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Photoactivatable cytotoxic agents derived from mitochondria-targeting luminescent iridium(III) poly(ethylene glycol) complexes modified with a nitrobenzyl linkage

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Electronic Supplementary Information

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General Information

All solvents were of analytical grade and purified according to standard procedures.[1] All buffer components were of biological grade and used as received. Benzyl bromide and acetovanillone were purchased from J&K Scientific. Acetic anhydride, triethylamine, triethylene glycol, 2-phenylquinoline, 4,4'-dimethyl-2,2'-bipyridine, $IrCl_3 \cdot 3H_2O$, poly(ethylene glycol) methyl ether (mPEG), and MTT were purchased from Sigma-Aldrich. Potassium iodide, potassium carbonate, *N*,*N*'-disuccinimidyl carbonate, sodium borohydride, propargyl bromide, potassium hexafluorophosphate, sodium azide, toluene-4-sulfonyl chloride, and trifluoroacetic acid were purchased from Acros. Nitric acid (70%) was supplied by Fisher Scientific. PD-10 size-exclusion columns were received from GE Healthcare. Autoclaved Milli-Q water was used for the preparation of the aqueous solutions. The ligand bpy-CH_2NH_2[2] and the dinuclear iridium(III) precursor complexes $[Ir_2(pq)_4Cl_2]$[3] were synthesized according to literature procedures. HeLa cells were obtained from American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), phenol red-free DMEM, fetal bovine serum (FBS), phosphate-buffered saline (PBS) at pH 7.2, trypsin-EDTA, MitoTracker Deep Red FM, CellROX® Deep Red, and penicillin/streptomycin were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin.

Photoactivation Treatment

HeLa cells were prepared using a conventional trypsinization procedure with trypsin/EDTA and incubated in flat-bottom 96-well culture plates for cell viability assessment. After being rested for 24 h, the cell cultures were washed with PBS to remove non-adherent dead cells (< 5%) and incubated with a culture medium

containing an iridium(III) complex for 1 h at 37°C under a 5% CO_2 atmosphere. The cells were gently washed with PBS prior to further incubation in phenol red-free medium. The cells were then irradiated at 365 nm using a 6 W UV-A lamp (Spectronic) placed at 6 cm above the cell culture for different time intervals. After irradiation, the culture medium was replaced with a fresh growth medium and further incubated of 24 h. The cell viability was assessed using the MTT assay as described below.

Cytotoxicity Assays

MTT assays[4] were conducted in 96-well, flat-bottomed microtiter plates. The cell cultures were treated with the complexes as mentioned above. After the treatments, MTT in PBS (5 mg mL^{-1}, 10 μL) was added to each well and the microtiter plate was incubated for 3 h. The medium was removed carefully and DMSO (200 μL) was added to each well. The absorbance of all the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corporation). The IC_{50} values of the complexes were evaluated based on the percentage cell survival in a dose-dependent manner relative to the controls. The presence of the DMSO did not affect the viability of cells and cytotoxic activity of the complexes in the studies.

Photolysis Studies

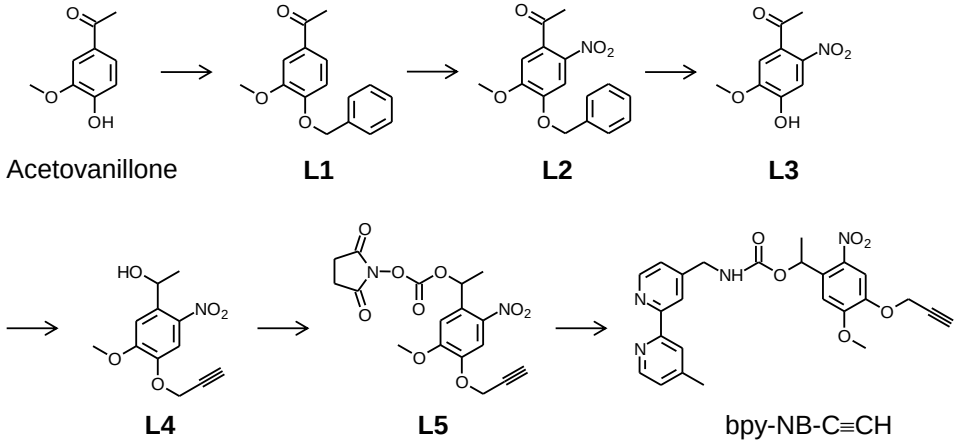
Complex **1** (50 μM) was dissolved in deionized water and irradiated for different time intervals (*t* min) under the same settings used in the biological studies. Photolysis of the complex was monitored using an HPLC system, which was composed of a UV detector (Waters 996 photodiode array) set to monitor at 280 nm, and a Waters 600 pump (Waters Corp.) equipped with a Rheodyne 7725i injector (Rohnert Park) with a

