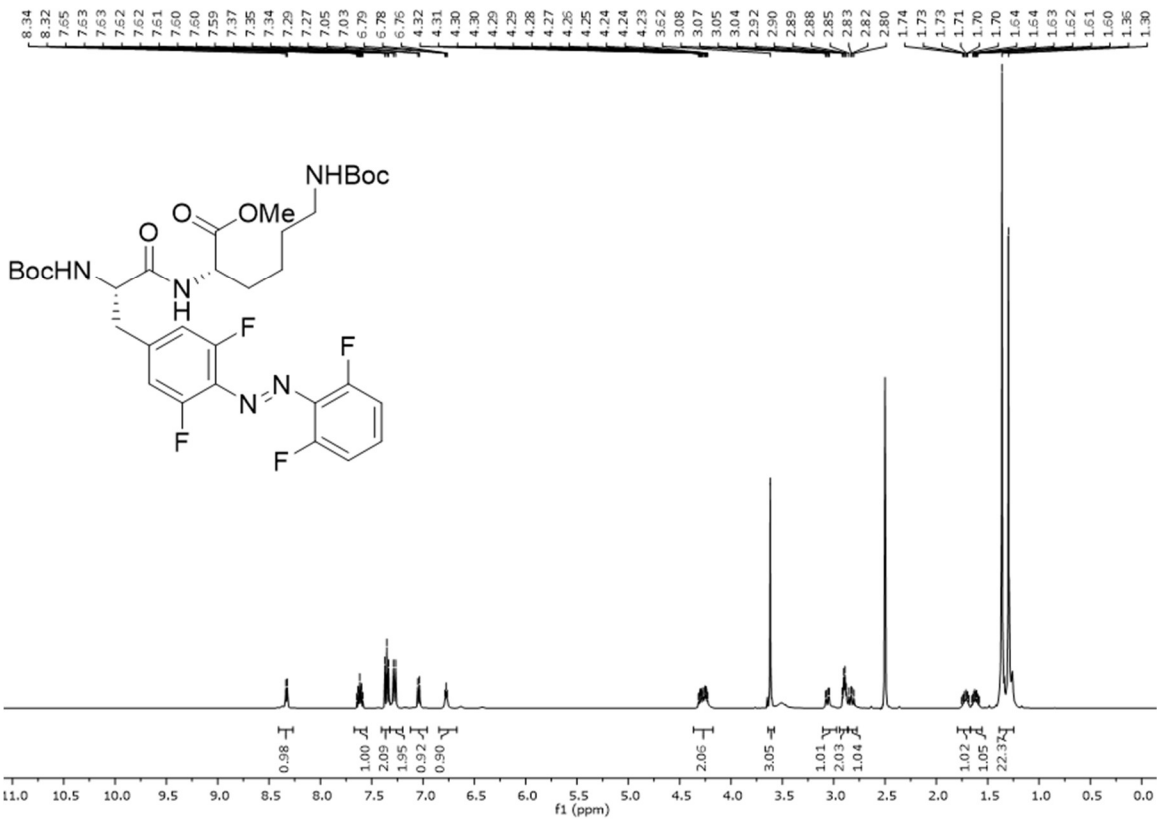


Figure S21. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **10**.

