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Supporting Information

Light-Controlled Cell-Cycle Arrest and Apoptosis

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Supporting Information

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Materials and Methods

Chemistry

General

Reagents and solvents were obtained from *abcr*, *Merck*, *Sigma-Aldrich* or *TCI* in the qualities *puriss.*, *p.a.*, or *purum* and used as received. Technical solvents were distilled prior to use for column chromatography and extraction on a rotary evaporator (*Heidolph Laborota* 4000 and 4001). Reactions were monitored on *Merck* Silica 60 F254 TLC plates and detection was done by irradiation with UV light (254 nm or 366 nm).

Column chromatography was performed on silica gel (*Merck*, particle size 0.040 - 0.063 mm or *ACROS*, 0.035 – 0.070 mm) using distilled technical solvents.

High Performance Liquid Chromatography (HPLC) was performed on a *Shimadzu* HPLC system consisting of a LC-20AP solvent delivery module, a CTO-20A column oven, a SPD-M20A photodiode array UV/Vis detector, and a CBM-20A system controller using a preparative Silica column from Diacel and HPLC grade solvents (EtOAc and *n*-heptane) from *Sigma-Aldrich* and *ROTH*.

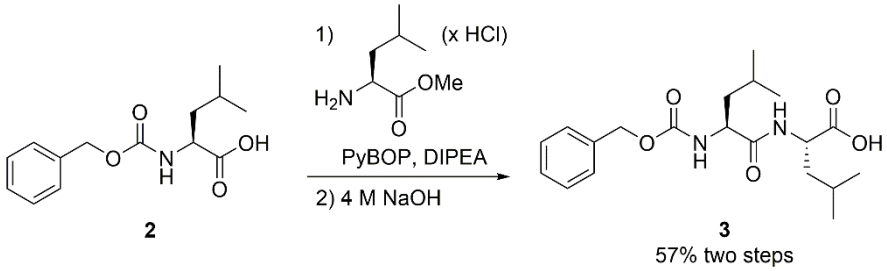
^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL ECX 400 (400 MHz), Bruker AVANCE III HD 400 (400 MHz), Varian VNMRS 400 (400 MHz), Varian VNMRS 600 (600 MHz), or Bruker AVANCE III HD 800 (800 MHz) NMR spectrometer. Deuterated solvents were obtained from *Cambridge Isotope Laboratories* and used without further purification. Chemical shifts (δ) are given relative to tetramethylsilane as external standard. Residual solvent signals in the ^{1}H and ^{13}C NMR spectra were used as internal reference. $CDCl_3$: δ_H = 7.260 ppm, δ_C = 77.160 ppm; CD_2Cl_2: δ_H = 5.320 ppm, δ_C = 54.000 ppm. Resonance multiplicity is indicated as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Chemical shifts are given in parts per million (ppm) on the delta scale (δ) and the coupling constant values (J) are in hertz (Hz). Signal assignments are given in the experimental part with the arbitrary numbering indicated.

Electrospray ionisation (ESI) mass spectra were measured on a *Thermo Finnigan LTQ-FT* mass spectrometer.

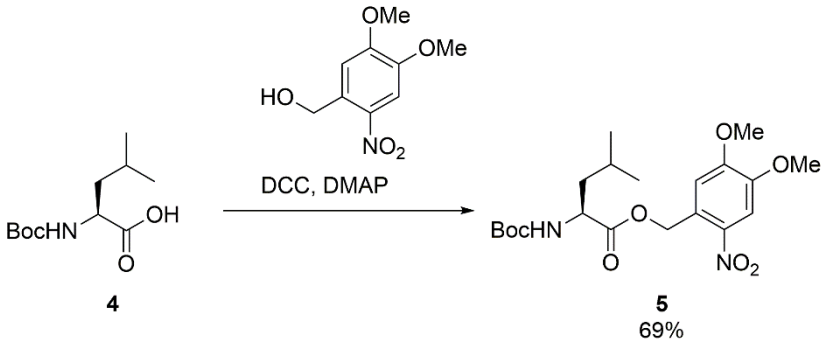
Infrared spectra were recorded on a *Perkin Elmer Spectrum BX-FT-IR* instrument equipped with a *Smith DuraSamplIR II* ATR-device. Transmittance values are qualitatively described by wavenumber (cm^{-1}) as very strong (vs), strong (s), medium (m), and weak (w).

Synthesis

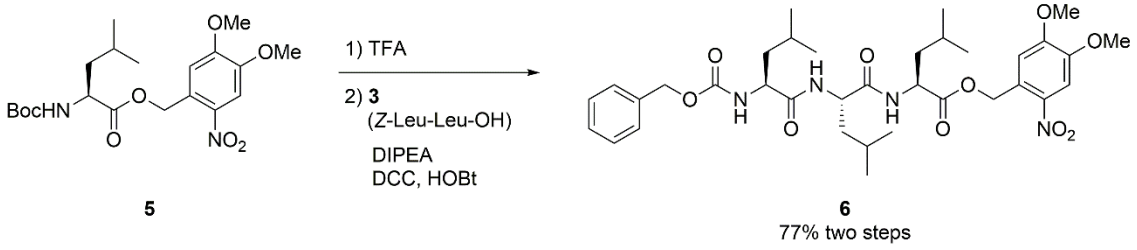
Precursor **3** was prepared according to a published procedure.[1]



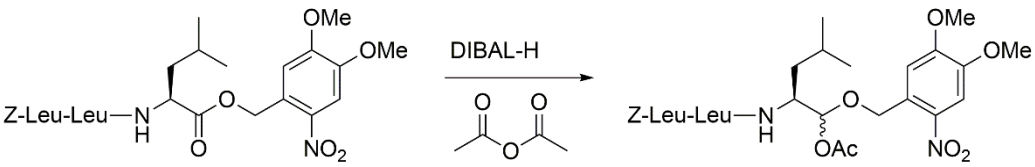
Scheme S1 Synthesis of dipeptide **3**.[1] Conditions: 1) Leu-OMe · HCl (1.0 eq), PyBOP (1.0 eq), DIPEA (2.0 eq), CH_2Cl_2, 4 h, 23 °C, 74%. 2) 4 M NaOH (1.3 eq), 1,4-dioxane:MeOH (3:1), 4 h, 23 °C, 77%; 57% over two steps.



Scheme S2 Synthesis of photocaged leucine **5**. Conditions: 1) 4,5-Dimethoxy-2-nitrobenzyl alcohol (1.0 eq), DCC (1.1 eq), DMAP (0.1 eq), CH_2Cl_2, 20 h, 23 °C, 69%.



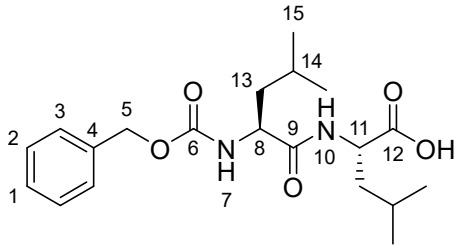
Scheme S3 Synthesis of photocaged tripeptide **6**. Conditions: 1) TFA, CH_2Cl_2, 0 °C – 23 °C, 2 h; 2) *Z*-Leu-Leu-OH (**3**, 1.0 eq), HOBt (1.1 eq), DCC (1.1 eq), DIPEA (2.0 eq), THF, 0 °C to 23 °C, 16 h, 77% over two steps.



1
MG132-Cage
17%

Scheme S4 Synthesis of photocaged MG132 (**1**). Conditions: DIBAL-H (1 M in hexanes, 2.0 eq), CH_2Cl_2, –78 °C, 45 min then pyridine (3.0 eq), DMAP (0.7 eq), acetic anhydride (6.0 eq), 20 h, 17%.

((Benzyloxy)carbonyl)-L-leucyl-L-leucine (3)



[378.469]

$C_{20}H_{30}N_2O_5$

A solution of *Z*-Leu-OH (2.80 g, 10.8 mmol, 1.0 eq) and Leu-OMe · HCl (1.96 g, 10.8 mmol, 1.0 eq) in CH_2Cl_2 (40 mL) was treated with PyBOP (5.60 g, 10.8 mmol, 1.0 eq) and DIPEA (3.65 mL, 21.5 mmol, 2.0 eq) and stirred for 4 h at 23 °C. The reaction mixture was extracted with aqueous 1 M HCl, saturated $NaHCO_3$ solution and brine. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. After flash column chromatography (SiO_2, CH_2Cl_2/methanol 97:3, R_f = 0.16 and SiO_2, *i*-Hex/EtOAc 7:3, R_f = 0.50) *Z*-Leu-Leu-OMe **3** (3.14 g, 8.00 mmol, 74%) was obtained as a colorless oil that was used directly in next step.

To a solution of *Z*-Leu-Leu-OMe (3.12 g, 7.95 mmol, 1.0 eq) in 1,4-dioxane (30 mL) and methanol (10 mL) was added sodium hydroxide (420 mg, 10.5 mmol, 1.32 eq) as aqueous 4 M solution. After stirring for 4 h at 23 °C the reaction mixture was acidified to pH 2 using aqueous 1 M HCl and concentrated *in vacuo*. The residue was taken up in EtOAc. The organic phase was extracted with water and brine, dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. After recrystallization from *i*-Hex/EtOAc compound **3** (2.32 g, 6.12 mmol, 77%) was obtained as a colorless solid.

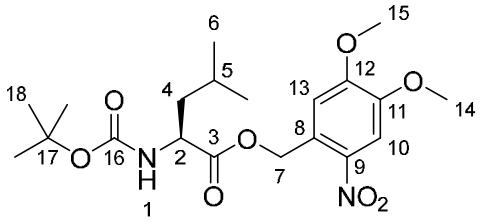
^{1}H NMR (599 MHz, $CDCl_3$, 27 °C) δ = 7.36 – 7.28 (m, 5H, H-C(1), H-C(2) and H-C(3)), 6.86 (d, *J* = 8.2 Hz, 1H, H-N(10)), 5.73 (d, *J* = 8.7 Hz, 1H, H-N(7)), 5.09 (s, 2H, H-C(5)), 4.59 (td, *J* = 8.6, 4.6 Hz, 1H, H-C(11)), 4.32 – 4.26 (m, 1H, H-C(8)), 1.73 – 1.47 (m, 6H, H-C(13) and H-C(14)), 0.94 – 0.85 (m, 12H, H-C(15)).

^{13}C NMR (151 MHz, $CDCl_3$, 27 °C) δ = 176.00 (C-12), 172.88 (C-9), 156.63 (C-6), 136.21 (C-4), 128.66 (C-2), 128.33 (C-1), 128.07 (C-3), 67.29 (C-5), 53.54 (C-8), 50.96 (C-11), 41.27 (C-13), 24.94 (C-14), 24.70 (C-14), 22.92 (C-15), 22.23 (C-15), 21.96 (C-15).

IR: $\tilde{\nu}$ = 3298 (w), 2957 (m), 2871 (w), 1700 (vs), 1637 (vs), 1536 (s), 1232 (s), 1211 (s), 1151 (m), 1042 (s), 1028 (m), 736 (m), 695 (s).

HR-MS (ESI$^+$), $[M+H]^+$ calc. for $[C_{20}H_{31}N_2O_5]$: 379.2227, found: 379.2229.

4,5-Dimethoxy-2-nitrobenzyl (*tert*-butoxycarbonyl)-L-leucinate (5)



[426.466]

$C_{20}H_{30}N_2O_8$

Boc-Leu-OH (1.00 g, 4.32 mmol, 1.0 eq), 4,5-dimethoxy-2-nitrobenzyl alcohol (992 mg, 4.32 mmol, 1.0 eq), DCC (981 mg, 4.76 mmol, 1.1 eq) and DMAP (52.8 mg, 4.76 mmol, 0.1 eq) in CH_2Cl_2 (30 mL) were stirred for 20 h at 23 °C in the dark. The reaction mixture was filtered and partitioned between EtOAc and 10% citric acid and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. After flash column chromatography (SiO_2, *i*-Hex/EtOAc 8:2, R_f = 0.26) compound **5** (1.28 g, 3.00 mmol, 69%) was obtained as a yellow solid that crystallized under high vacuum.

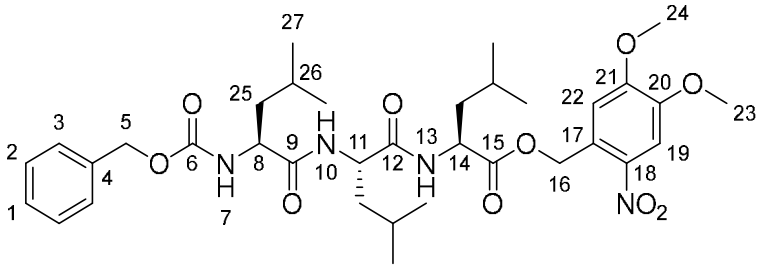
^{1}H NMR (599 MHz, $CDCl_3$, 27 °C) δ = 7.73 (s, 1H, H-C(10)), 7.08 (s, 1H, H-C(13)), 5.66 (d, *J* = 15.3 Hz, 1H, H-C(7)), 5.52 (d, *J* = 15.0 Hz, 1H, H-C(7)), 4.87 (d, *J* = 8.5 Hz, 1H, H-N(1)), 4.40 (td, *J* = 9.1, 5.5 Hz, 1H, H-C(2)), 4.03 (s, 3H, H-C(15)), 3.96 (s, 3H, H-C(14)), 1.78 – 1.71 (m, 1H, H-C(5)), 1.66 (ddd, *J* = 13.7, 8.2, 5.5 Hz, 1H, H-C(4)), 1.61 – 1.51 (m, 1H, H-C(4)), 1.42 (d, *J* = 1.6 Hz, 9H, H-C(18)), 0.97 (m, 6H, H-C(6)).

^{13}C NMR (151 MHz, $CDCl_3$, 27 °C) δ = 173.38 (C-3), 155.70 (C-16), 154.01 (C-12), 148.27 (C-11), 139.66 (C-8), 127.47 (C-9), 109.99 (C-13), 108.28 (C-10), 80.20 (C-17), 63.96 (C-7), 56.79 (C-14), 56.56 (C-15), 52.49 (C-2), 41.59 (C-4), 28.45 (C-18), 25.04 (C-5), 23.00 (C-6), 22.00 (C-6).