20 μL sample loop. The reverse-phase separation was performed on a C18 column (Grace Alltima^{TM} C18 Rocket^{TM} 53 × 7 mm, 3 μm) using MeOH (85%) with 0.1% TFA as an eluent (flow rate: 1.0 mL min^{-1}).

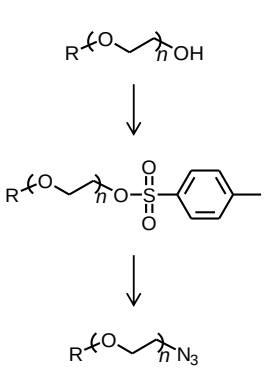
Cellular Uptake Measurements

HeLa cells were grown in a 60 mm tissue culture dish and incubated at 37°C under a 5% CO_2 atmosphere for 48 h. The culture medium was removed and replaced with medium/DMSO (99:1, *v*/*v*) containing the complex at [Ir] = 20 μM. After incubation for 1 h, the medium was removed, and the cell layer was washed gently with PBS (1 mL × 3). Then, the cell layer was trypsinized and digested in 65% HNO_3 (2 mL) at 70 °C for 2 h for ICP-MS (PerkinElmer SCIEX, ELAN DRC Plus) analysis.

Scheme S1 Synthesis of bpy-NB-C≡CH.