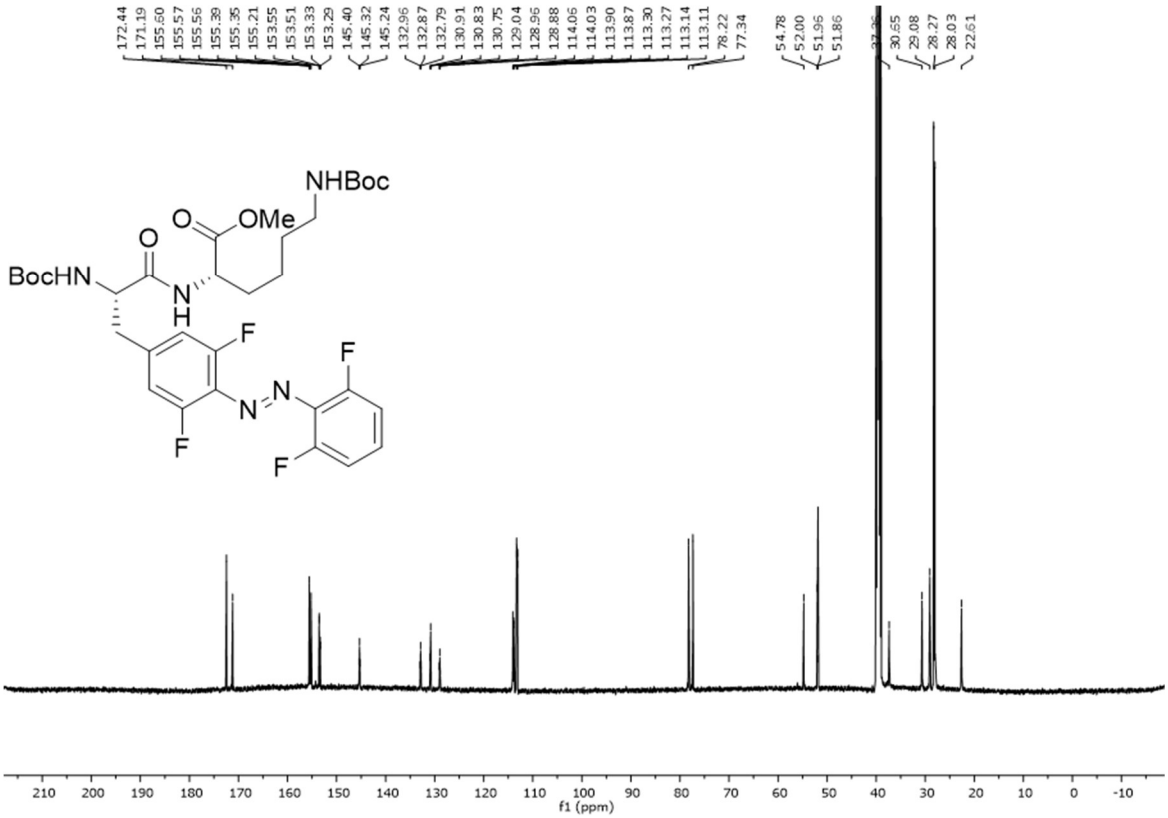


Figure S22. ^{13}C NMR spectrum (101 MHz, DMSO-d_6) of compound **10**

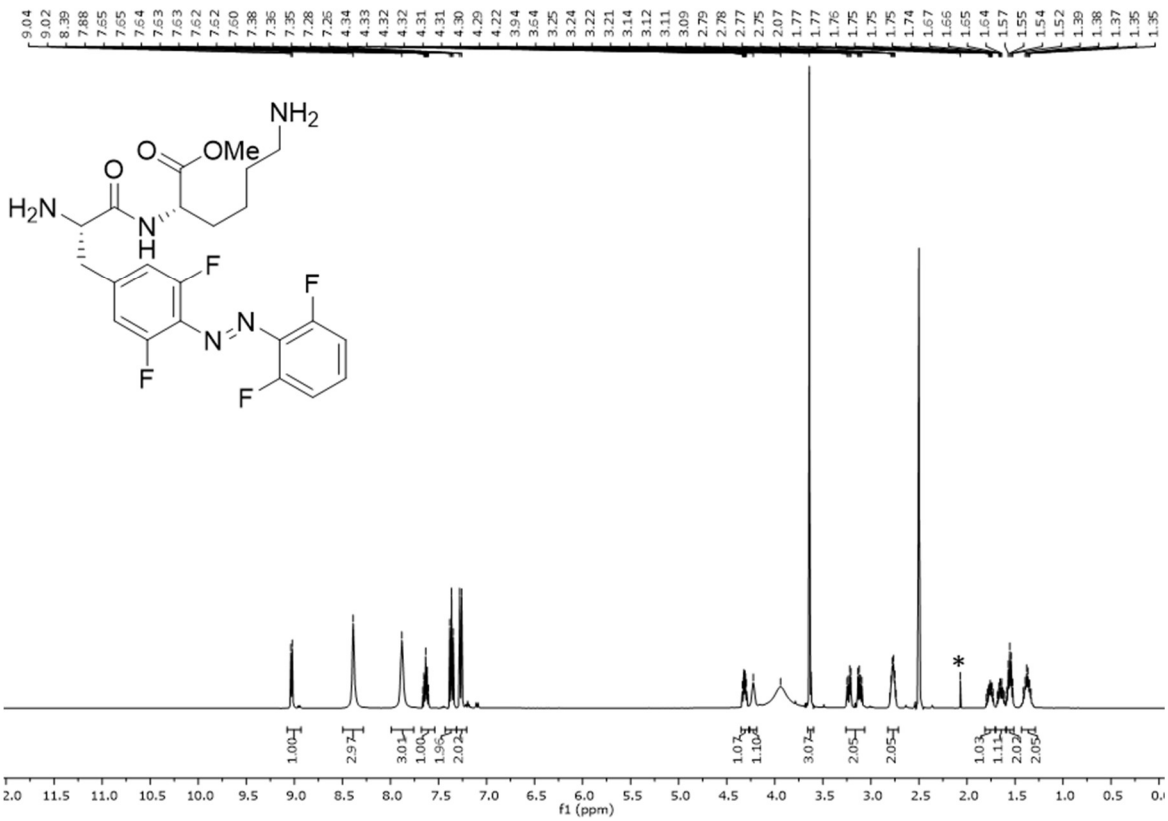


Figure S23. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **11**.