IR: $\tilde{\nu}$ = 3450 (w), 2963 (w), 1747 (m), 1706 (s), 1522 (m), 1502 (s), 1325 (m), 1277 (m), 1226 (s), 1156 (vs), 1067 (m), 1051 (m), 987 (m), 887 (m), 853 (m), 796 (m), 757 (m).

HR-MS (ESI$^+$), $[M+NH_4]^+$ calc. for $[C_{20}H_{34}N_3O_8]$: 444.2340, found: 444.2349.

4,5-Dimethoxy-2-nitrobenzyl ((benzyloxy)carbonyl)-L-leucyl-L-leucyl-L-leucinate (6)



[686.803]

$C_{35}H_{50}N_4O_{10}$

Trifluoroacetic acid (1.0 mL) was added to a stirring solution of compound **5** (500 mg, 1.17 mmol, 1.0 eq) in CH_2Cl_2 (4.0 mL) at 0 °C. The solution was stirred for 2 h at 23 °C and the solvent was evaporated *in vacuo* to give the deprotected TFA-Leu-NVOC (**7**). The amount of residual trifluoroacetic acid was calculated from the difference to 100% yield and neutralized in the following step with additional DIPEA (1:1).

TFA-Leu-NVOC (**7**) (1.17 mmol, 1.0 eq), Cbz-Leu-Leu (**3**) (433 mg, 1.17 mmol, 1.0 eq), HOBt · H_2O (197 mg, 1.29 mmol, 1.1 eq) and DCC (266 mg, 1.29 mmol, 1.1 eq) in THF (5.0 mL) were cooled to 0 °C. DIPEA (408 mL, 303 mg, 2.34 mmol, 2.0 eq) was added and the reaction mixture was stirred at 23 °C for 16 h. The mixture was partitioned between EtOAc and 10% citric acid and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. After flash column chromatography (SiO_2, CH_2Cl_2:MeOH 97:3, R_f = 0.33) compound **6** (620 mg, 903 µmol, 77%) was obtained as a colorless solid.

^{1}H NMR (599 MHz, $CDCl_3$, 27 °C) δ = 7.71 (s, 1H, H-C(19)), 7.37 – 7.27 (m, 5H, H-C(1) to H-C(3)), 7.03 (s, 1H, H-C(22)), 6.87 (d, *J* = 7.8 Hz, 1H, H-N(13)), 6.63 (d, *J* =

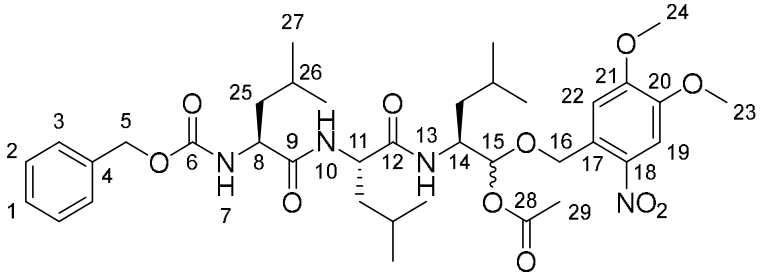
7.9 Hz, 1H, H-N(10)), 5.60 – 5.51 (m, 2H, H-C(16)), 5.38 (d, *J* = 8.1 Hz, 1H, H-N(7)), 5.12 – 5.05 (m, 2H, H-C(5)), 4.66 – 4.59 (m, 1H, H-C(14)), 4.50 – 4.43 (m, 1H, H-C(11)), 4.22 – 4.16 (m, 1H, H-C(8)), 4.01 (s, 3H, H-C(24)), 3.94 (s, 3H, H-C(23)), 1.72 – 1.54 (m, 7H, H-C(25) and H-C(26)), 1.52 – 1.44 (m, 2H, H-C(25)), 0.94 – 0.81 (m, 18H, H-C(27)).

^{13}C NMR (151 MHz, $CDCl_3$, 27 °C) δ = 172.43 (C-9), 172.35 (C-15), 171.89 (C-12), 156.43 (C-6), 153.91 (C-21), 148.33 (C-20), 139.74 (C-18), 136.15 (C-4), 128.67 (C-2), 128.37 (C-1), 128.09 (C-3), 127.08 (C-17), 110.27 (C-22), 108.28 (C-19), 67.26 (C-5), 64.10 (C-16), 56.78 (C-24), 56.52 (C-23), 53.66 (C-8), 51.72 (C-11), 51.10 (C-14), 41.33 (C-25), 41.02 (C-25), 40.57 (C-25), 24.95 (C-26), 24.80 (C-26), 22.98 (C-27), 22.83 (C-27), 22.22 (C-27), 22.12 (C-27), 21.84 (C-27).

IR: $\tilde{\nu}$ = 3324 (m), 2927 (s), 2850 (m), 1704 (w), 1645 (m), 1625 (vs), 1572 (s), 1523 (vs), 1436 (m), 1311 (m), 1273 (s), 1242 (s), 1221 (s), 1185 (m), 1156 (m), 1087 (m), 1067 (s), 1045 (m), 983 (w), 891 (m), 870 (w), 796 (w), 733 (w).

HR-MS (ESI$^+$), $[M+NH_4]^+$ calc. for $[C_{35}H_{54}N_5O_{10}]$: 704.3865, found: 704.3876.

(5*S*,8*S*,11*S*)-14-(4,5-dimethoxy-2-nitrophenyl)-5,8,11-triisobutyl-3,6,9-trioxo-1-phenyl-2,13-dioxa-4,7,10-triazatetradecan-12-yl acetate – MG132-Cage (1)



[730.856]

$C_{37}H_{54}N_4O_{11}$

Compound **6** (222 mg, 323 mmol, 1.0 eq) in CH_2Cl_2 (2 mL) was cooled to –78 °C and DIBAL-H (1 M in hexanes, 646 µL, 646 µmol, 2.0 eq) was added dropwise. 45 min after complete addition pyridine (76.7 mg, 970 µmol, 3.0 eq), DMAP solution (26.0 mg, 213 µmol, 0.66 eq in 1 mL CH_2Cl_2) and acetic anhydride (198 mg, 1.94 mmol, 6.0 eq) were added. The reaction mixture was stirred for another 20 h at –78 °C before it was warmed to 0 °C. After another 35 min the reaction was stopped by addition of saturated ammonium chloride solution and saturated Rochelle salt solution. The mixture was stirred for 50 min at 22 °C and then extracted with CH_2Cl_2. The combined organic phases were washed with ice cold 1 M potassium bisulfate solution, saturated sodium bicarbonate solution and brine. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo*. After flash column chromatography (SiO_2, *i*-Hex/EtOac 3:1, R_f = 0.14) **MG132-Cage** (**1**) (mixture of diastereomers (6:4), 40 mg, 54.7 µmol, 17%) was obtained as an off-white solid.

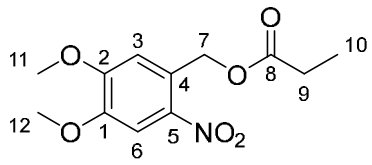
^{1}H NMR (800 MHz, CD_2Cl_2, 27 °C) δ = 7.68 (s, 1H, H-C(19)), 7.38 – 7.31 (m, 5H, H-C(1) to H-C(3)), 7.27 (s, 1H, H-C(22)), 7.21 (s, 1H, H-C(22)), 6.32 – 6.25 (m, 2H, H-N(10) and H-N(13)), 5.90 (d, *J* = 3.6 Hz, 1H, H-C(15)), 5.87 (d, *J* = 3.2 Hz, 1H, H-C(15)), 5.15 – 4.98 (m, 5H, H-C(5), H-N(7) and H-C(16)), 4.36 – 4.29 (m, 2H, H-C(11) and H-C(14)), 4.10 – 4.03 (m, 1H, H-C(8)), 3.99 (s, 3H, H-C(24)), 3.91 (s, 2H, H-C(23)), 3.90 (s, 1H, H-C(24)), 2.09 (s, 1H, H-C(29)), 2.05 (s, 2H, H-C(29)), 1.73 – 1.40 (m, 9H, H-C(25) and H-C(26)), 0.95 – 0.83 (m, 18H, H-C(27)).

^{13}C NMR (201 MHz, CD_2Cl_2, 27 °C) δ = 172.52 (C-9), 171.97 (C-12), 171.15 (C-28), 154.52 (C-21), 154.39 (C-21), 148.63 (C-20), 139.94 (C-18), 136.88 (C-4), 129.49 (C-

17), 129.09 (C-2), 128.80 (C-1), 128.53 (C-3), 128.50 (C-3), 110.75 (C-22), 110.68 (C-22), 108.60 (C-19), 108.49 (C-19), 97.93 (C-15), 97.82 (C-15), 69.38 (C-16), 69.14 (C-16), 67.67 (C-5), 57.02 (C-24), 56.94 (C-24), 56.84 (C-23), 56.81 (C-23), 52.59 (C-11 or C-14), 52.48 (C-11 or C-14), 50.37 (C-11 or C-14), 50.12 (C-11 or C-14), 41.66 (C-25), 41.06 (C-25), 40.93 (C-25), 39.06 (C-25), 39.01 (C-25), 25.32 (C-26), 25.28 (C-26), 25.11 (C-26), 25.01 (C-26), 23.87 (C-27), 23.76 (C-27), 23.26 (C-27), 23.23 (C-27), 23.11 (C-27), 22.26 (C-27), 22.13 (C-27), 22.09 (C-27), 22.00 (C-27), 21.91 (C-27), 21.38 (C-29).

HR-MS (ESI$^+$), $[M+NH_4]^+$ calc. for $[C_{35}H_{58}N_5O_{11}]$: 748.4127 found: 748.4133.

4,5-dimethoxy-2-nitrobenzyl propionate (Propionic acid-Cage 8)



[269.253]

$C_{12}H_{15}NO_6$

TEA (405 mg, 4.00 mmol, 2.0 eq) and propionyl chloride (204 mg, 2.20 mmol, 1.1 eq) were added to a stirring solution of 6-nitroveratryl alcohol (426 mg, 2.00 mmol, 1.0 eq) and DMAP (24.4 mg, 0.20 mmol, 0.1 eq) in CH_2Cl_2 (20.0 mL). The solution was stirred for 3 d at 23 °C before it was transferred to a separatory funnel charged with aqueous 2 M HCl solution. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and the solvent was evaporated *in vacuo*. After flash column chromatography (SiO_2, *i*-Hex:EtOAc 3:2) compound **8** (398 mg, 1.48 mmol, 74%) was obtained as a pale yellow solid.

^{1}H NMR (599 MHz, $CDCl_3$, 27 °C) δ = 7.72 (s, 1H, H-C(6)), 7.00 (s, 1H, H-C(3)), 5.51 (s, 2H, H-C(7)), 3.98 (s, 3H, H-C(11)), 3.96 (s, 3H, H-C(12)), 2.48 – 2.42 (m, 2H, H-C(9)), 1.22 – 1.19 (m, 3H, H-C(10)).

^{13}C NMR (151 MHz, $CDCl_3$, 27 °C) δ = 173.86 (C-8), 153.58 (C-2), 148.37 (C-1), 140.18 (C-5), 127.33 (C-4), 110.56 (C-3), 108.41 (C-6), 63.24 (C-7), 56.57 (C-11), 56.51 (C-12), 27.75 (C-9), 9.32 (C-10).

HR-MS (ESI$^+$), $[M+NH_4]^+$ calc. for $[C_{12}H_{19}N_2O_6]$: 287.1243, found: 287.1239.

NMR Spectra

Compound 3

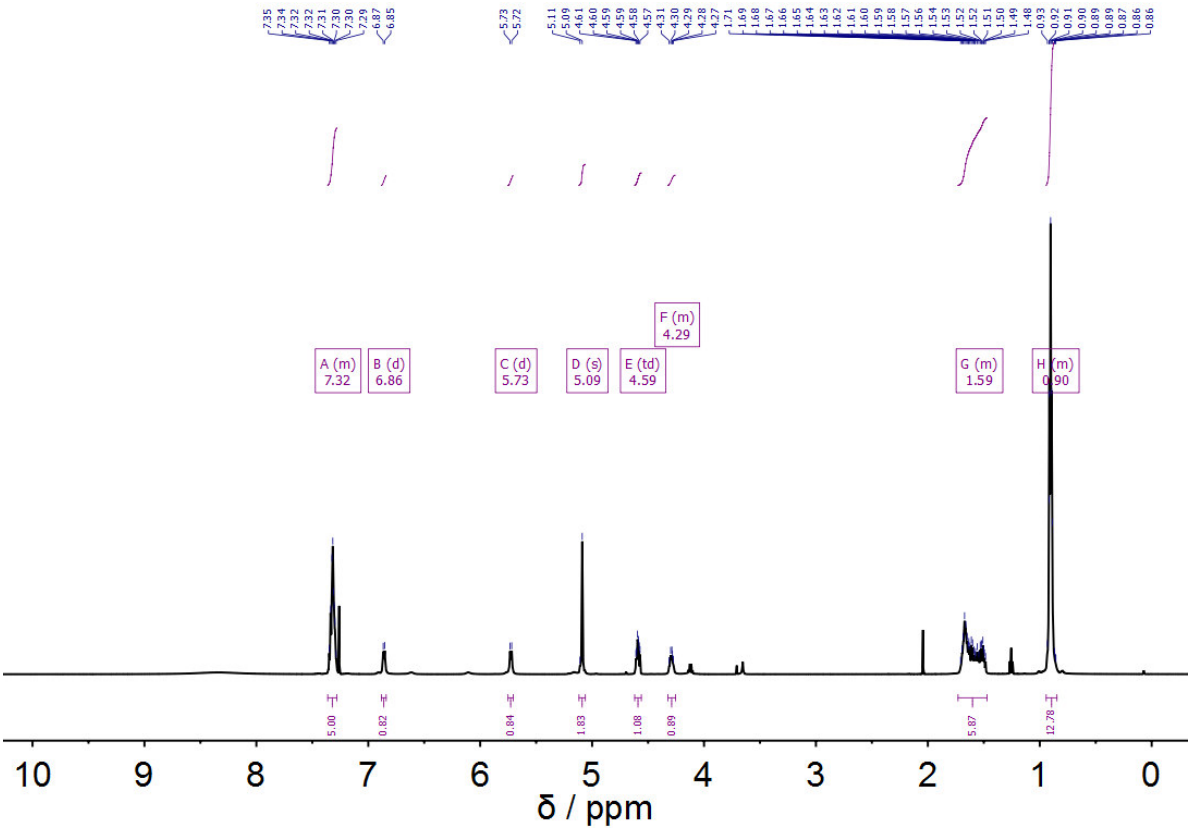


Figure S1 ^{1}H NMR spectrum (599 MHz, $CDCl_3$, 27 °C) of compound **3**.