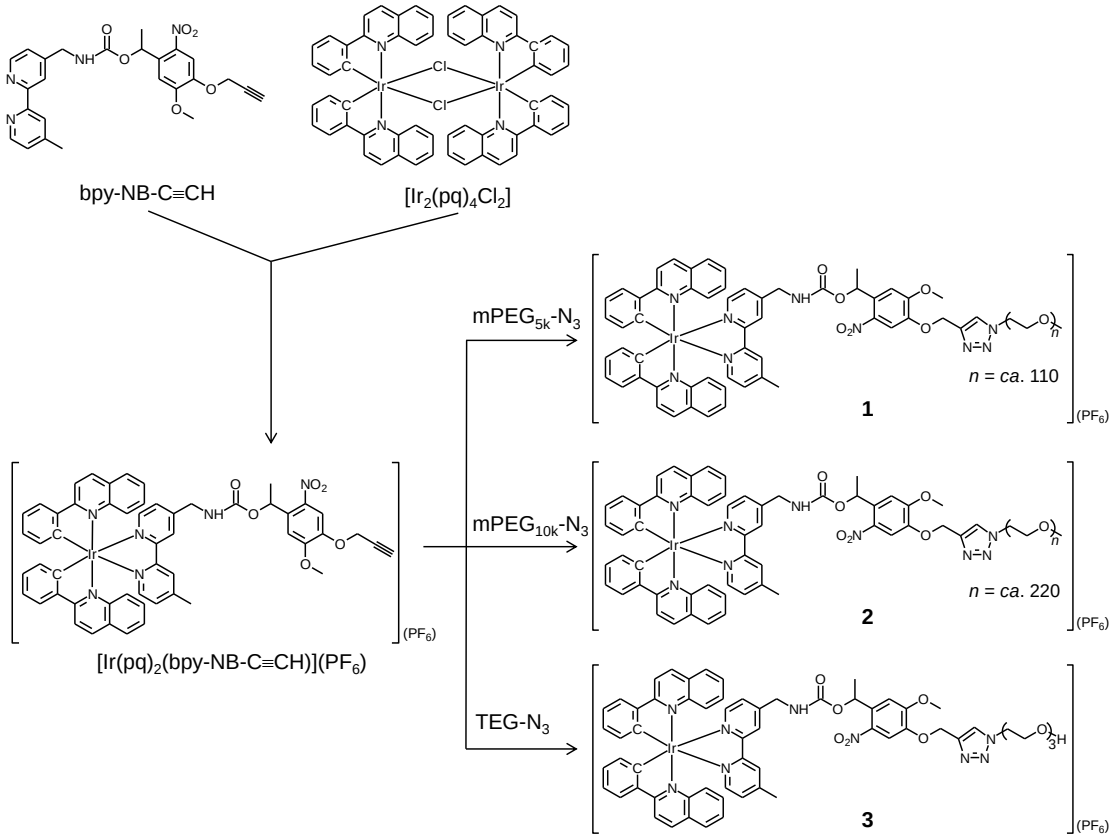


Scheme S2 Synthesis of mPEG-N_3 and TEG-N_3.



mPEG$_{5k}$-OH:	R = CH_3, *n* = *ca*. 110
mPEG$_{10k}$-OH:	R = CH_3, *n* = *ca*. 220
TEG:	R = H, *n* = 3
mPEG$_{5k}$-Ts:	R = CH_3, *n* = *ca*. 110
mPEG$_{10k}$-Ts:	R = CH_3, *n* = *ca*. 220
TEG-Ts:	R = H, *n* = 3
mPEG$_{5k}$-N_3:	R = CH_3, *n* = *ca*. 110
mPEG$_{10k}$-N_3:	R = CH_3, *n* = *ca*. 220
TEG-N_3:	R = H, *n* = 3

Scheme S3 Synthesis of $[Ir(pq)_2(bpy\text{-}NB\text{-}C{\equiv}CH)](PF_6)$ and complexes **1** – **3**.



Synthesis and Characterization

Benzyl 4-acetyl-2-methoxyphenyl ether (L1)

Benzyl bromide (6.20 g, 36.25 mmol) was added to a suspension of acetovanillone (5.01 g, 30.14 mmol), potassium iodide (250 mg, 1.51 mmol), and potassium carbonate (9.10 g, 65.84 mmol) in CH_3CN (70 mL). The pale yellow mixture was heated to reflux under an inert atmosphere of N_2 for 12 h. The suspension was filtered and the filtrate was evaporated to dryness. The yellow crude product was purified by column chromatography on silica gel. The desired product was eluted with *n*-hexane/EtOAc (4:1, *v*/*v*) and finally isolated as a yellow solid. Yield: 7.11 g (92%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.55–7.31 (m, 7H, H on benzyl ring, H3 and H5 of phenyl ring), 6.90 (d, J = 8.0 Hz, 1H, H6 of phenyl ring), 5.21 (s, 2H, CH_2 of benzyl), 3.92 (s, 3H, CH_3 of methoxy), 2.53 ppm (s, 3H, CH_3 of acetyl). Positive-ion ESI-MS ion clusters at *m*/*z* 279 $[M + Na^+]^+$.

Benzyl 4-acetyl-2-methoxy-5-nitrophenyl ether (L2)

L1 (2.10 g, 8.20 mmol) dissolved in acetic anhydride (7 mL) was added dropwise to a solution of 70% nitric acid (47 mL) and acetic anhydride (17 mL) at 0°C. The resultant orange mixture was stirred for 3 h at room temperature. Cold deionized water (300 mL) was added to the mixture, which was then stirred for 2 h at 0°C. The orange-red precipitate was collected by suction filtration and purified by column chromatography on silica gel. The desired product was eluted with *n*-hexane/EtOAc (4:1, *v*/*v*) and finally isolated as a yellow solid. Yield: 1.45 g (58%). ^{1}H NMR (300 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.69 (s, 1H, H6 of phenyl ring), 7.48–7.37 (m, 5H, H on benzyl ring), 6.79 (s, 1H, H3 of phenyl ring), 5.24 (s, 2H, CH_2 of benzyl), 4.02 (s, 3H, CH_3 of methoxy), 2.51 ppm (s, 3H, CH_3 of acetyl).

Positive-ion ESI-MS ion clusters at *m*/*z* 301 $[M + H^+]^+$.