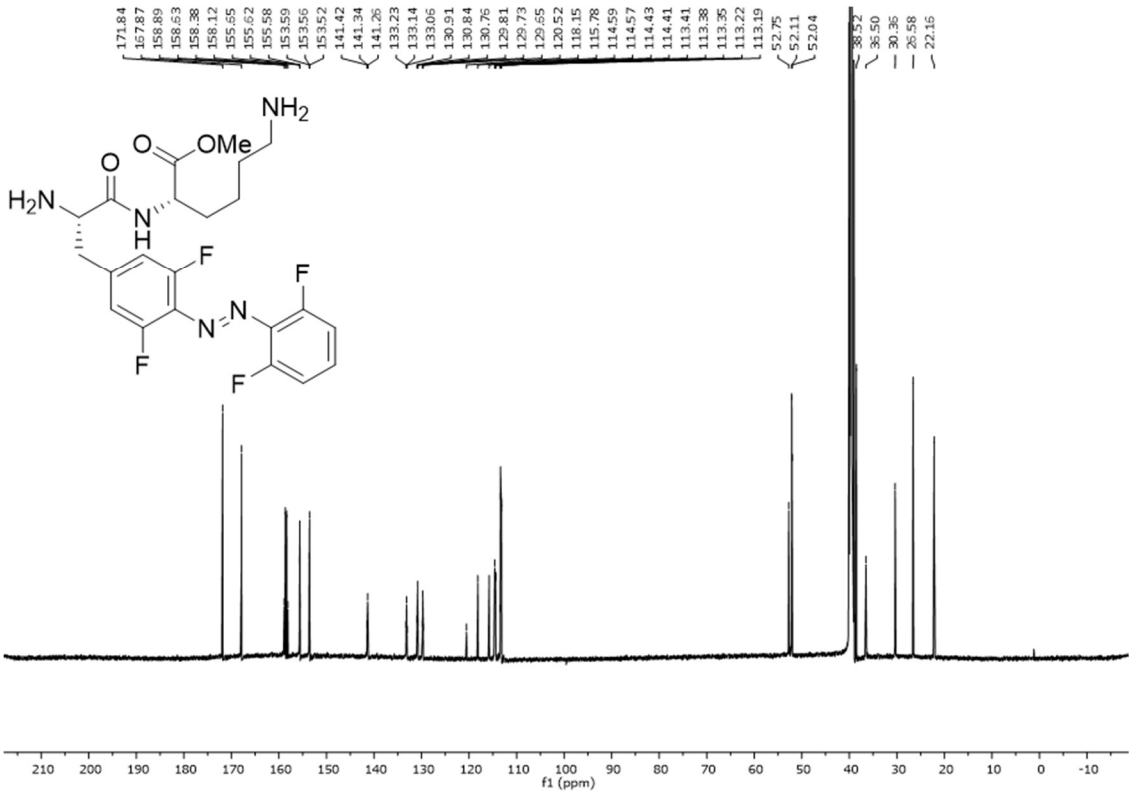


Figure S24. ^{13}C NMR spectrum (101 MHz, DMSO-d_6) of compound **11**

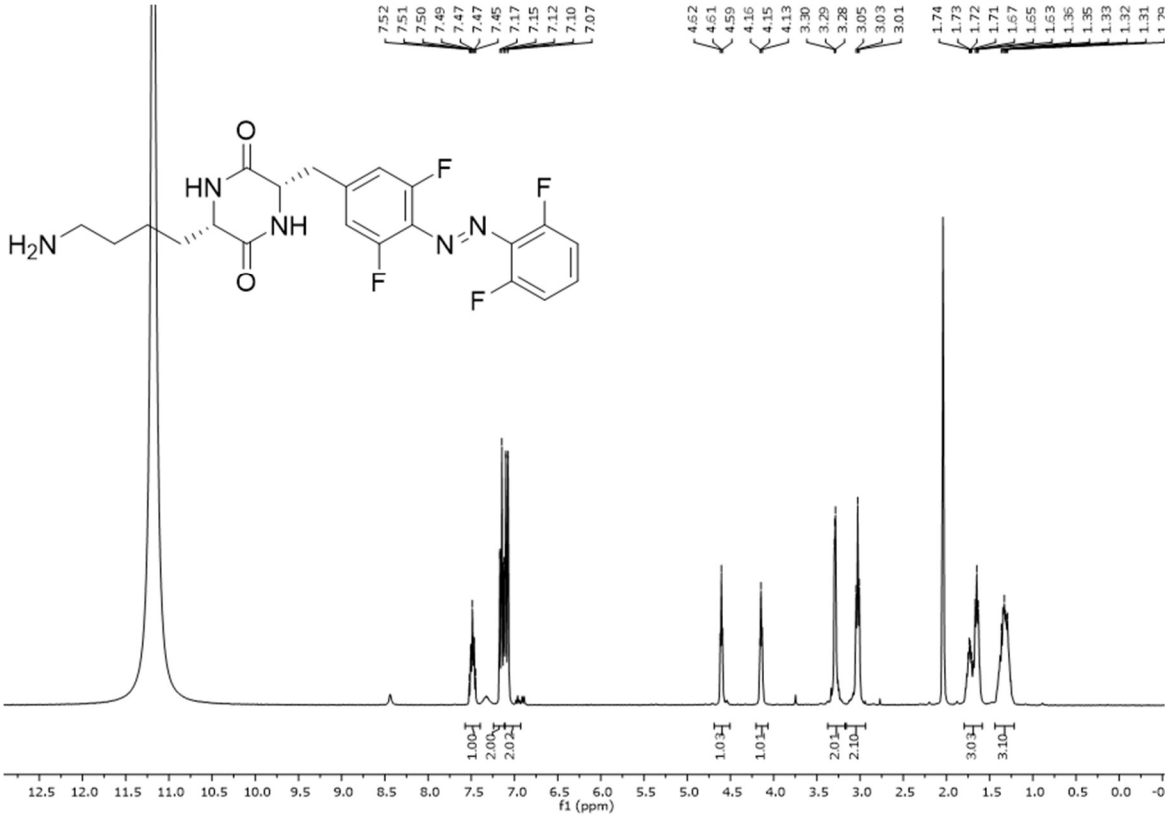


Figure S25. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of compound **1**. (isolated as TFA salt from HPLC)