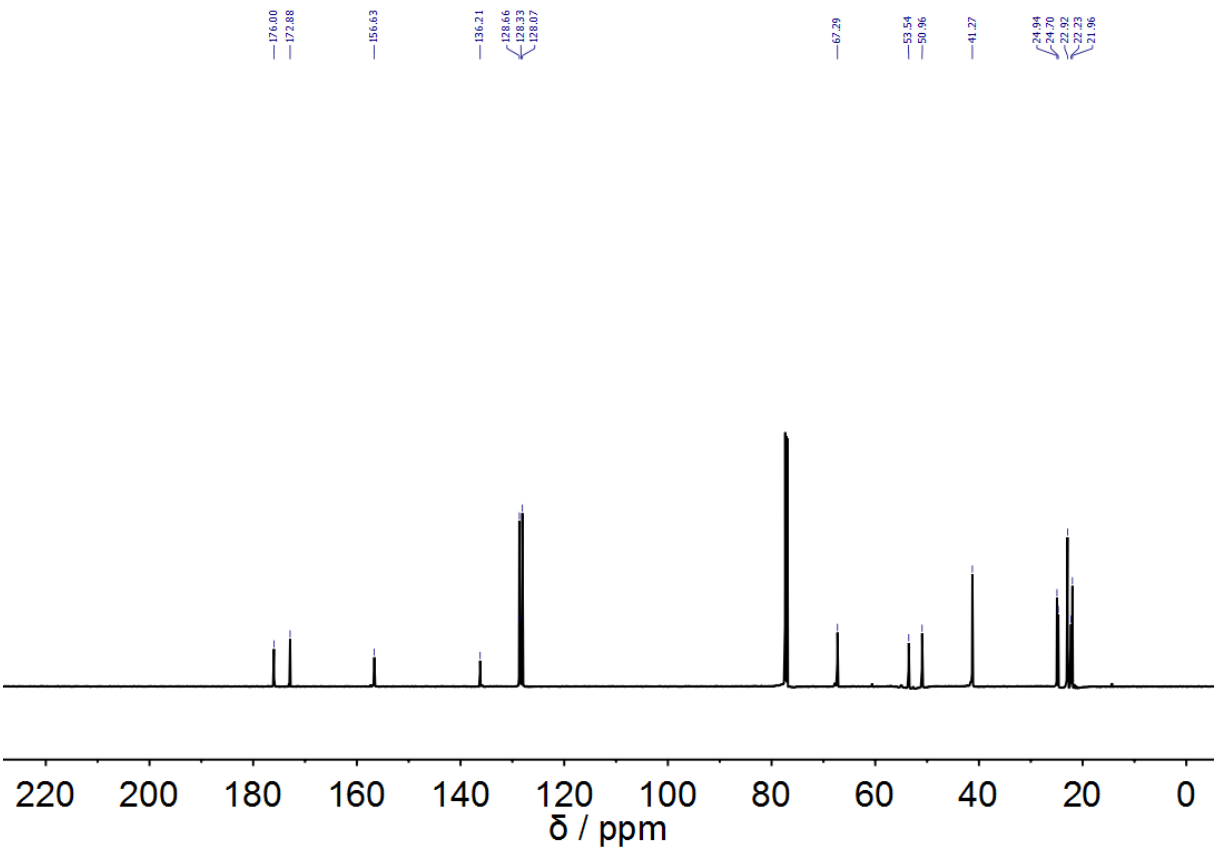


Figure S2 ^{13}C NMR spectrum (151 MHz, $CDCl_3$, 27 °C) of compound **3**.

Compound 5

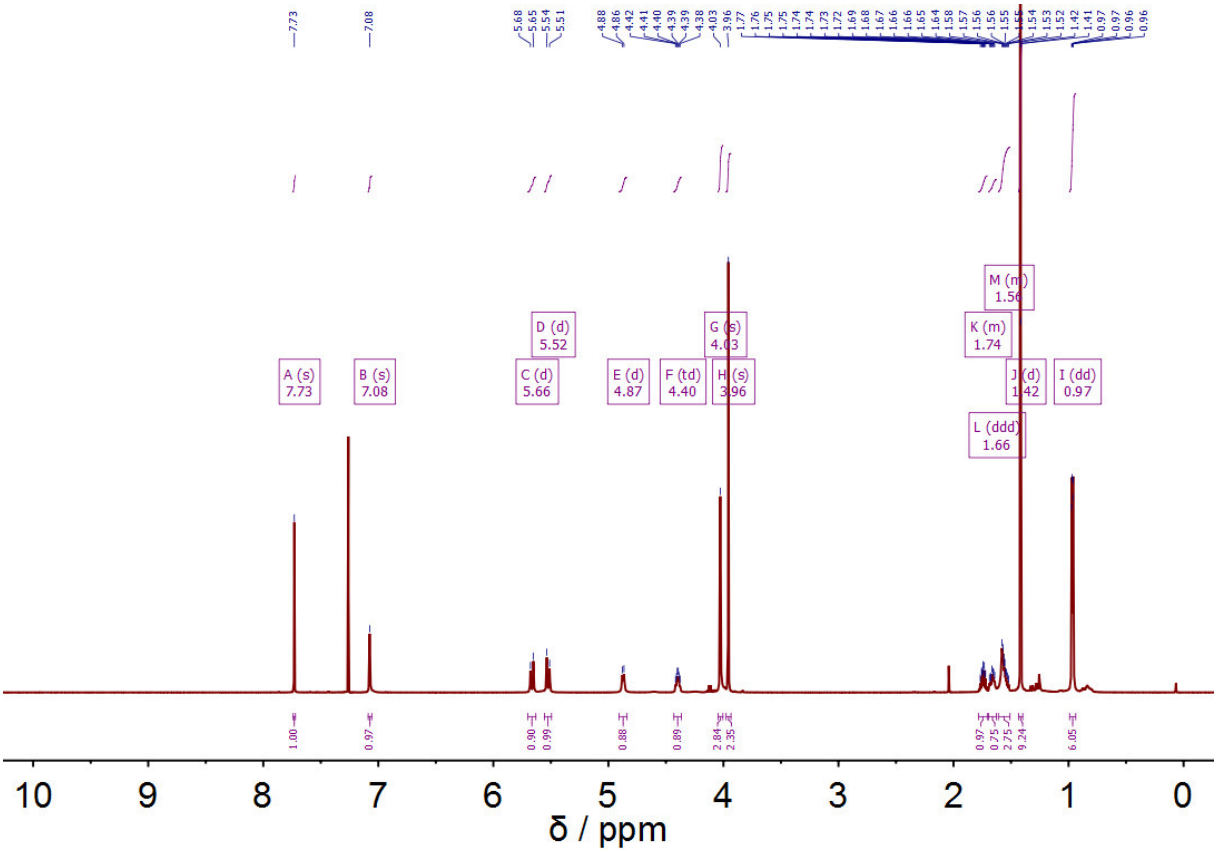


Figure S3 ^{1}H NMR spectrum (599 MHz, $CDCl_3$, 27 °C) of compound **5**.

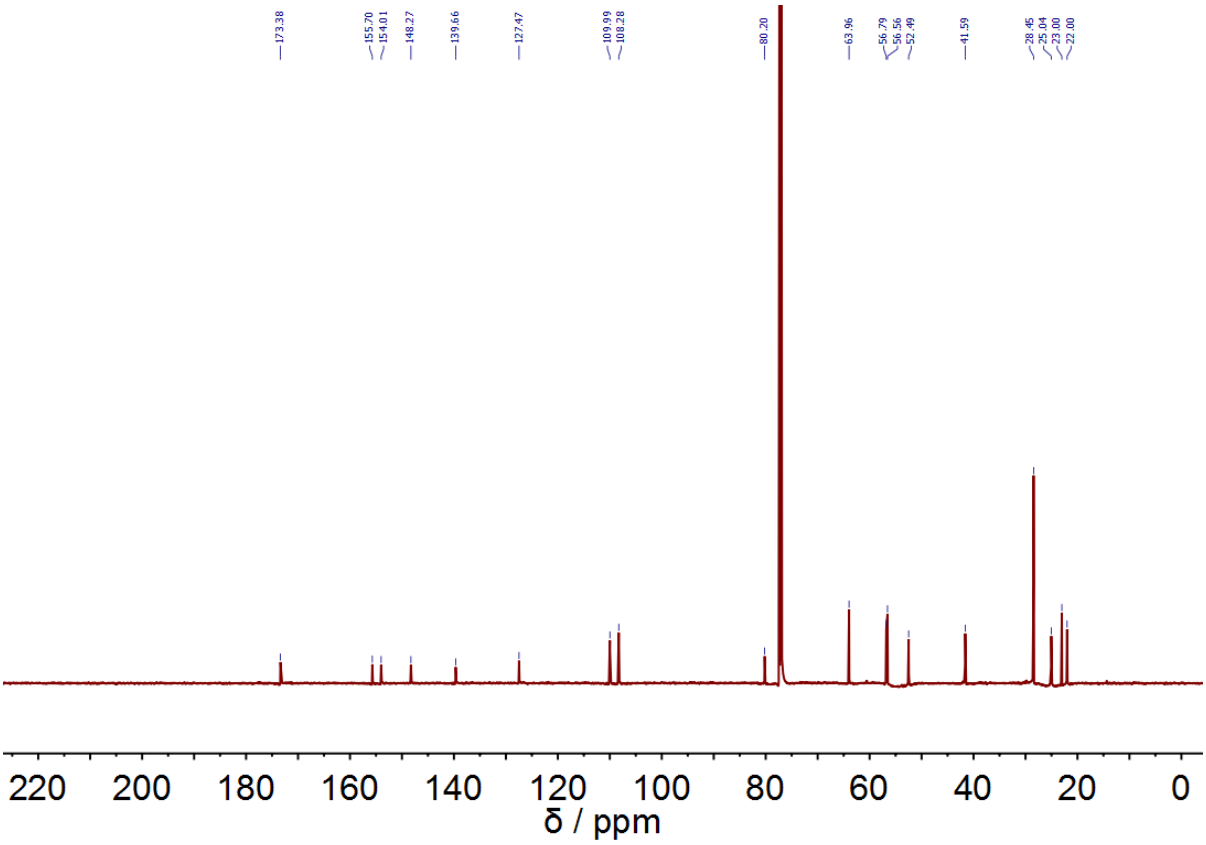


Figure S4 ^{13}C NMR spectrum (151 MHz, $CDCl_3$, 27 °C) of compound **5**.

Compound 6

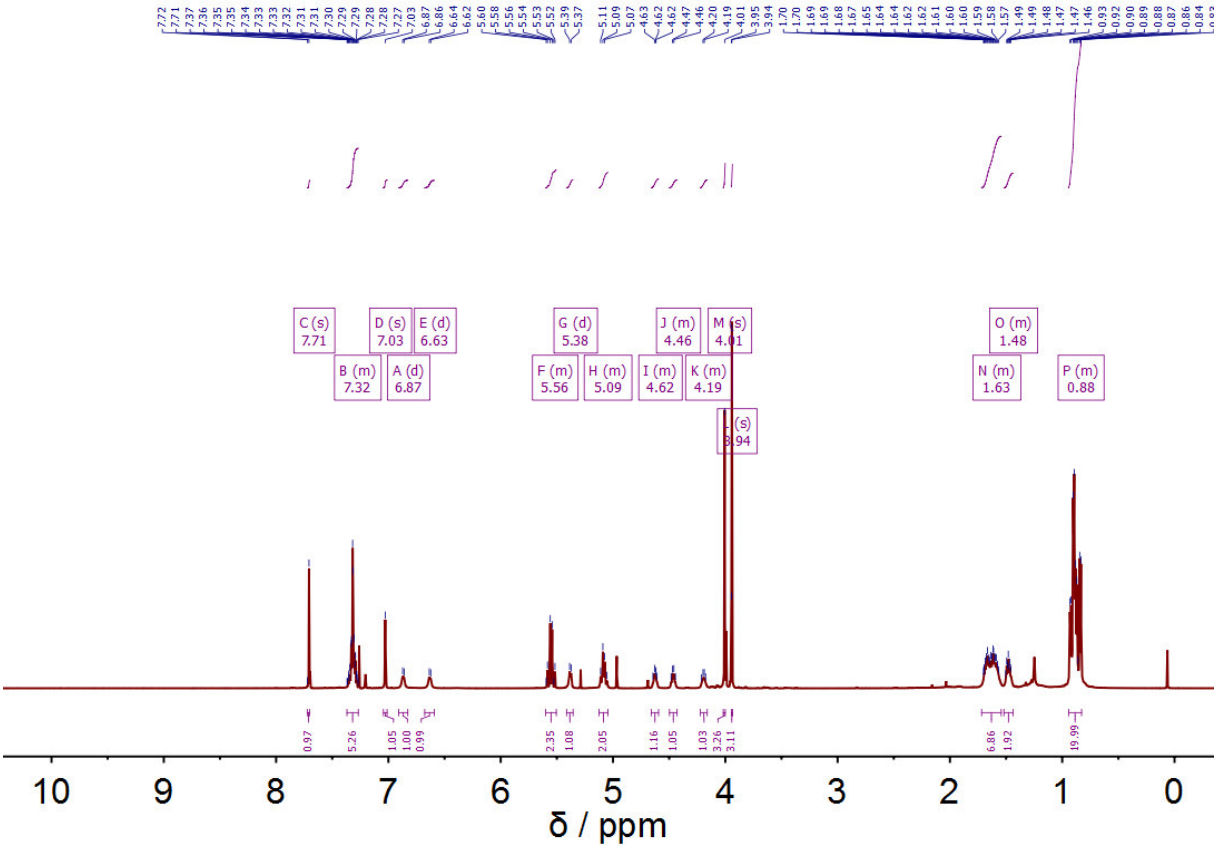


Figure S5 ^{1}H NMR spectrum (599 MHz, $CDCl_3$, 27 °C) of compound **6**.