4-Acetyl-2-methoxy-5-nitrophenol (L3)

L2 (500 mg, 1.66 mmol) was dissolved in trifluoroacetic acid (3.35 g, 29.38 mmol) and the yellow solution was stirred under an inert atmosphere of N_2 for 24 h. The solution was evaporated to dryness under reduced pressure. The yellow crude product was dissolved in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (30 mL × 2) and saturated aqueous NaCl (30 mL × 2). The CH_2Cl_2 portion was dried over $MgSO_4$ and the solvent was removed by rotary evaporation. The yellow crude product was purified by column chromatography on silica gel. The desired product was eluted with *n*-hexane/EtOAc (1:1, *v*/*v*) and finally isolated as a yellow solid. Yield: 303 mg (86%). ^{1}H NMR (300 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.69 (s, 1H, H6 of phenyl ring), 6.82 (s, 1H, H3 of phenyl ring), 5.98 (s, OH), 4.04 (s, 3H, CH_3 of methoxy), 2.51 ppm (s, 3H, CH_3 of acetyl). Positive-ion ESI-MS ion clusters at *m*/*z* 234 $[M + Na^+]^+$.

Propargyl 4-(1-hydroxyethyl)-2-methoxy-5-nitrophenyl ether (L4)

Propargyl bromide (110 mg, 0.92 mmol) was added to a white suspension of **L3** (98 mg, 0.46 mmol) and potassium carbonate (140 mg, 1.01 mmol) in CH_3CN (20 mL). The mixture was heated to reflux under an inert atmosphere of N_2 for 2 h. The suspension was filtered by suction filtration and the filtrate was evaporated to dryness under reduced pressure. The yellow crude product was purified by column chromatography on silica gel, and eluted with *n*-hexane/EtOAc (5:1, *v*/*v*) and finally isolated as a pale yellow solid. Yield: 94 mg (82%). Positive-ion ESI-MS ion clusters at *m*/*z* 272 $[M + Na^+]^+$. The solid (94 mg, 0.38 mmol) was dissolved in

THF/MeOH (27 mL, 1:8, *v*/*v*) and cooled at 0°C for 5 min. Sodium borohydride (101 mg, 2.66 mmol) was added to the solution. The mixture was stirred for 2 h at 0°C under an inert atmosphere of N_2. The reaction was quenched by deionized water (5 mL) and the pale yellow crude product was extracted with CH_2Cl_2 (30 mL × 3). The organic CH_2Cl_2 portion was concentrated by rotary evaporation and purified by column chromatography on silica gel. The desired product was eluted with *n*-hexane/EtOAc (5:1, *v*/*v*) and finally isolated as a pale yellow solid. Yield: 85 mg (90%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.75 (s, 1H, H6 of phenyl ring), 7.35 (s, 1H, H3 of phenyl ring), 5.61–5.56 (m, 1H, CH of hydroxyethyl), 4.83 (d, J = 2.4 Hz, 2H, CH_2 of propargyl), 4.01 (s, 3H, CH_3 of methoxy), 2.59 (s, 1H, CH of propargyl), 2.36 (s, 1H, OH), 1.57 ppm (d, J = 6.3 Hz, 3H, CH_3 of hydroxyethyl). Positive-ion ESI-MS ion clusters at *m*/*z* 274 $[M + Na^+]^+$.

1-(5-Methoxy-2-nitro-4-propargyloxyphenyl)ethyl *N*-succinimidyl carbonate (L5)

N,*N*'-Disuccinimidyl carbonate (76 mg, 0.30 mmol), **L4** (15 mg, 0.06 mmol), and triethylamine (31 mg, 0.31 mmol) were dissolved in dry CH_3CN (5 mL). The mixture was then stirred under an inert atmosphere of N_2 for 24 h. The solvent was removed by rotary evaporation. The yellow crude product was purified by column chromatography on silica gel. The desired product was eluted with *n*-hexane/EtOAc (2:1, *v*/*v*) and finally isolated as a yellow solid. Yield: 20 mg (85%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.82 (s, 1H, H3 of phenyl ring), 7.10 (s, 1H, H6 of phenyl ring), 6.53–6.48 (m, 1H, CH of ethyl), 4.84–4.82 (m, 2H, CH_2 of propargyl), 4.06 (s, 3H, CH_3 of methoxy), 2.80 (s, 4H, H on *N*-succinimidyl), 2.60 (s, 1H, CH of propargyl), 1.77 ppm (d, J = 6.4 Hz, 3H, CH_3 of ethyl).