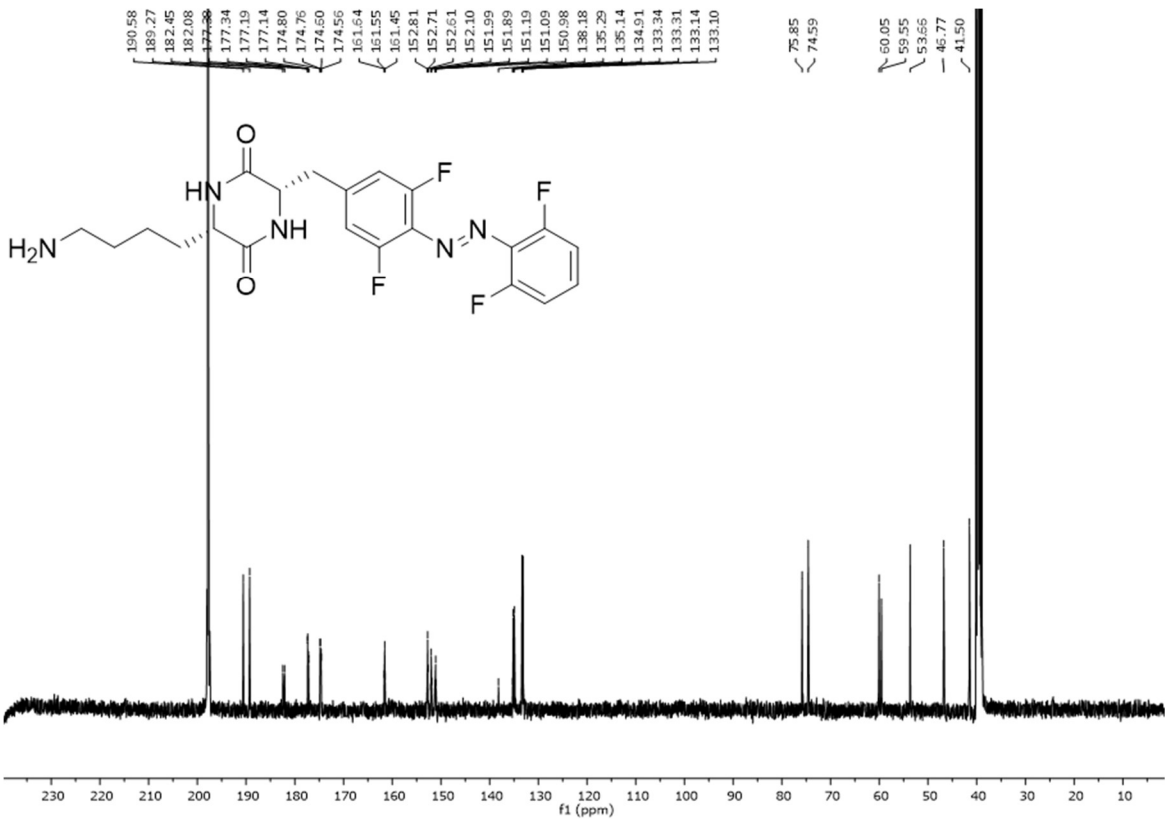


Figure S26. ^{13}C NMR spectrum (101 MHz, DMSO-d_6) of compound **1**

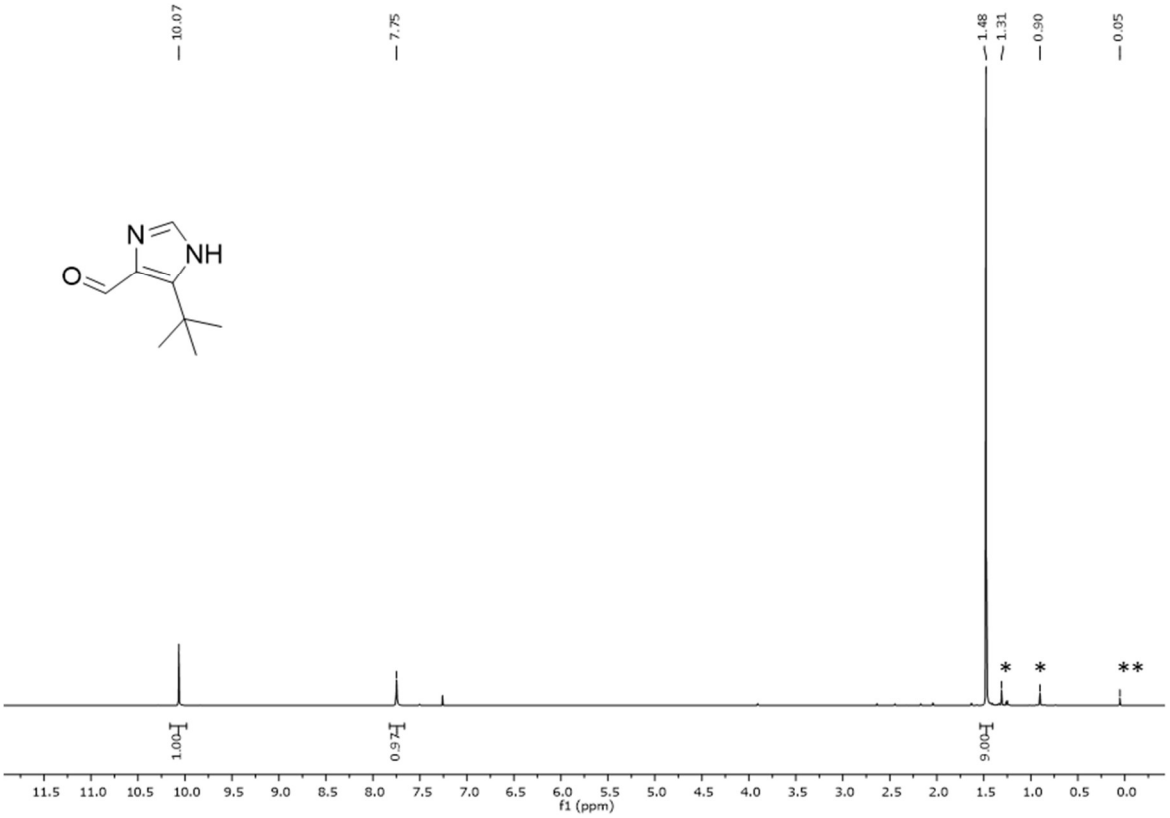


Figure S27. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **13**. *substrate, **silicon grease.