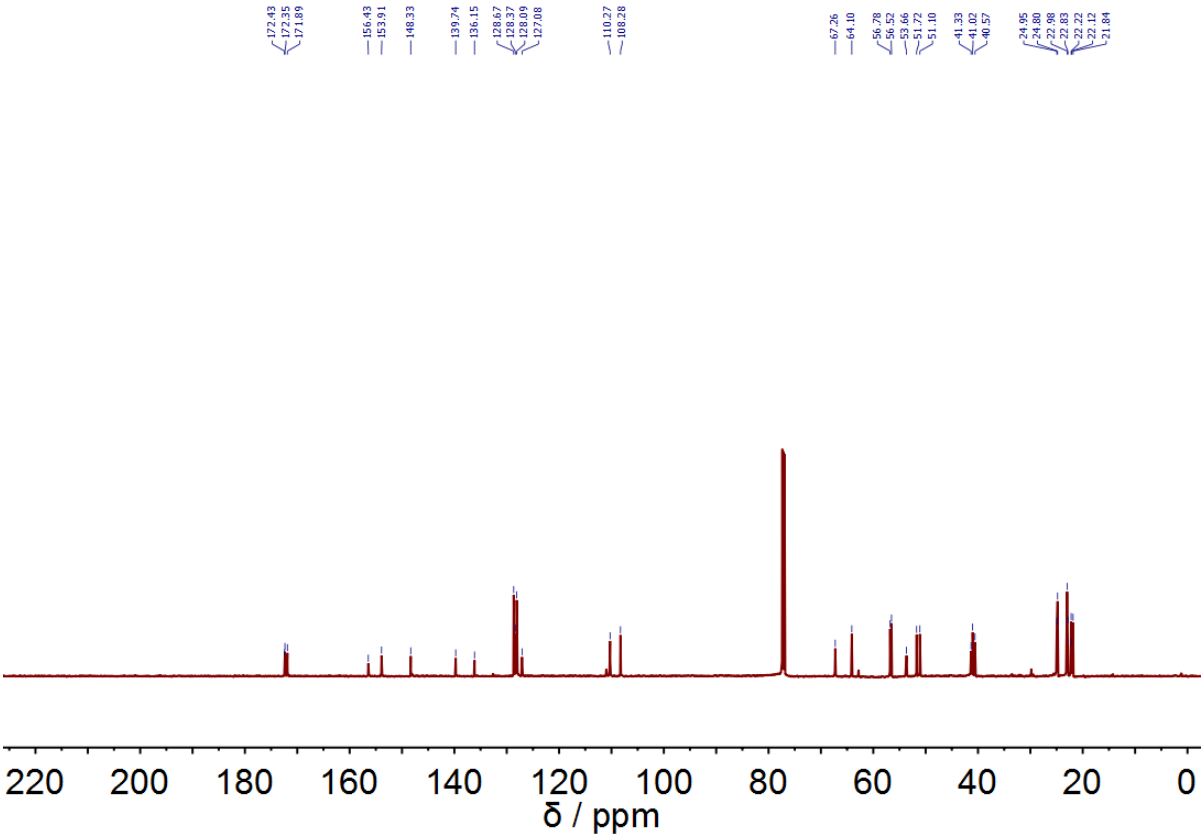


Figure S6 ^{13}C NMR spectrum (151 MHz, $CDCl_3$, 27 °C) of compound **6**.

Compound 1

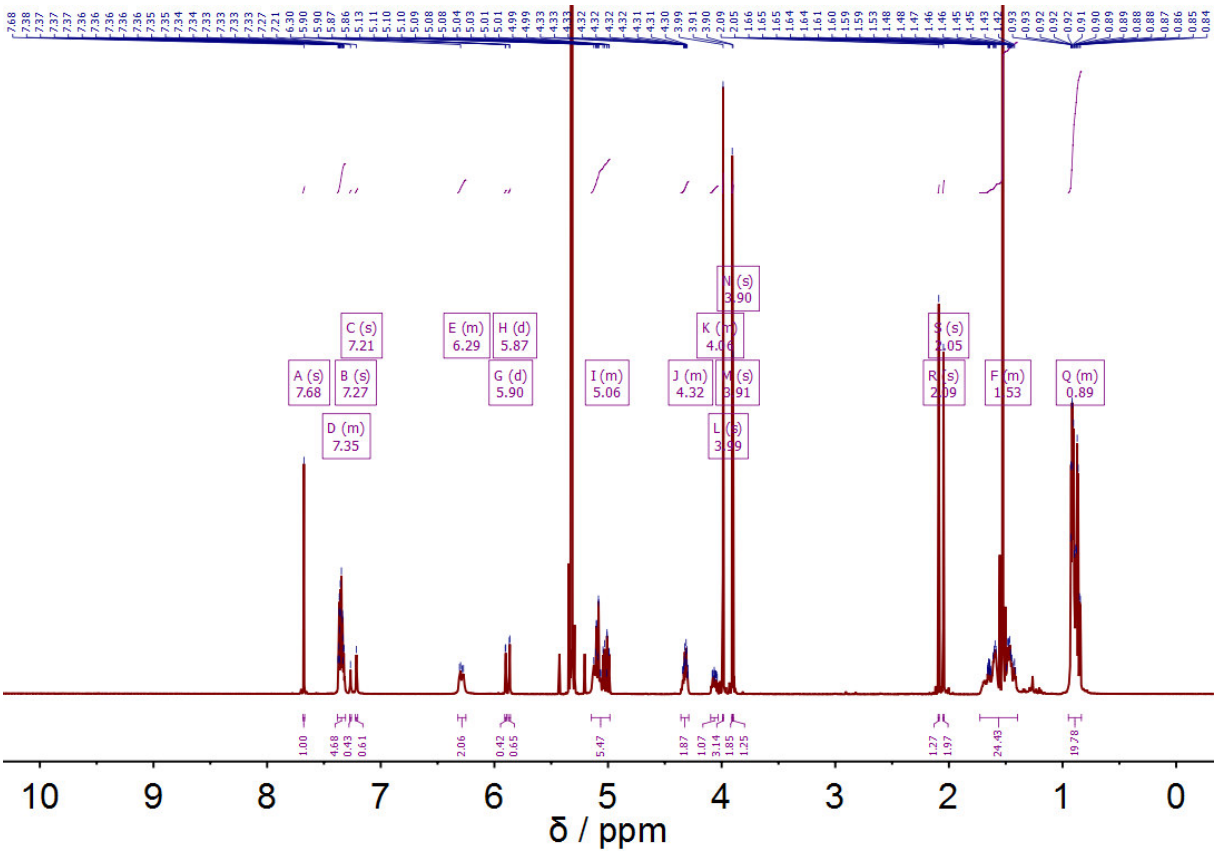


Figure S7 ^{1}H NMR spectrum (800 MHz, CD_2Cl_2, 27 °C) of compound **1**.

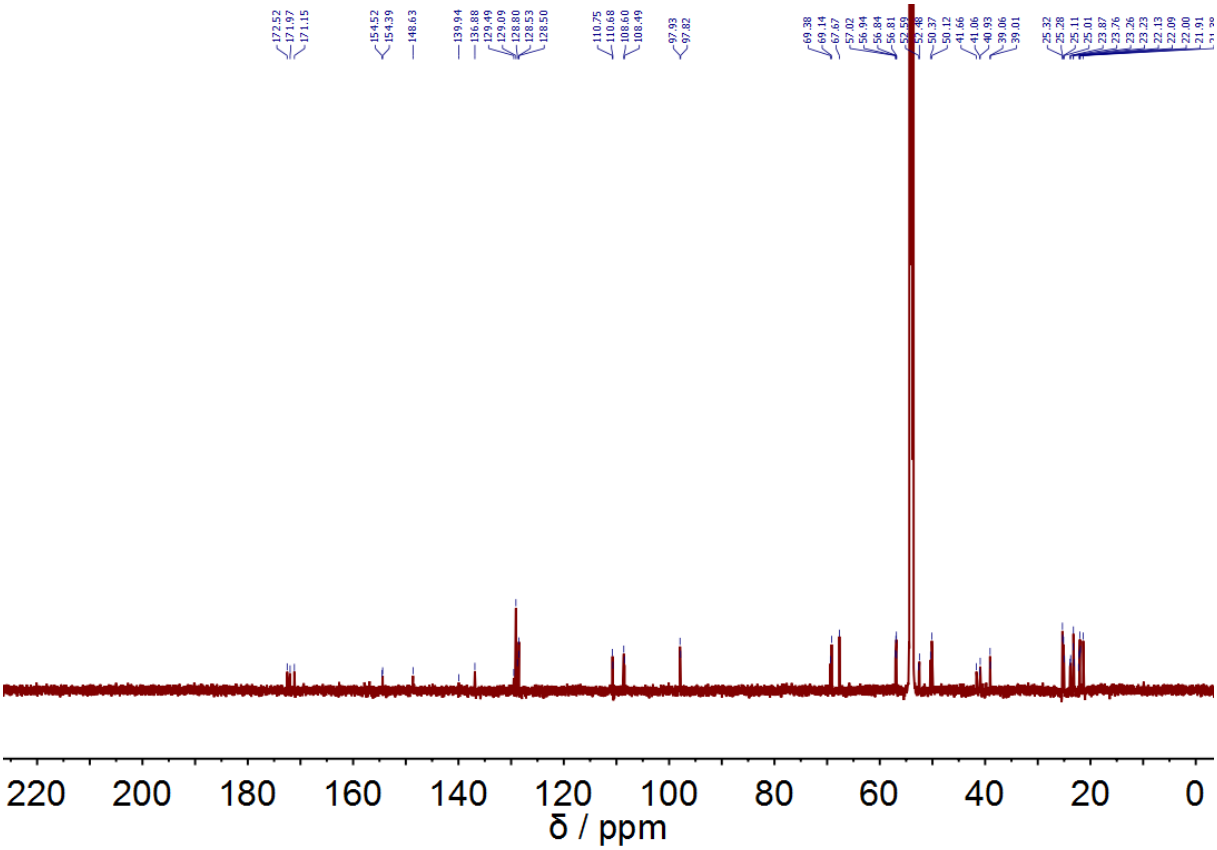


Figure S8 ^{13}C NMR spectrum (201 MHz, CD_2Cl_2, 27 °C) of compound **1**.

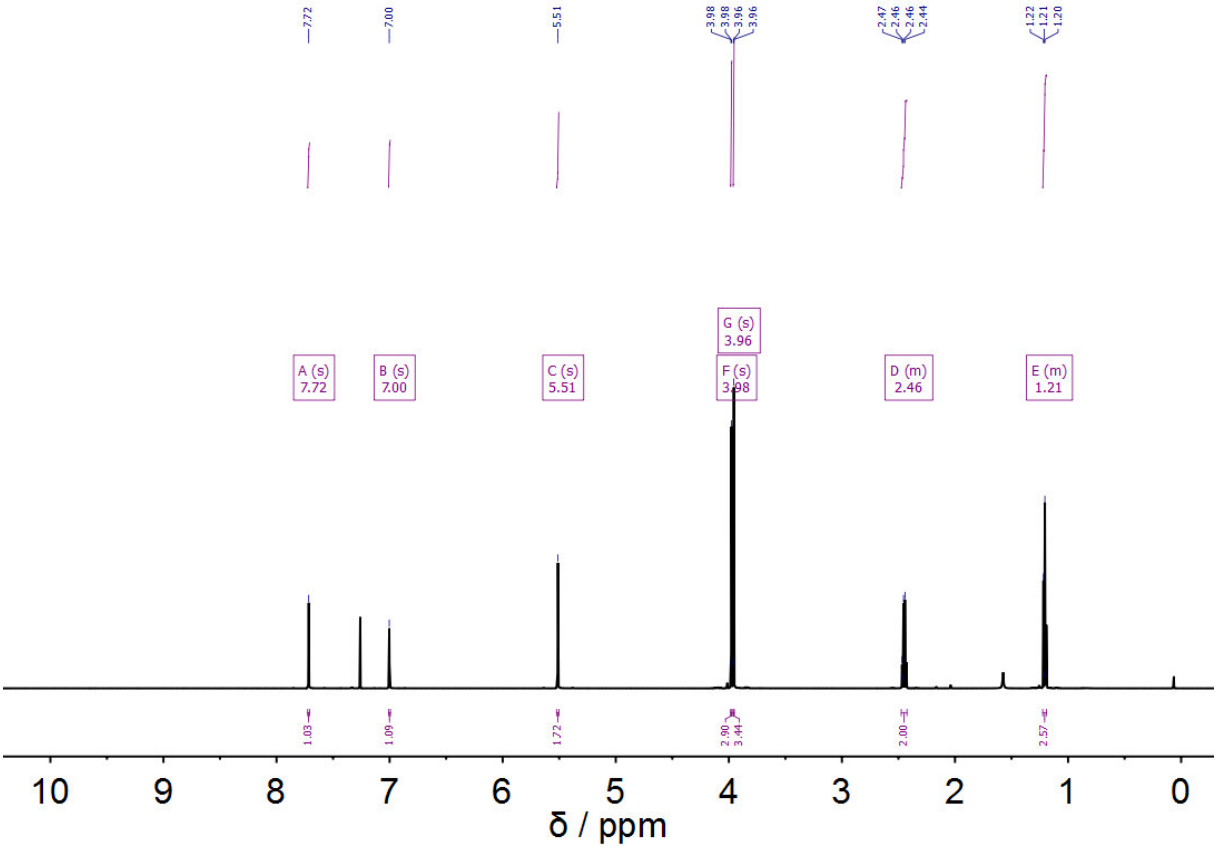


Figure S9 ^{1}H NMR spectrum (599 MHz, $CDCl_3$, 27 °C) of compound **8**.