4-(*N*-(1-(5-methoxy-2-nitro-4-propargyloxyphenyl)ethoxy)carbonyl)aminomethyl -4'-methyl-2,2'-bipyridine (bpy-NB-C≡CH)

Triethylamine (64 mg, 0.64 mmol) was added to a solution of **L5** (20 mg, 0.05 mmol) and bpy-CH_2NH_2 (10 mg, 0.05 mmol) in dry DMF (1 mL). The solution was stirred in the dark at room temperature under an inert atmosphere of N_2 for 24 h. The yellow crude product was extracted with CH_2Cl_2 and then purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (20:1, *v*/*v*) and finally isolated as a pale yellow solid. Yield: 19 mg (80%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 8.42 (d, *J* = 5.2 Hz, 1H, H6 of bpy), 8.35 (d, *J* = 5.2 Hz, 1H, H6' of bpy), 8.11 (s, 1H, H3 of bpy), 8.05 (s, 1H, H3' of bpy), 7.61 (s, 1H, H3 of phenyl ring), 7.06–7.00 (m, 3H, H5 and H5' of bpy, H6 of phenyl ring), 6.55–6.53 (m, 1H, NH), 6.29–6.25 (m, 1H, CH of ethyl), 4.67–4.66 (m, 2H, CH_2 of propargyl), 4.24–4.27 (m, 2H, bpy-CH_2), 3.77 (s, 3H, CH_3 of methoxy), 2.81 (s, 3H, CH_3 on bpy), 2.52 (s, 1H, CH of propargyl), 1.47 ppm (d, *J* = 6.4 Hz, 3H, CH_3 of ethoxy). Positive-ion ESI-MS ion clusters at *m*/*z* 499 [M + Na^+]$^+$.

[Ir(pq)$_2$(bpy-NB-C≡CH)](PF$_6$)

A mixture of [$Ir_2(pq)_4Cl_2$] (83 mg, 0.07 mmol) and bpy-NB-C≡CH (62 mg, 0.13 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature under an inert atmosphere of N_2 in the dark for 24 h. Then, KPF_6 (40 mg, 0.2 mmol) was added and the mixture was stirred for 1 h. The solvent was removed by rotary evaporation. The red crude product was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (10:1, *v*/*v*). Subsequent recrystallization of the product from CH_2Cl_2/diethyl ether afforded the complex as an orange solid. Yield:

114 mg (72%). ^{1}H NMR (400 MHz, methanol-d_4, 298 K, relative to TMS): δ = 8.44–8.29 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline of pq), 8.24–8.02 (m, 6H, H3, H3', H6, H5 of bpy and H4 of quinoline of pq), 7.81–7.70 (m, 3H, H8 of quinoline of pq and H6' of bpy), 7.42–7.29 (m, 6H, H5, H7 of quinoline of pq, H3 of nitrophenyl ring and H5' of bpy), 7.18–7.14 (m, 3H, H6 of quinoline of pq and H6 of nitrophenyl ring), 7.03–6.99 (m, 2H, H4 of phenyl ring of pq), 6.81–6.75 (m, 2H, H5 of phenyl ring of pq), 6.52–6.47 (m, 2H, H6 of phenyl ring of pq), 6.27–6.21 (m, 1H, CH on C2 of ethoxy), 4.90–4.86 (m, 2H, CH_2 of propargyl), 4.35–4.22 (m, 2H, bpy-CH_2), 3.86 (s, 1.5 H, methoxy), 3.81 (s, 1.5 H, methoxy), 2.43 (s, 3H, CH_3 on bpy), 1.62 ppm (d, J = 6.4 Hz, 3H, CH_3 of ethoxy). Positive-ion ESI-MS ion clusters at m/z 1077 $[M - PF_6^-]^+$.