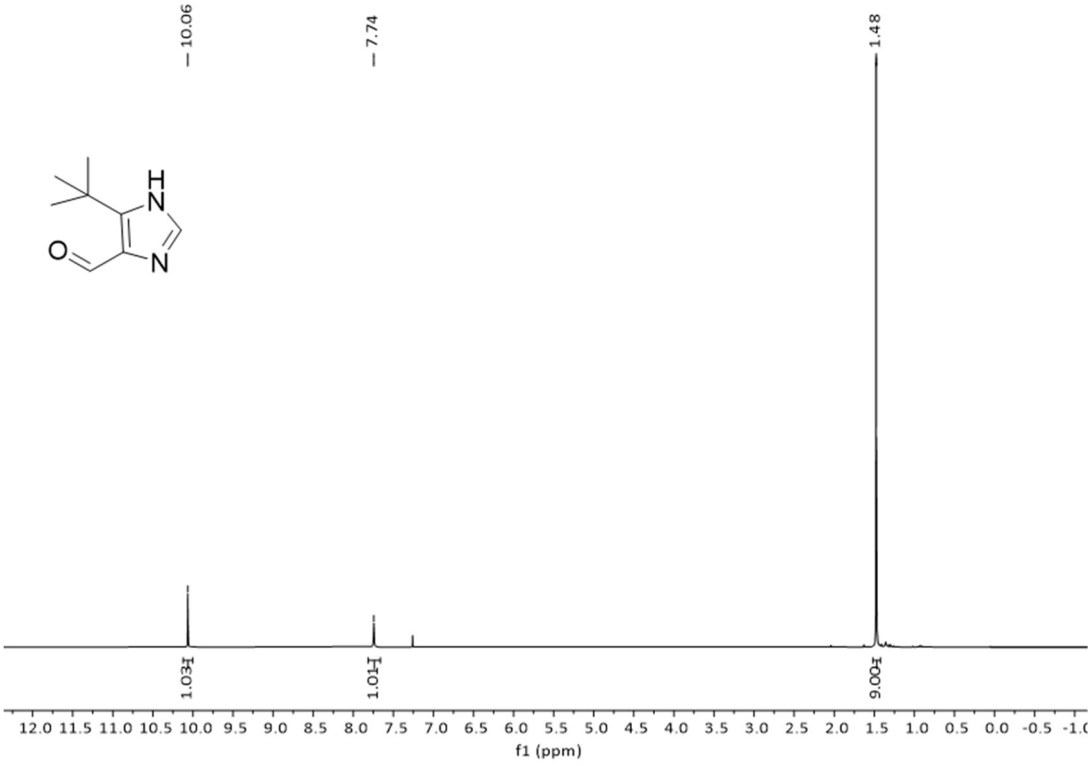


Figure S28 – ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **13**.

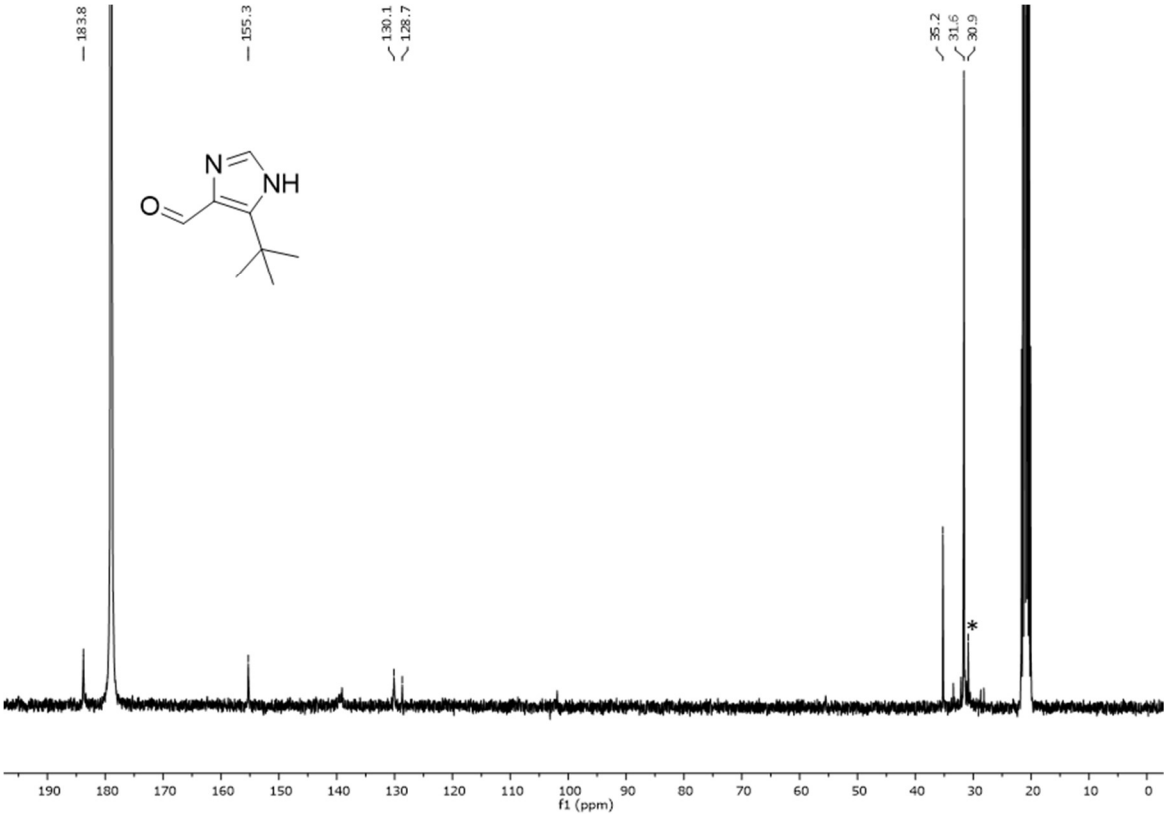


Figure S29. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of compound **13**.