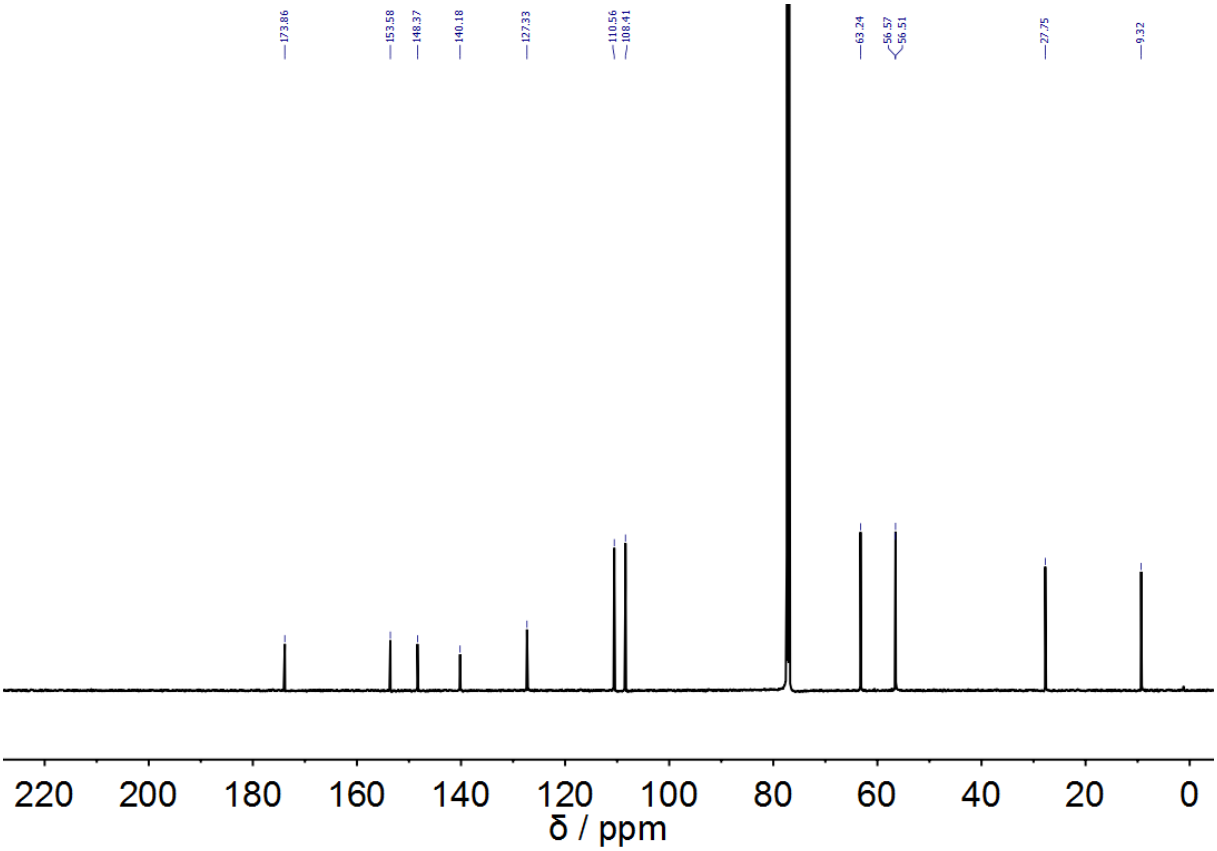


Figure S10 ^{13}C NMR spectrum (151 MHz, $CDCl_3$, 27 °C) of compound **8**.

Photodeprotection of Propionic Acid-Cage, MG132-Ester-Cage, and MG132-Cage upon irradiation with 405 nm light

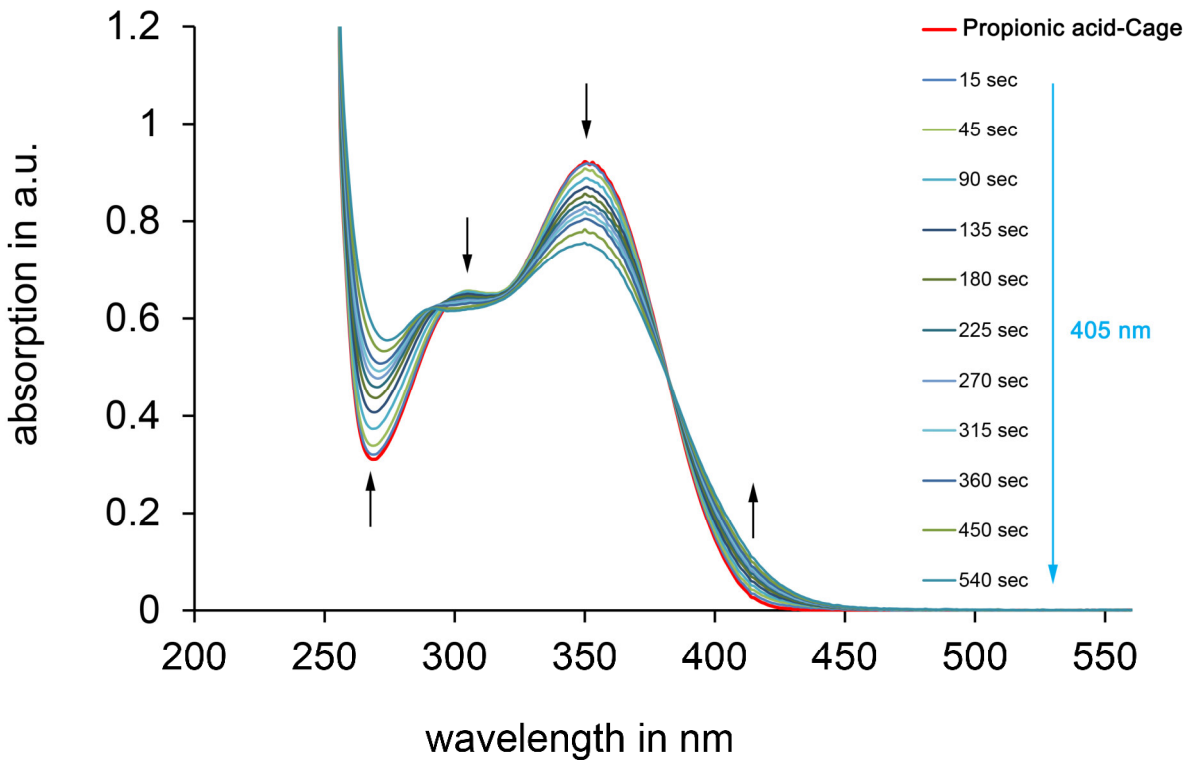


Figure S11 Changes of the UV/Vis absorption recorded in intervals during 405 nm irradiation of Propionic-Acid-Cage in DMSO solution at 20 °C.

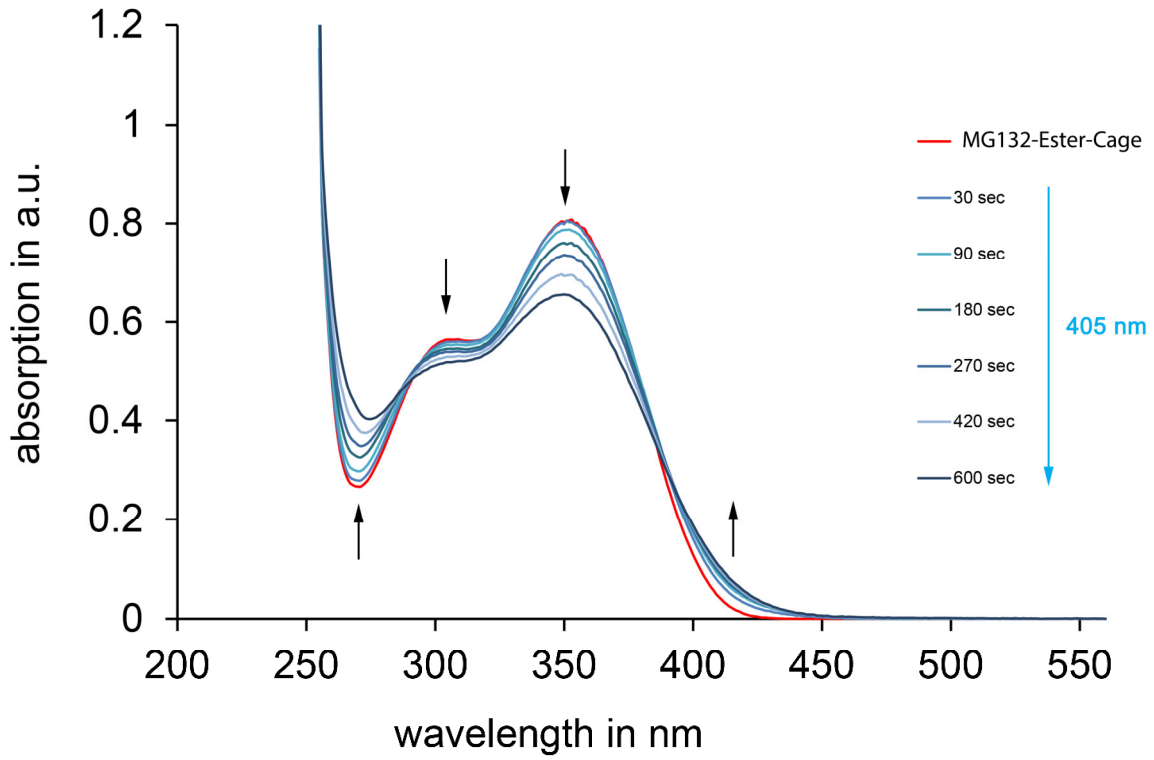


Figure S12 Changes of the UV/Vis absorption recorded in intervals during 405 nm irradiation of MG132-Ester-Cage in DMSO solution at 20 °C.

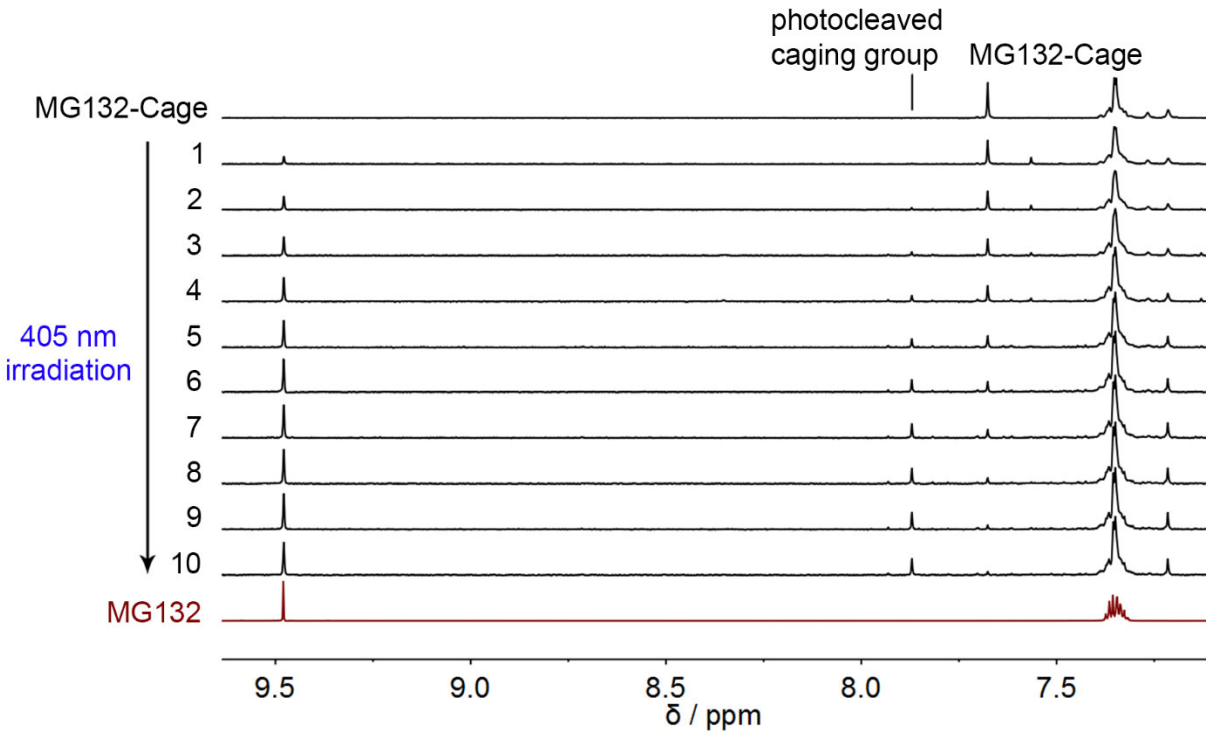


Figure S13 Aromatic part of the ^{1}H NMR spectra of a solution of MG132-Cage (400 MHz, CD_2Cl_2, 22 °C) before (top) and during (spectra 1-10) irradiation with 405 nm light. Spectra were taken in intervals of 1 min. A spectrum of pure MG132 (bottom) is shown for comparison.

Quantum yield measurement

Quantum yield measurements were conducted in DMSO solution because of the limited solubility of MG132-Cage in buffered solutions.

For the quantum yield measurement the molar absorption coefficients have to be determined first in order to relate spectral changes to photoreaction turnover in a quantitative way. The molar absorption coefficients for MG132-Cage are depicted in Figure S14.