α-Tosyl-ω-methoxypoly(ethylene glycol), 5 kDa (mPEG$_{5k}$-Ts)

To a solution of poly(ethylene glycol) methyl ether (mPEG$_{5k}$-OH) (1.0 g, 0.20 mmol) in THF (10 mL) at 0°C, sodium hydride (15 mg, 0.63 mmol) was added. The suspension was stirred for 5 min and *p*-toluenesulfonyl chloride (115 mg, 0.60 mmol) was added to the suspension. The mixture was stirred at room temperature under an inert atmosphere of N_2 for 48 h. The solvent was removed by rotary evaporation. The monotosylated poly(ethylene glycol) (mPEG$_{5k}$-Ts) was purified by column chromatography on silica gel. mPEG$_{5k}$-Ts was eluted with CH_2Cl_2/MeOH (10:1, *v*/*v*) and finally isolated as a white solid. Yield: 721 mg (70%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.78 (d, J = 6.4 Hz, 2H, H3 and H5 of toluene-4-sulfonyl), 7.34 (d, J = 8.0 Hz, 2H, H2 and H6 of toluene-4-sulfonyl), 4.15–4.12 (t, J = 4.8 Hz, 2H, OCH_2), 3.81–3.79 (t, J = 4.8 Hz, 2H, OCH_2), 3.50–3.70 (m, ca. 475H, H of PEG), 3.43–3.46 (t, J = 4.8 Hz, 2H, OCH_2), 3.36 (s, 3H, CH_3 of PEG),

2.43 ppm (s, 3H, CH_3 of toluene).

α-Azido-ω-methoxypoly(ethylene glycol), 5 kDa ($mPEG_{5k}$-N_3)

Sodium azide (46 mg, 0.71 mmol) was added to a solution of the $mPEG_{5k}$-Ts (711 mg, 0.14 mmol) in dry DMF (5 mL). The mixture was heated to reflux under an inert atmosphere of N_2 for 48 h. After the removal of DMF by vacuum distillation, the white crude product was dissolved in CH_2Cl_2 and the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure and the white solid was dissolved in deionized water (2 mL), and loaded onto a PD-10 size-exclusion column that had been equilibrated with deionized water. The desired product was collected and immediately lyophilized to yield a white solid. Yield: 500 mg (70%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ 3.83–3.80 (m, 2H, OCH_2), 3.70–3.64 (m, ca. 475H, H of PEG), 3.56–3.54 (m, 2H, OCH_2), 3.47–3.45 (m, 2H, OCH_2), 3.40–3.37 ppm (m, 5H, OCH_2, CH_3 of PEG). MALDI-TOF-MS: M_n = 4948.02, M_w = 5004.93, PDI = 1.012.

α-Azido-ω-methoxypoly(ethylene glycol), 10 kDa ($mPEG_{10k}$-N_3)

$mPEG_{10k}$-N_3 was synthesized similarly according to the synthetic procedure of $mPEG_{5k}$-N_3 except that $mPEG_{10k}$-OH (1.0 g, 0.10 mmol) was used instead of $mPEG_{5k}$-OH, and $mPEG_{5k}$-Ts (430 mg, 0.04 mmol) was replaced by $mPEG_{10k}$-Ts. $mPEG_{10k}$-Ts: Yield: 570 mg (57%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.78 (d, J = 6.4 Hz, 2H, H3 and H5 of toluene-4-sulfonyl), 7.34 (d, J = 8.0 Hz, 2H, H2 and H6 of toluene-4-sulfonyl), 4.15–4.12 (t, J = 4.8 Hz, 2H, OCH_2), 3.82–3.37 (m, ca. 910H, H of PEG), 3.43–3.46 (t, J = 4.8 Hz, 2H, OCH_2), 3.37 (s, 3H, CH_3 of PEG), 2.45 ppm (s, 3H, CH_3 of toluene-4-sulfonyl).

$mPEG_{10k}$-N_3: Yield: 180 mg (42%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ 3.89–3.80 (m, 2H, OCH_2), 3.70–3.37 ppm (m, ca. 910H, H of PEG). MALDI-TOF-MS: M_n = 9774.79, M_w = 9792.95, PDI = 1.002.