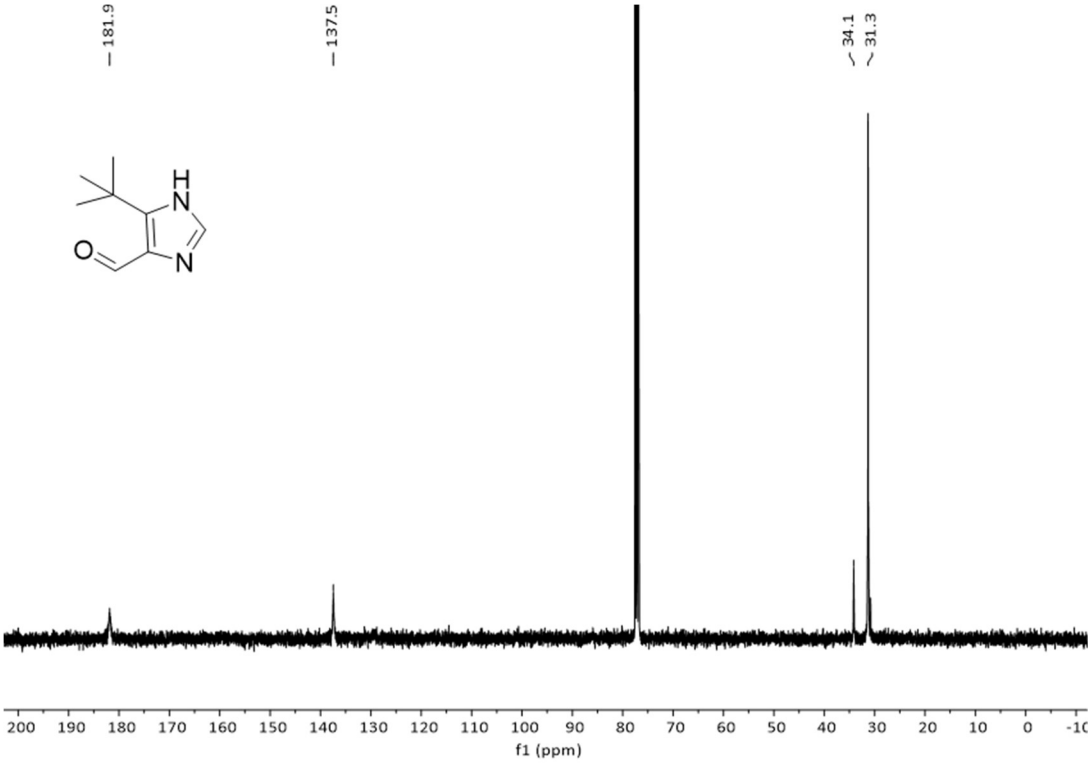


Figure S30. – ^{13}C NMR spectrum (101 MHz, DMSO-d_6) of compound **13**.

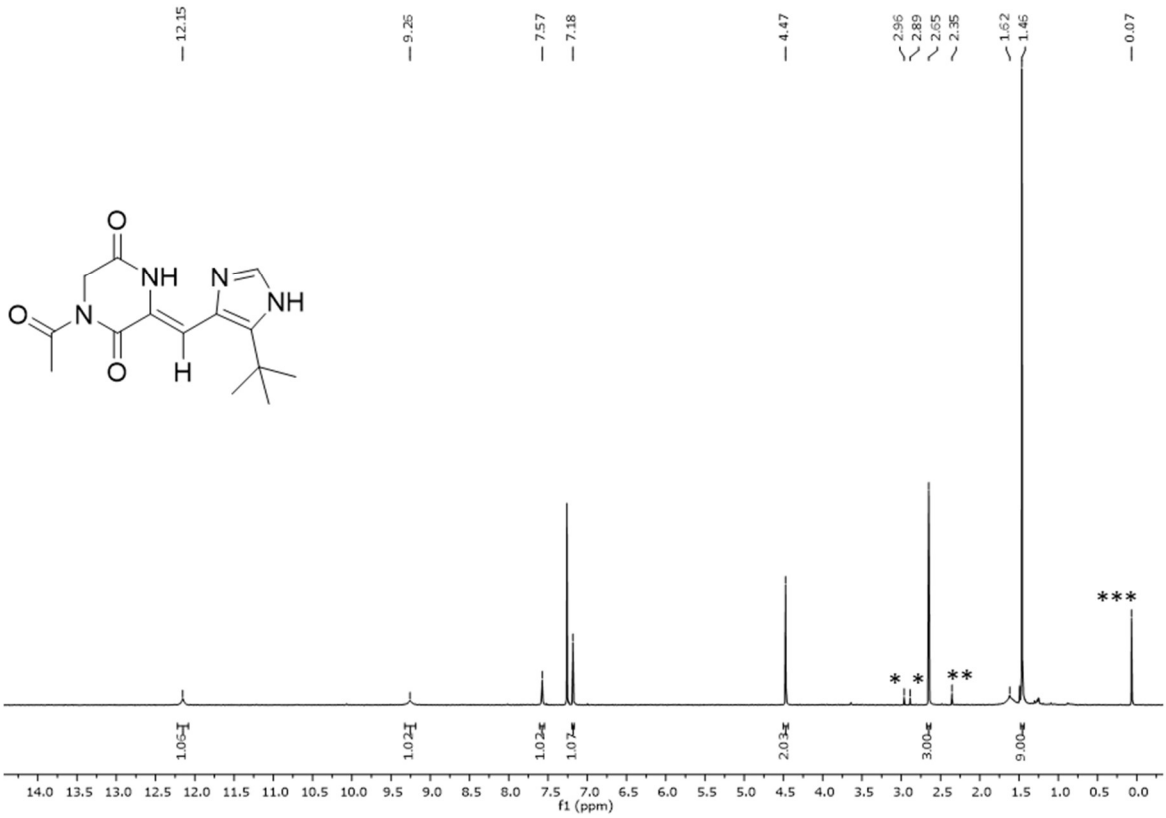


Figure S31. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **15**. *DMF, ***silicon grease.