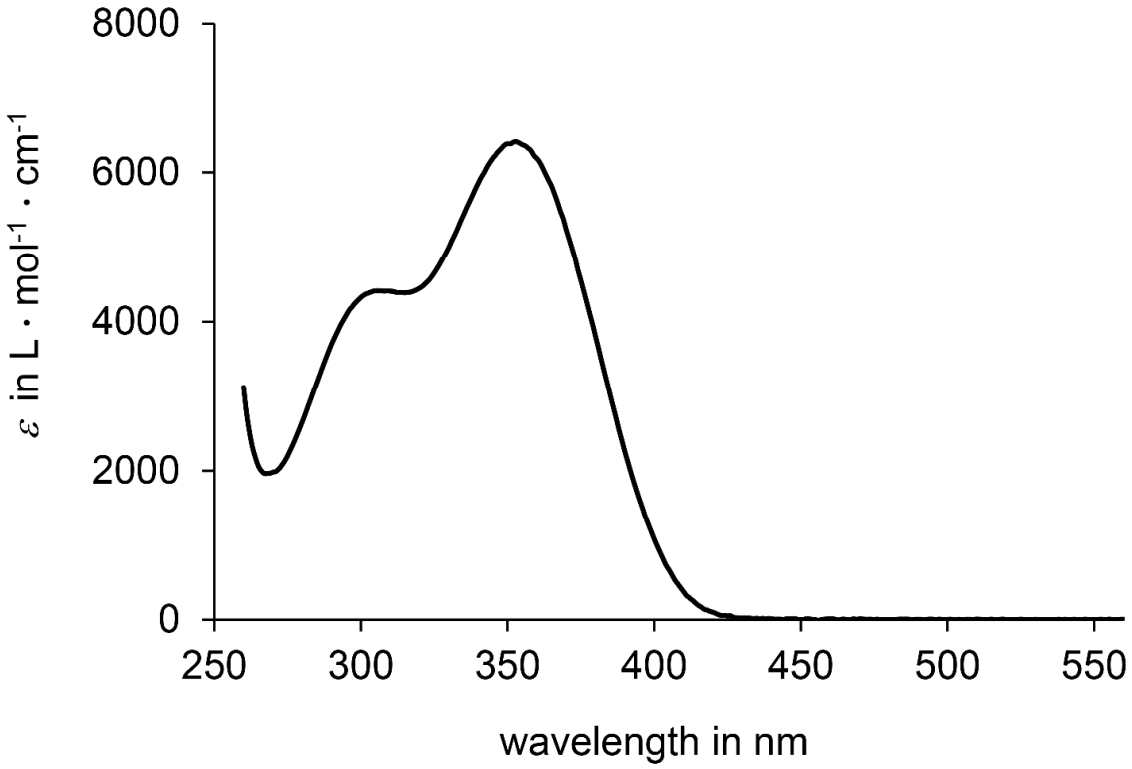


Figure S14 Molar absorption coefficients of MG132-Cage in DMSO solution.

To obtain the molar absorption coefficients of the photoreaction product a solution of MG132-Cage was prepared in DMSO-d_6 and irradiated for a short time period resulting in exactly 20% conversion as determined by ^{1}H NMR spectroscopy. A sample of this resulting NMR solution was diluted in DMSO and the corresponding UV/Vis spectrum was recorded. Normalization of this UV/Vis spectrum to the known isosbestic points

(see Figure 2b in the manuscript) allowed to relate absorption changes occurring during irradiation quantitatively to molecular conversion (Figure S15).

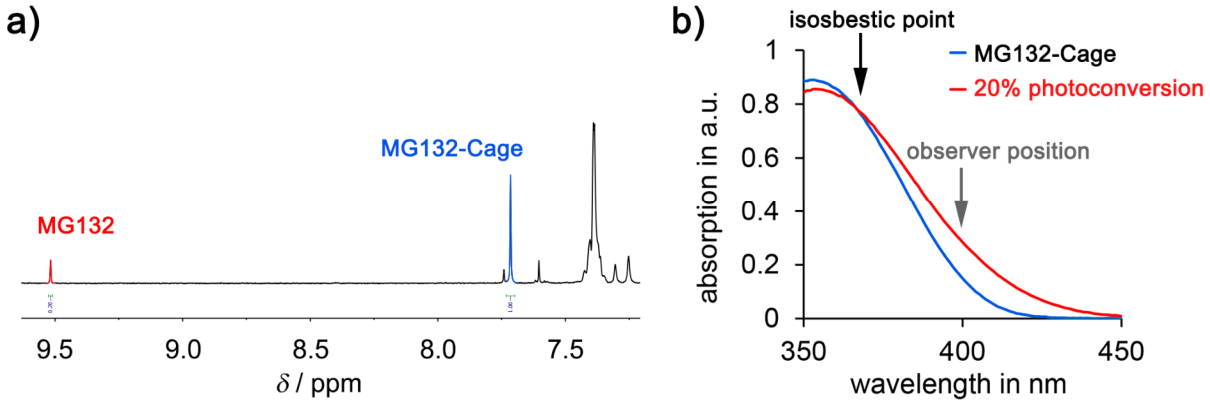


Figure S15 a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 27 °C) of MG132-Cage obtained after short irradiation with 405 nm light. 20% of MG132-Cage are photodeprotected as judged by integration of the indicated signals. b) Corresponding UV/Vis spectrum of a diluted sample of the same NMR solution obtained after short irradiation with 405 nm light with known ration between MG132-Cage and released MG132 (red). The 20% conversion spectrum was normalized to the isosbestic point at 368 nm (black arrow) occurring during photodeprotection of MG132-Cage to obtain absolute molar absorptions. The spectrum of pure MG132-Cage in DMSO solution is shown for comparison (blue) with the observer position at 400 nm indicated (grey arrow).

The photochemical quantum yield of the photoconversion reactions was calculated as the ratio between the numbers of isomerized molecules and the number of absorbed photons according to eq. 1.

$$\Phi = \frac{n(isomerized\ molecules)}{n(absorbed\ photons)} \qquad \text{(eq. 1)}$$

To determine the quantum yield Φ, a solution of pure MG132-Cage in DMSO, with known concentration and volume, was irradiated within a cuvette with a focused light beam of a 405 nm LED within the published instrumental setup from the group of *E. Riedle*.[2] To determine the number of photo-uncaged molecules UV-Vis absorption spectra were taken after different irradiation periods as shown in Figure S16. Using the

known molar absorption coefficients (see above) the change in concentration of MG132-Cage and therefore the number of uncaged molecules could be determined for each irradiation step.

The number of absorbed photons over time was measured directly at the thermal photometer according to eq. 2.

$$n(h\nu) = \frac{\Delta P \cdot \lambda_{\mathrm{ex}} \cdot t}{c \cdot h} \qquad \text{(eq. 2)}$$

Where c is the speed of light ($2.99792.10^8$ m·s^{-1}), h is the Planck's constant ($6.62607 \cdot 10^{-34}$ J·s), λ_{ex} is the excitation wavelength in m, t is the elapsed time during irradiation, and ΔP is the difference in power readouts at the thermal photometer between a cuvette filled with solvent (P_0) and a cuvette containing the sample solution (P_t) during the initial period in Watt (eq. 3). The power read out during the irradiation period did not change substantially (~4%) and was averaged over the sixteen steps that were used for the calculations.

$$\Delta P = P_{\mathrm{t}} - P_0 \qquad \text{(eq. 3)}$$

At the start of the experiment only the pure MG132-Cage is converted resulting in a linear behavior. Therefore the first sixteen steps of the measurement were used to determine the quantum yield Φ (Figure S12).

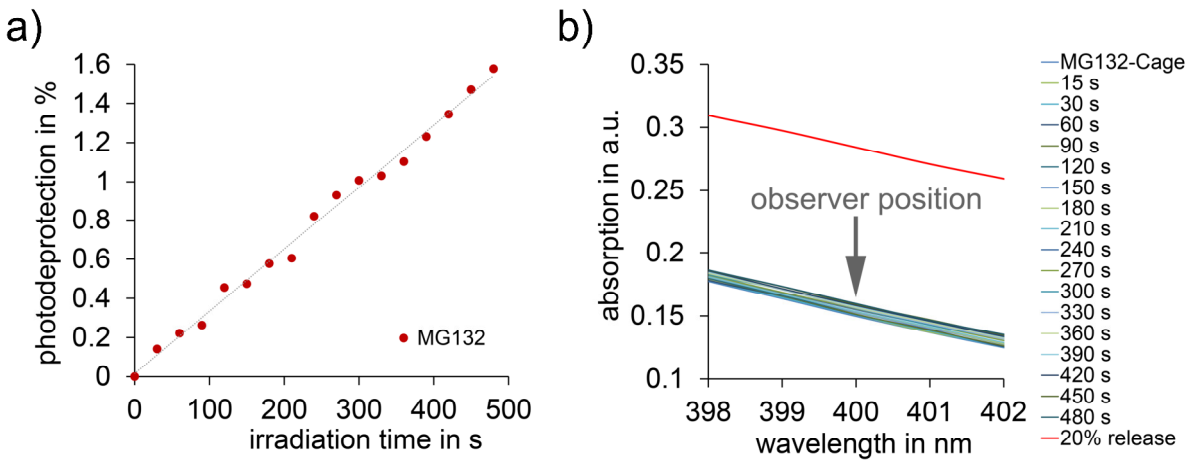


Figure S16 Quantum yield measurement for photodeprotection of MG132-Cage. a) Quantification of the concentration changes of MG132-Cage induced by irradiation with 405 nm light. Concentrations were calculated after different time intervals of irradiation from UV-Vis spectra at 400 nm spectral position using the molar absorption coefficients. The first sixteen data points were used for the quantum yield determination. b) Absorption increase occurring during the quantum yield measurements around the observer position at 400 nm (grey arrow, bottom spectra) and corresponding spectrum recorded after 20% deprotection (red).

The determined quantum yield is 1.0% for 405 nm irradiation, which is in very good agreement with the previously reported quantum yield of 1.0% (350 nm irradiation) for the photodeprotection of an alcohol bearing the same DMNB photocage.[3]

Biology

Cell culture and cell line generation

HeLa cells were grown at 37 °C in 5% CO_2 in Dulbecco's growth medium (DMEM, Life Technologies, 2172747) supplemented with 10% FCS (Merck, 2017-103299) and 1% penicillin/streptomycin (Merck, A2213). The HeLa cell lines EZ56, which expressed mKate2-α-tubulin and anillin-GFP, and Flp-In T-Rex[4] were used. The Flp-In T-Rex HeLa cell line was used for the annexin V and propidium iodide (PI) staining and the EZ56 HeLa cell line for all other experiments. For the EZ56 cell line mKate2-α-tubulin was cloned in the pcDNA6 mammalian expression vector and integrated in the HeLa cell line stably expressing anillin-GFP.[5] Sequences encoding mKate,[6] α-tubulin,[7] and the pcDNA6 plasmid backbone including blasticidin[8] resistance were amplified by polymerase chain reaction (PCR) and assembled together by Gibson cloning (NEB, E2611). The generated plasmid encoding mKate-α-tubulin was transfected into anillin-GFP expressing HeLa cells using X-tremeGENE 9 (06365779001, Roche). After selection with blasticidin (InvivoGen, ant-bl-1) stable clones co-expressing anillin-GFP and mKate-α-tubulin were isolated by fluorescence-activated cell sorting (FACS).

Experimental setup for compound treatment and 405 nm light exposure

Stock solutions for proteasome-inhibitor MG132 (Calbiochem, 474790) and MG132-Cage were prepared in DMSO (Sigma, D8418) with a concentration of 10 mM. To analyze cell cycle arrest, cells were synchronized with a double thymidine block. Cells were seeded at 50% confluency in 12-well plates (Greiner, 665180) containing 18 mm poly-L-lysine (Sigma, Y1251-100G) coated glass coverslips (Menzel, P233.1) for immunostainings; in 6-well plates (Greiner, 657160) for live-cell imaging; and in 8-well ibidiTreat chambers (Ibidi, 80826) for the prewash experiments. After cell attachment fresh medium containing 0.5 mM thymidine (Sigma, T1895) was added and cells were incubated for 15 h. Cells were released from the first thymidine block by washing 3x for 5 min with 1 mL DMEM/10%FCS medium. 8-10 h after the release, a second thymidine block was performed by adding fresh medium containing 0.5 mM thymidine.

After 15 h incubation, cells were released from the second thymidine block by washing 3x for 5 min with 1 mL DMEM/10%FCS medium.

8 h after release from the second thymidine block the DMEM/10%FCS medium was removed and fresh DMEM medium (Biochrom, F0475) supplemented with 10% FCS, 2 mM L-Glutamine (Life Technologies, 25030149) and containing 5 or 10 µM MG132-Cage, 5 or 10 µM MG132, 0.1% DMSO, or no additive was added. Immediately after addition of the different compounds 12-well plates were irradiated for 5 or 10 min using two 405 nm LEDs (Roithner H2A1-H405 405 nm, 105 mW; and Thorlabs M405D2 405 nm run at ca. 375 mW, FWHM = 12 nm) in a self-made dark chamber at room temperature. For irradiation two LED lights were placed approximately 2 cm above the wells of the plates. Control cells without irradiation were protected from light during the experiment until fixation. For inhibitor-washout experiments, cells were treated with MG132-Cage, MG132, or 0.1% DMSO 6 h after the release from the second thymidine block, exposed to 10 min 405 nm light, incubated for 2 h, washed 3x with 1 mL DMEM/10%FCS/2mM L-Glutamine medium and fixed after another 2 h incubation.

For prewash experiments cells were seeded in 8-well ibidiTreat chambers (Ibidi, 80826) and the cell cycle was synchronized with a double thymidine block as described above. 8 h after the release from the second thymidine block the medium was removed and 80 µL prewarmed DMEM medium (Biochrom, F0475) supplemented with 10% FCS, 2 mM L-Glutamine (Life Technologies, 25030149) and containing 10 µM MG132-Cage, or 10 µM MG132 was added and cells were incubated for 1 h in the dark. Prior to 10 min 405 nm LED irradiation, the MG132-Cage- or MG132-containing medium was removed and 80 µL prewarmed DMEM medium without MG132-Cage or MG132 was added. After another 2 h incubation cells were fixed and stained for F-actin and Hoechst in the chambers.

To determine the number of healthy cells HeLa cells were seeded in DMEM/10%FCS on 18 mm poly-L-lysine coated coverslips. One day later the culture medium was exchanged for fresh DMEM (Biochrom, F0475) supplemented with 10% FCS/2 mM L-Glutamine and containing 10 µM MG132-Cage, 10 µM MG132, 0.1% DMSO, or no additive. Plates were exposed to 405 nm LED light for 10 min or maintained in the dark as described above. Subsequently cells were fixed and stained 0 h, 12 h, 18 h, and 24 h after 405 nm light exposure.

For annexin V and propidium iodide (PI) labeling cells were seeded in 8-well ibidiTreat chambers (Ibidi, 80826) and one day later incubated with 10 µM MG132-Cage, 10 µM MG132, or 0.1% DMSO and exposed for 10 min to 405 nm LED light or maintained in the dark. After 20 h incubation cells were labeled for annexin V, PI and Hoechst in the chambers.