8-Tosyl-3,6-dioxaoctanol (TEG-Ts)

To a solution of triethylene glycol (TEG) (1.4 g, 9.59 mmol) in CH_3CN (10 mL), *p*-toluenesulfonyl chloride (833 mg, 4.36 mmol) and triethylamine (441 mg, 4.36 mmol) were added. The resulting solution was stirred under an inert atmosphere of N_2 for 24 h. The solvent was removed by rotary evaporation. The monotosylated triethylene glycol (TEG-Ts) was purified by column chromatography on silica gel. TEG-Ts was eluted with CH_2Cl_2/MeOH (10:1, *v*/*v*) and finally isolated as a colorless oil. Yield: 2.0 g (68%). ^{1}H NMR (300 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 7.75 (d, *J* = 6.4 Hz, 2H, H3 and H5 of toluene-4-sulfonyl), 7.28 (d, *J* = 8.0 Hz, 2H, H2 and H6 of toluene-4-sulfonyl), 5.26–5.22 (m, 1H, OH), 4.12–4.09 (m, 2H, OCH_2), 3.72–3.48 (m, 10H, OCH_2), 3.36 (s, 3H, CH_3 of PEG), 2.35 ppm (s, 3H, CH_3 of toluene). Positive-ion ESI-MS ion clusters at *m*/*z* 327 $[M + Na^+]^+$.

8-Azido-3,6-dioxaoctanol (TEG-N_3)

Sodium azide (3 mg, 0.04 mol) was added to a solution of TEG-Ts (2.0 g, 6.56 mmol) in dry DMF (3 mL). The mixture was heated to reflux under an inert atmosphere of N_2 for 48 h. After removal of DMF by vacuum distillation, the oily crude product was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (10:1, *v*/*v*) and finally isolated as a colorless oil. Yield: 1.1 g (95%). ^{1}H NMR (400 MHz, chloroform-*d*, 298 K, relative to TMS): δ = 4.28–4.25 (m, 1H, OH), 3.68–3.63 (m, 8H, OCH_2), 3.56–3.51 (m, 2H, OCH_2), 3.40–3.36

ppm (m, 2H, OCH_2). Positive-ion ESI-MS ion clusters at *m*/*z* 198 $[M + Na^+]^+$.

$[Ir(pq)_2$(bpy-NB-PEG5k)$](PF_6)$ (1)

A solution of ascorbic acid (14 mg, 0.08 mmol) and anhydrous copper(II) sulfate (7 mg, 0.04 mmol) in H_2O (1 mL) was added to a solution of $[Ir(pq)_2$(bpy-NB-C≡CH)$](PF_6)$ (27 mg, 0.02 mmol) and $mPEG_{5k}$-N_3 (100 mg, 0.02 mmol) in MeOH (5 mL). The resulting solution was stirred at room temperature under an inert atmosphere of N_2 in the dark for 24 h. The solvent was removed by rotary evaporation. The orange crude product was dissolved in CH_2Cl_2 and the solution was filtered. The filtrate was evaporated to dryness under reduced pressure and the orange solid was dissolved in deionized water (1.5 mL), and loaded onto a PD-10 size-exclusion column that had been equilibrated with deionized water. The desired product was collected and purified using a Centricon-10 ultrafiltration device. The orange residue was immediately lyophilized to yield an orange solid. Yield: 77 mg (63%). ^{1}H NMR (400 MHz, methanol-d_4, 298 K, relative to TMS): δ = 8.40–8.33 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline of pq), 8.25–8.05 (m, 6H, H3, H3', H6, H5 of bpy and H4 of quinoline of pq), 7.84–7.76 (m, 3H, H8 of quinoline of pq and H6' of bpy), 7.45–7.29 (m, 6H, H5, H7 of quinoline of pq, H3 of nitrophenyl ring and H5' of bpy), 7.19–7.13 (m, 3H, H6 of quinoline of pq and H6 of nitrophenyl ring), 7.04–7.00 (m, 2H, H4 of phenyl ring of pq), 6.82–6.77 (m, 2H, H5 of phenyl ring of pq), 6.51–6.47 (m, 2H, H6 of phenyl ring of pq), 6.24–6.21 (m, 1H, CH on C2 of ethoxy), 5.31 (s, 2H, CH_2 of propargyl), 4.36–4.29 (m, 2H, bpy-CH_2), 3.87–3.80 (m, 5H, OCH_2 of TEG and CH_3 of methoxy), 3.64–3.36 (m, ca. 475H, H of PEG), 2.44 (s, 3H, CH_3 on bpy), 1.62 ppm (d, *J* = 6.4 Hz, 3H, CH_3 of ethoxy). IR (KBr): ν = 3432 (br, N–H), 1466 (m, N–O), 1342 (m, N–O), 1110 (s, C–O), 842 cm^{-1}

(s, PF_6^-). MALDI-TOF-MS: M_n = 5996.86, M_w = 6073.06, PDI = 1.013.