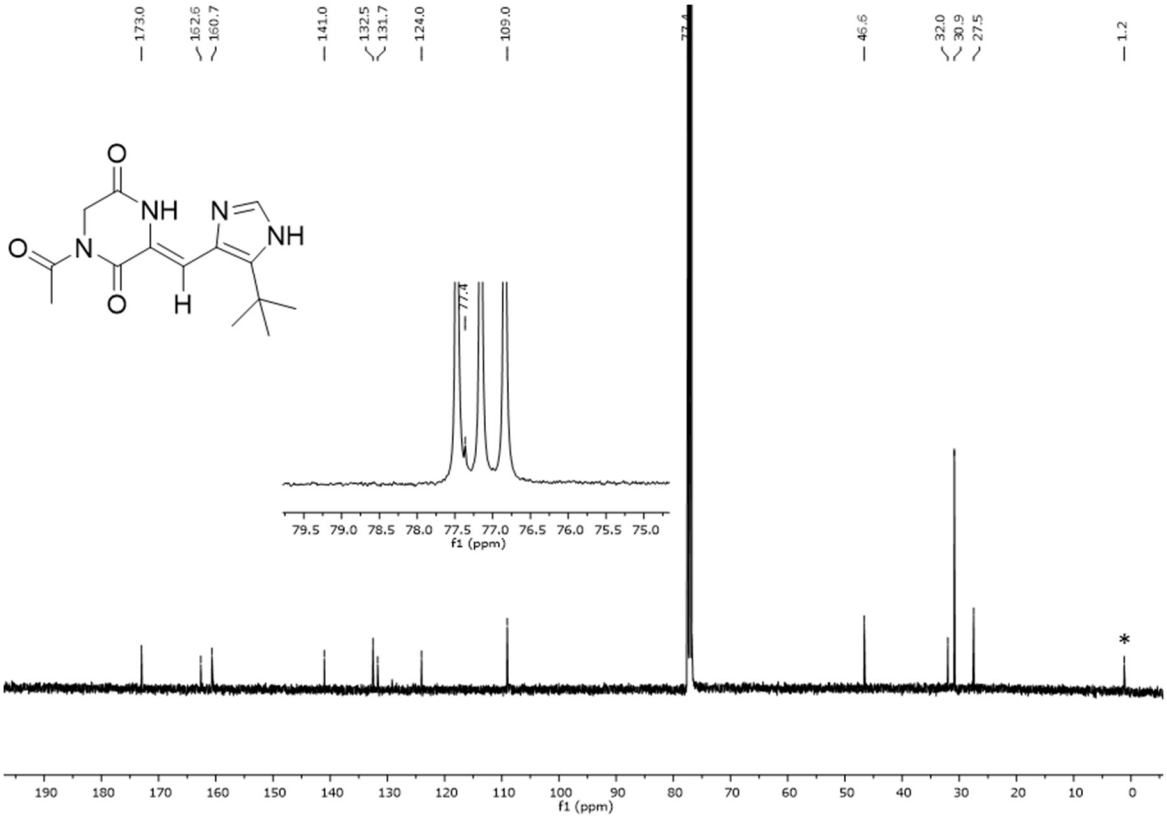


Figure S32. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **15**. *silicon grease.

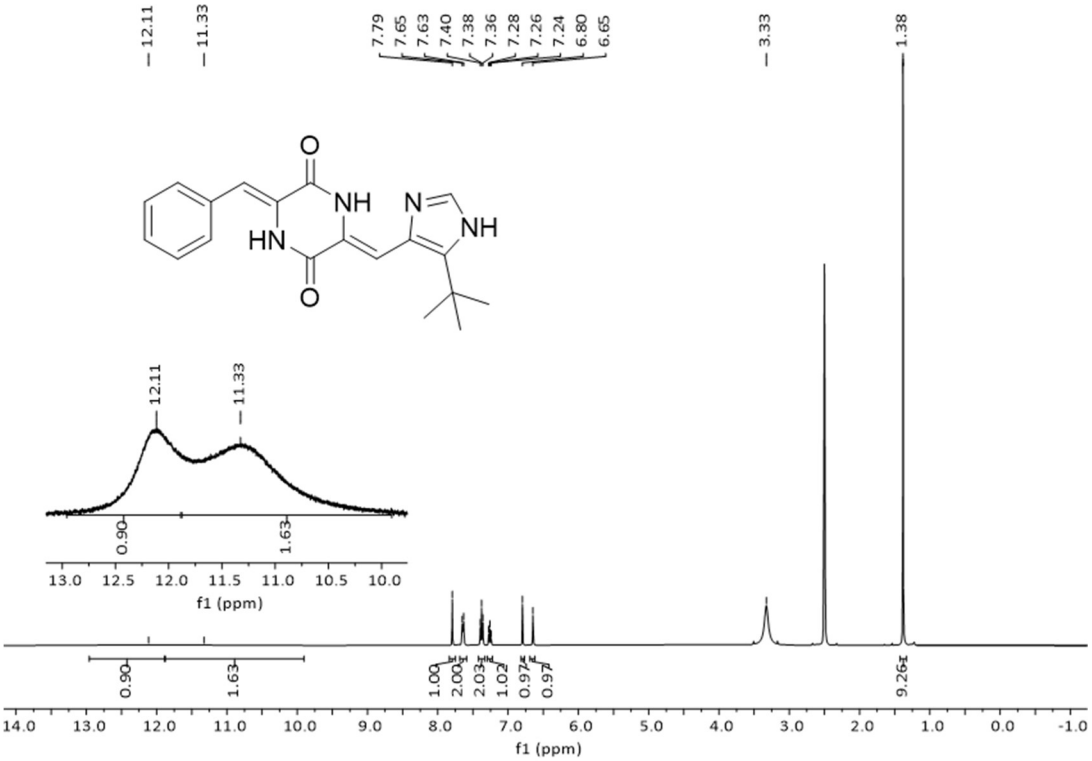


Figure S33. ^{1}H NMR spectrum (101 MHz, DMSO-d_6) of compound **2** (Plinabulin).