Immunostaining

For immunostaining, coverslips or ibidiTreat chambers were washed once with 1xPBS (Phosphate buffered saline), fixed in 4% PFA/1x PBS (formaldehyde, 28908 Thermo Scientific) for 15 min on ice and blocked with 4% BSA/1x PBS/0.1% Triton™ X-100 at 4 °C over night. The primary anti α-tubulin (1:500, T6199, Sigma) antibody was incubated for 2 h at room temperature (RT) in 4% BSA/1x PBS/0.1% Triton™ X-100. Afterwards, coverslips were washed 3x for 5 min with 1x PBS/0.1% Triton™ X-100. Subsequently, the secondary antibodies Alexa Fluor™ 635 goat anti-mouse (1:500, Life Technologies, A31574), Alexa Fluor™ phalloidin 568 (1:500, Life Technologies, A12380) and Hoechst 33258 (1:1,000, Sigma-Aldrich, 86140) in 4% BSA/1x PBS/0.1% Triton™ X-100 were incubated for 1 h at 22 °C. After incubation, coverslips were washed 3x for 5 min in 1x PBS/0.1% Triton™ X-100, placed on glass slides, covered with mounting medium (1x PBS/4% n-Propyl-Gallate, 90% Glycerol) and stored at -20 °C until imaging.

For annexin V and PI labeling (ab14085, Abcam) the medium was removed 20 h after light exposure and 100 µL 1x Binding Buffer containing 1 µL FITC-annexin V,1 µL PI and 1 µL Hoechst (1:1,000) was added and incubated for 10 min at 22 °C in the dark. Subsequently, cells were fixed with 4% PFA/1x Binding Buffer for 15 minutes on ice and washed once with 1x Binding buffer. Images were acquired by confocal microscopy within 36 h.

Immunoblotting

Cells were seeded in 6-well plates at 40% confluence and one day later 10 µM MG132-Cage, 10 µM MG132, or 0.1% DMSO were added. Plates were irradiated for 10 min with 405 nm LED or maintained in the dark. Cells were collected 14 h after light irradiation in ice-cold PBS, washed 3x with 1x PBS and incubated in RIPA buffer (50

mM Tris-HCl pH=7.5, 150 mM NaCl, 0.5% Na-deoxycholate, 1% Triton X 100, 1% SDS, 1% protease inhibitor cocktail) rotating for 30 min at 4 °C. 2x Sample buffer (0.1M Tris, 4% SDS, 1.4% ß-mercaptoethanol, 20% glycerol, 1% Bromophenol blue) was added, samples were sonicated in a water bath for 20 min, heated at 95 °C for 5 min and resolved on a 10% SDS-page. As primary antibodies mouse anti-actin (1:10,000, #A5316, Sigma) and mouse anti-PARP (1:1,000, #9542, Cell Signaling) were used; and as secondary antibodies HRP-conjugated mouse (1:10,000 for actin, 1:3,000 for PARP, 170-6516; Bio-Rad). Antibody-probed membranes were developed using ECL™ Prime Western Blotting (GE Healthcare, RPN22236) for PARP and ECL™ Western Blotting (GE Healthcare, RPN2106) for actin. Membranes were imaged on a Molecular Imager ChemiDoc™ XRS+ (Bio-Rad).

Resazurin-based cell viability measurements

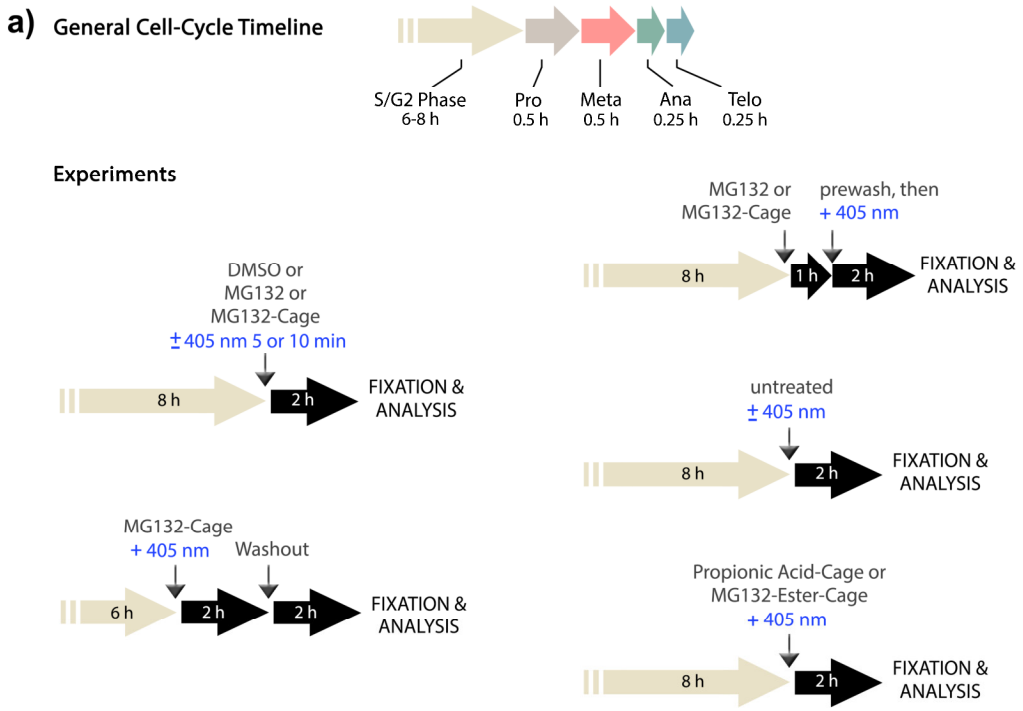
To measure cell viability 15,000 cells per well were seeded into black 96-well plates (655090, Greiner). One day later medium was removed and 100 µL medium containing no additive, 0.1% DMSO, 10 µM MG132-Cage, 10 µM MG132, 10 µM Propionic Acid-Cage, or 10 µM MG132-Ester-Cage were added in triplicates. To correct for background fluorescence medium was added to empty wells. Plates with MG132-Cage, Propionic Acid-Cage, or MG132-Ester-Cage were exposed to 405 nm LED light for 10 min. 28 h after addition of MG132 or light exposure, 10 µL of CellTiter Blue® reagent containing resazurin (G8080, Promega) was added to each well. Plates were shaken for 2 min and centrifuged at 1,000 rpm for 1 min. Fluorescence intensity (excitation 560 nm/emission 590 nm) was measured every 30 min using a Tecan Infinite Pro2000 (bottom reading) until saturation of the signal.[9] For each well the mean background fluorescence intensity for wells without cells was subtracted and for each condition the mean fluorescence intensity for each triplicate was calculated. All values were normalized to the mean fluorescence intensity of control cells.

Microscopy and image analysis

Immunofluorescence images (Figure 3, 4) were acquired on a Leica laser scanning confocal TCS SP5 microscope which was controlled by the Leica Application Suite Software 2.7.2, and equipped with a 63x 1.4-NA Plan-Apochromat oil immersion objective and 405-, 594- and 635-nm lasers. Live-cell movies (Figure 5, S22) and dose-response images (Figure S20a) were acquired on a Nikon eclipse Ti spinning disk confocal microscope.

For live-cell imaging cells were seeded in 6-well plates and the cell cycle was synchronized with a double thymidine block as described above. Cells were transferred into 2-well ibidiTreat chambers (Ibidi, 80286) at the start of the second thymidine block one day before filming. During live-cell imaging cells were maintained at 37 °C in CO_2-independent medium (18045054, Thermo Fischer) containing 10% FCS/1% penicillin/streptomycin/2mM L-Glutamine. Transmission and mKate-α-tubulin live-cell movies were acquired on a Nikon eclipse Ti spinning disk confocal microscopy, which was controlled by the NIS Elements 4.51 software. For transmission images a 470 nm cut-off filter was placed in the light path to exclude the blue parts of the imaging light. The Nikon eclipse was outfitted with a 60x Apo TIRF oil immersion DIC N2 and an Apo LWD 40x WI Lambda S DIC N2 objective and an Andor DU-888 X-11056 camera. Exposure to 405 nm LED light for 5 min was performed on the microscope stage using one LED (either Roithner H2A1-H405 or Thorlabs M405D2) without removing the imaging chamber.

To analyze the mitotic stage and the cell number of the fixed cells, images were randomly acquired on each coverslip. For analyzing the immunofluorescence images and live-cell movies, the open-source software Fiji[10] was used. For all live-cell mKate-α-tubulin images a maximum z-projection of three z-planes 4 µm apart is shown. To quantify the intensities of the PARP and cleaved PARP bands on western blot the Biorad Image Lab™ software (Version 5.2.1) was used. Figures were assembled in Affinity Designer or Photoshop and graphs were plotted with Prism (Graphpad) or Excel (Microsoft).



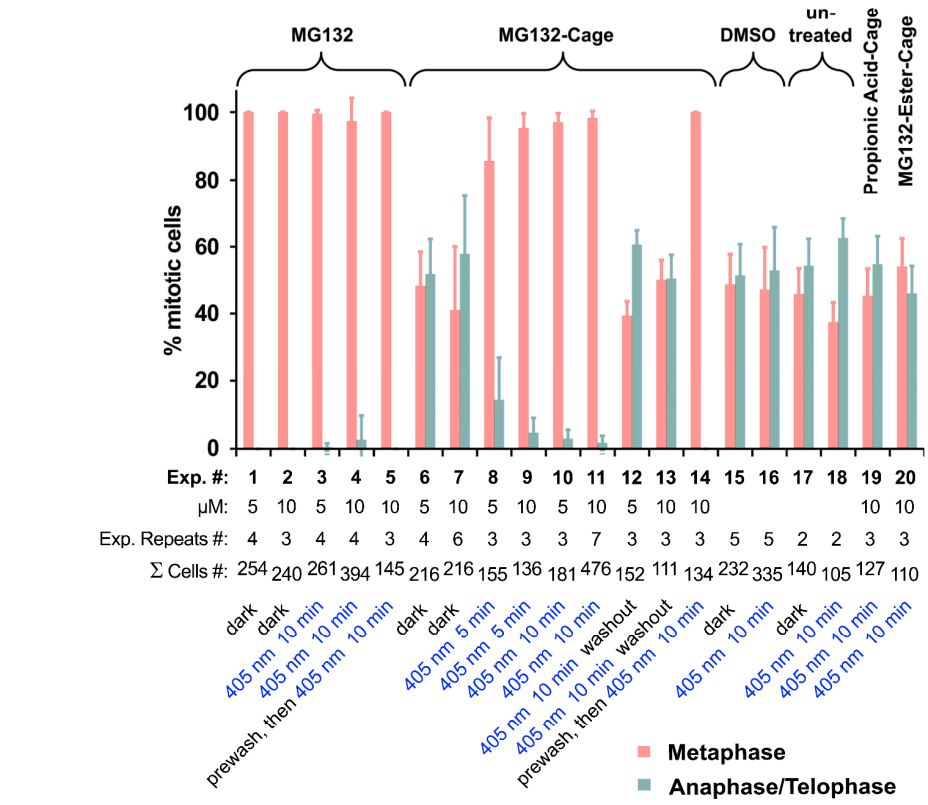


Figure S17 Cell-cycle control with blue light including control experiments. a) Experimental setups to quantify blue-light induced metaphase arrest and control experiments using MG132-Cage, MG132, Propionic Acid-Cage, MG132-Ester-Cage, DMSO, or no additive. b) Mean percentages of mitotic cells in metaphase and ana-/telophase for the indicated conditions. Mean number of independent experiments and the total number of cells analyzed is shown. Error bars represent standard deviation. Experiments # 2, 6, 7, 10, 11, 13, 15, 16, 20 are reproduced from Figure 3d in the main manuscript.

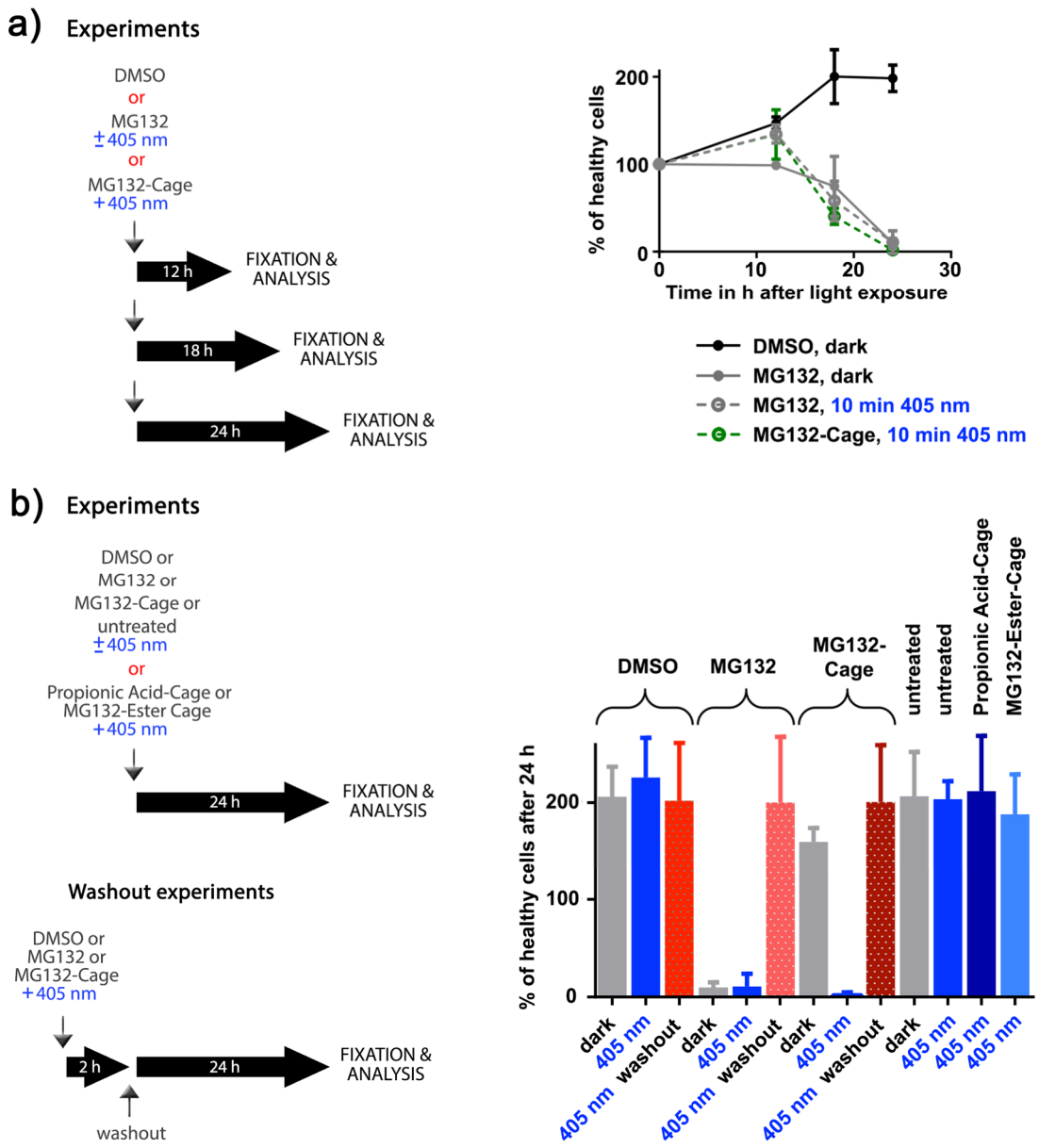


Figure S18 Induction of cell death in HeLa cells with blue light. a) Experimental setups to quantify blue-light induced cell death over time (left). Mean percentages of healthy cells relative to 0 h DMSO-treated cells for the indicated conditions (right). Uncaging of MG132-Cage results in complete cell death 24 hours after irradiation similar to addition of MG132 with or without irradiation. Data of the DMSO control and MG132-Cage with 405 nm are replica taken from Figure 4d in the main manuscript. Mean of 2 independent experiments is shown and error bars represent the standard deviation. b) Quantitative comparison of the % of healthy cells obtained under the indicated experimental conditions after 24 h. An additional washout experiment is included for DMSO, MG132, and MG132-Cage cells in which the washout was performed 2 h after light irradiation. After 24 h the cell number is the same as in the DMSO control showing reversibility of the effect of MG132-Cage unmasking by blue light. Cells were also incubated without any additive in the dark and with 405 nm 10 min light exposure and with 10 µM MG132-Ester-Cage and 10 µM Propionic-Acid-Cage with 405 nm 10 min light exposure. The means of 2-5 independent experiments are shown and between 104 and 782 cell were analyzed for each condition. Error bars represent standard deviation.

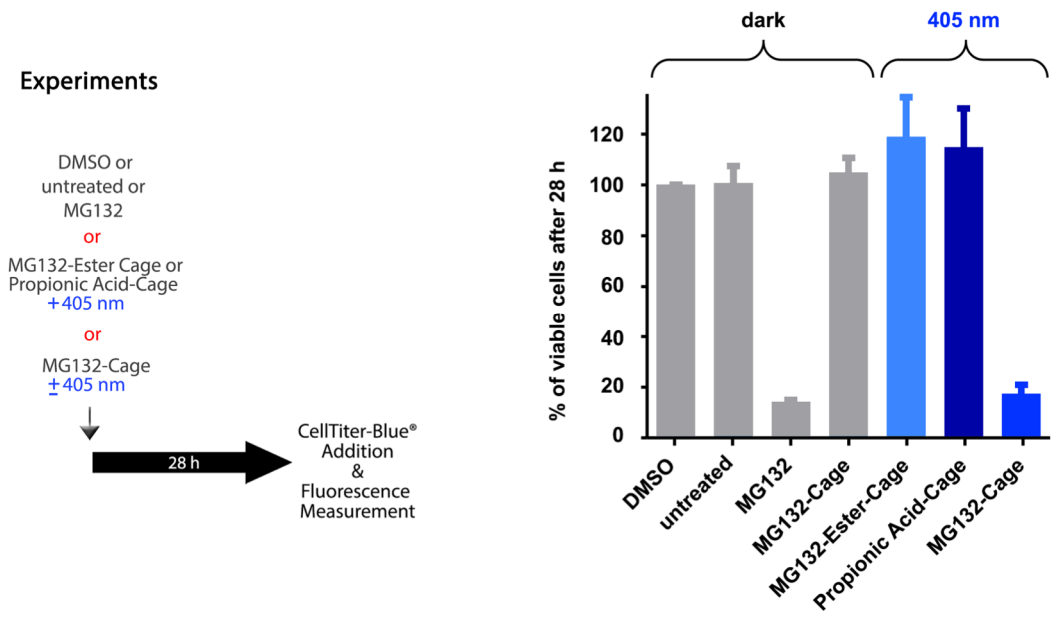


Figure S19 Blue light induced cellular toxicity. Cells were treated with 10 µM solutions of the different compounds as shown on the left, exposed to 405 nm light for 10 min or maintained in the dark, and cultured for 28 h in 96-well plates. After the addition of CellTiter Blue® (resazurin) the fluorescence intensity at 590 nm was measured in triplicate. For each condition the fluorescence intensity was normalized to 0.1% DMSO-treated cells maintained in the dark. Means of 2 independent experiments are shown and error bars represent standard deviation.

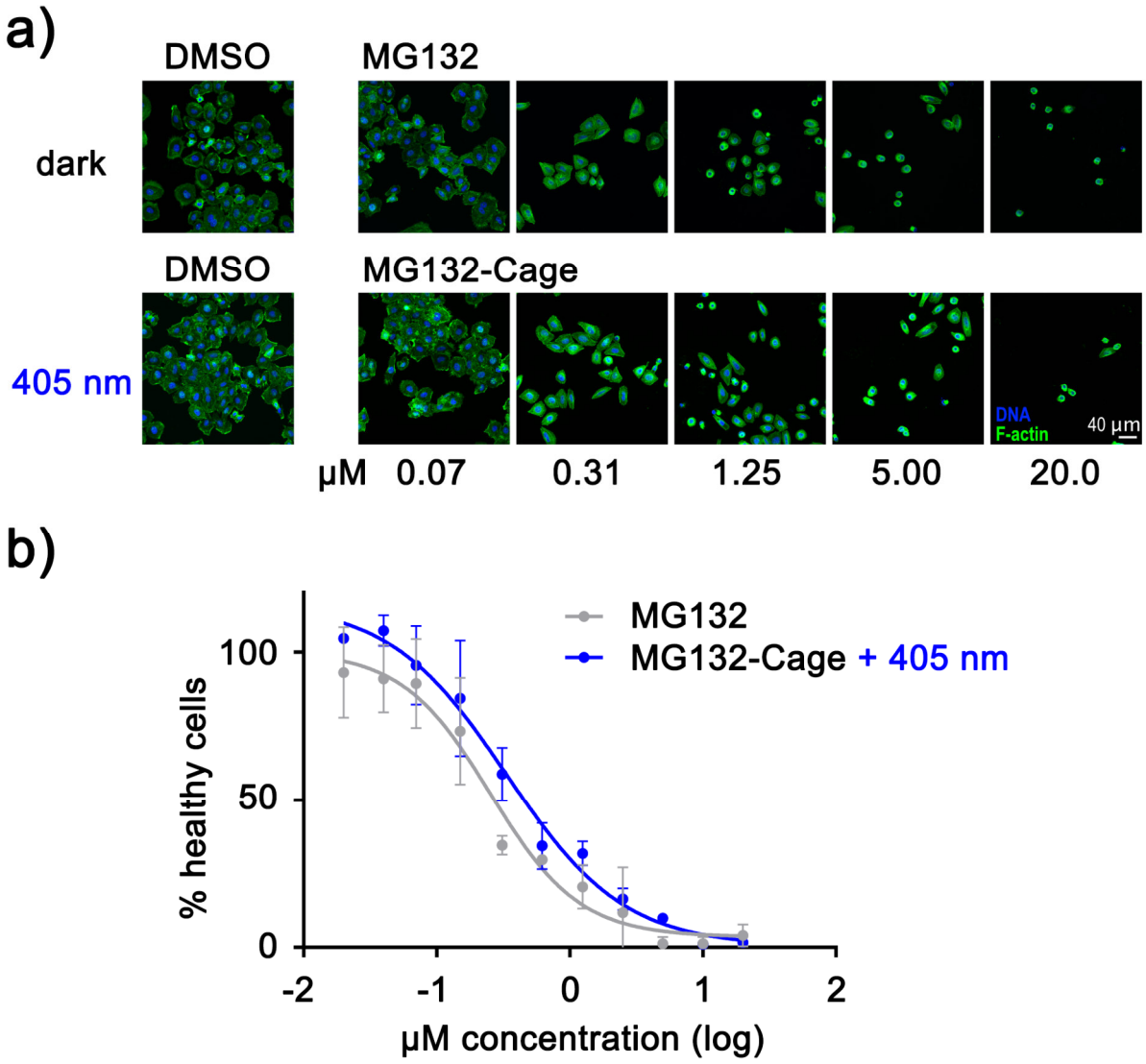


Figure S20 Dose-dependent induction of cell death in MG132-Cage treated cells exposed to 405 nm light for 10 min. a) HeLa cells were incubated in 0.1% DMSO and serial dilutions of MG132 (dark) or MG132-Cage (405 nm 10 min LED light). After 24 h cells were fixed and stained for F-actin (green) and DNA (blue) and the number of healthy cells was determined. Shown are maximum z-projections of confocal images. Scale bar represents 40 µm. b) The % of healthy cells relative to control cells is plotted for the indicated conditions. The means for 3 independent experiments are shown and error bars represent standard deviation.

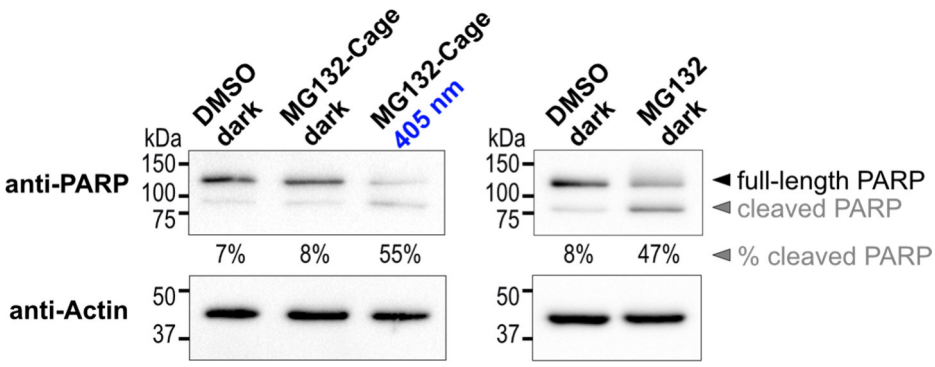


Figure S21 Cells were incubated with 0.1% DMSO, 10 μM MG132-Cage (with and without 405 nm light for 10 min), or 10 μM MG132 for 14 h**.** Immunoblots were probed with anti-actin as a loading control and anti-PARP antibodies. The percentage of cleaved PARP relative to uncleaved PARP is shown for each condition.

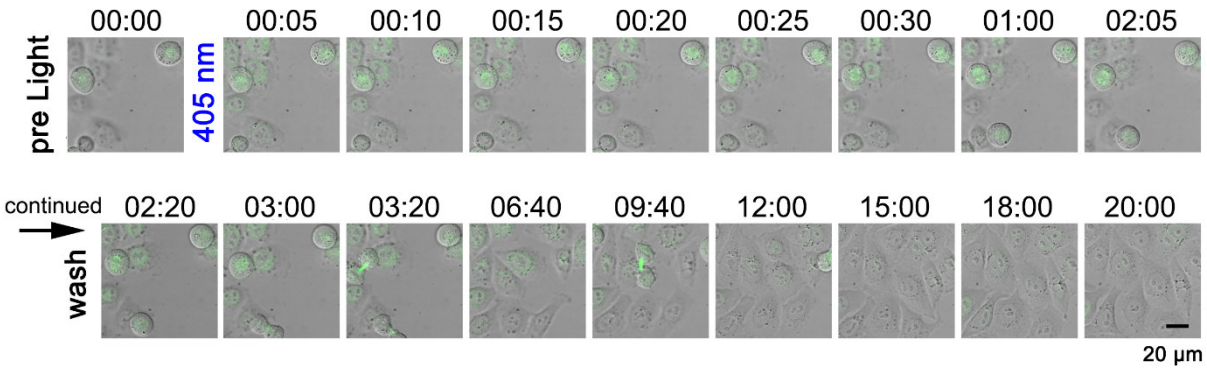


Figure S22 Live-cell imaging of reversible blue light induced metaphase arrest in HeLa cells with greater time resolution as compared to Figure 5c in the main manuscript. Selected time frames are taken from Suppl. Movie 3. Merged transmission and confocal mKate-α-tubulin (maximum z-projection shown in green) images for indicated time points are shown. Prometaphase cells exposed to 405 nm light arrested in metaphase. After 2 h inhibitor washout was performed and the cells continued to proliferate, thus establishing reversibility of the arrest. After 20 h no signs of apoptosis are seen. Scale bar represents 20 μm.

Supplementary Movie figure legends

Supplementary Movie 1 Merged transmission and confocal mKate-α-tubulin (maximum z-projection shown in green) images of HeLa cells incubated with 10 μM MG132-Cage and either exposed to 405 nm light for 5 min (left) or maintained in the dark (right). Images were acquired every 5 min with a 60x 1.45-NA Plan-Apochromat oil immersion objective on Nikon eclipse spinning disc confocal microscope.

Supplementary Movie 2 Merged transmission and confocal mKate-α-tubulin (maximum z-projection shown in green) images of HeLa cells incubated with 10 μM MG132-Cage and either exposed to 405 nm light for 5 min (left) or maintained in the dark (right). Images were acquired every 5 min for 20 h, as described for Suppl. Movie 1. After 1 h displayed time-intervals are increased to every 20 min.

Supplementary Movie 3 Merged transmission and confocal mKate-α-tubulin (maximum z-projection shown in green) images of HeLa cells incubated with 10 μM MG132-Cage and exposed to 405 nm light for 5 min. 2 h after light exposure the inhibitor was washed out and imaging was continued for 20 h. Images were acquired every 5 min for 20:20 (h:min) as described for Suppl. Movie 1. After 5 h displayed time-intervals are increased to every 20 min.

References

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