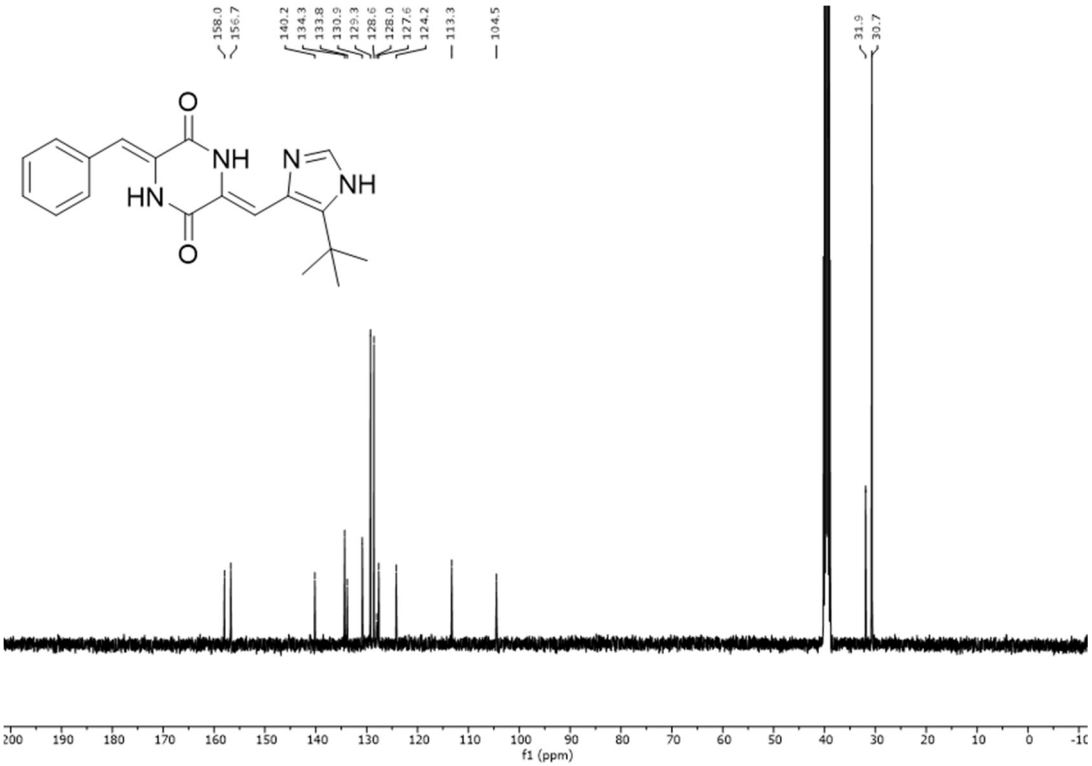


Figure S34. ^{13}C NMR spectrum (101 MHz, DMSO-d_6) of compound **2** (Plinabulin).

12. References

[1] H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512-7515.

[2] A. J. Ross, H. L. Lang, R. F. Jackson, *J. Org. Chem.* **2010**, *75*, 245-248.

[3] C. Knie, M. Utecht, F. L. Zhao, H. Kulla, S. Kovalenko, A. M. Brouwer, P. Saalfrank, S. Hecht, D. Bleger, *Chem. Eur. J.* **2014**, *20*, 16492-16501.

[4] Y. Yamazaki, K. Tanaka, B. Nicholson, G. Deyanat-Yazdi, B. Potts, T. Yoshida, A. Oda, T. Kitagawa, S. Orikasa, Y. Kiso, H. Yasui, M. Akamatsu, T. Chinen, T. Usui, Y. Shinozaki, F. Yakushiji, B. R. Miller, S. Neuteboom, M. Palladino, K. Kanoh, G. K. Lloyd, Y. Hayashi, *J. Med. Chem.* **2012**, *55*, 1056-1071.

[5] Z. Ding, H. Cheng, S. Wang, Y. Hou, J. Zhao, H. Guan, W. Li, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1416-1419.

[6] M. Cigl, A. Bubnov, M. Kaspar, F. Hampl, V. Hamplova, O. Pacherova, J. Svoboda, *J. Mater. Chem. C* **2016**, *4*, 5326-5333.

[7] M. Perez, C. Fourrier, I. Sigogneau, P. J. Pauwels, C. Palmier, G. W. John, J. P. Valentin, S. Halazy, *J. Med. Chem.* **1995**, *38*, 3602-3607.

[8] R. Pascal, R. Sola, F. Labeguere, P. Jouin, *Eur. J. Org. Chem.* **2000**, 3755-3761.

[9] D. Bleger, J. Schwarz, A. M. Brouwer, S. Hecht, *J. Am. Chem. Soc.* **2012**, *134*, 20597-20600.

[10] J. Karcher, Z. L. Pianowski, *Chem. Eur. J.* **2018**, *24*, 11605-11610.

[11] I. Coin, M. Beyermann, M. Bienert *Nat. Protocols* **2007**, *2,* 3247–3256

[12] J. E. Sheppeck II, H. Kar, H. Hong *Tet. Lett.,* **2000**, *41(28),* 5329-5333