[Ir(pq)$_2$(bpy-NB-PEG10k)](PF$_6$) (2)

Complexes **2** was synthetized similarly according to the synthetic procedure of complex **1** except that mPEG$_{10k}$-N$_3$ (100 mg, 0.01 mmol) was used instead of mPEG$_{5k}$-N$_3$. Yield: 78 mg (70%). ^{1}H NMR (400 MHz, methanol-d_4, 298 K, relative to TMS): δ = 8.43–8.36 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline of pq), 8.25–8.08 (m, 6H, H3, H3', H6, H5 of bpy and H4 of quinoline of pq), 7.87–7.77 (m, 3H, H8 of quinoline of pq and H6' of bpy), 7.42–7.30 (m, 6H, H5, H7 of quinoline of pq, H3 of nitrophenyl ring and H5' of bpy), 7.21–7.15 (m, 3H, H6 of quinoline of pq and H6 of nitrophenyl ring), 7.08–7.02 (m, 2H, H4 of phenyl ring of pq), 6.83–6.75 (m, 2H, H5 of phenyl ring of pq), 6.52–6.49 (m, 2H, H6 of phenyl ring of pq), 6.26–6.22 (m, 1H, CH on C2 of ethoxy), 5.29 (s, 2H, CH_2 of propargyl), 4.35–4.22 (m, 2H, bpy-CH_2), 3.89–3.37 (m, ca. 910H, H of PEG and CH_3 of methoxy), 2.43 (s, 3H, CH_3 on bpy), 1.62 ppm (d, J = 6.4 Hz, 3H, CH_3 of ethoxy). IR (KBr): ν = 347 (br, N–H), 1466 (m, N–O), 1342 (m, N–O), 1110 (s, C–O), 842 cm^{-1} (s, PF_6^-). MALDI-TOF-MS: M_n = 10781.10, M_w = 10788.95, PDI = 1.001.

[Ir(pq)$_2$(bpy-NB-TEG)](PF$_6$) (3)

Complexes **3** was synthetized similarly according to the synthetic procedure of complex **1** except that TEG-N$_3$ (9 mg, 0.05 mmol) was used instead of mPEG$_{5k}$-N$_3$. Yield: 52 mg (75%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, relative to TMS): δ = 8.39–8.31 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline of pq), 8.21–8.03 (m, 6H, H3, H3', H6, H5 of bpy and H4 of quinoline of pq), 7.83–7.73 (m, 3H, H8 of quinoline of pq and H6' of bpy), 7.44–7.30 (m, 6H, H5, H7 of quinoline of pq, H3 of

nitrophenyl ring and H5' of bpy), 7.19–7.13 (m, 3H, H6 of quinoline of pq and H6 of nitrophenyl ring), 7.06–7.00 (m, 2H, H4 of phenyl ring of pq), 6.83–6.75 (m, 2H, H5 of phenyl ring of pq), 6.52–6.47 (m, 2H, H6 of phenyl ring of pq), 6.26–6.22 (m, 1H, CH on C2 of ethoxy), 5.30 (m, 2H, CH_2 of propargyl), 4.35–4.22 (m, 2H, bpy-CH_2), 3.99–3.37 (m, 5H, OCH_2 of TEG and CH_3 of methoxy), 3.63–3.46 (m, 10H, OCH_2 of TEG), 2.43 (s, 3H, CH_3 on bpy), 1.62 ppm (d, J = 6.4 Hz, 3H, CH_3 of ethoxy). IR (KBr): ν = 3424 (br, N–H), 1517 (s, N–O), 1339 (m, N–O), 1275 (s, C–O), 845 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion clusters at m/z 1251 $[M - PF_6^-]^+$. Anal. Calc. for $C_{61}H_{57}N_9O_9PF_6Ir \cdot CH_3CN \cdot 1.5(CH_3CH_2)_2O$: C, 53.48; H, 4.88; N, 9.04. Found: C, 53.12; H, 5.23; N, 9.35%.

$[Ir(pq)_2(bpy\text{-}CH_2NH_2)](PF_6)$ (4)

A mixture of $[Ir_2(pq)_4Cl_2]$ (46 mg, 0.04 mmol) and bpy-CH_2NH_2 (12 mg, 0.06 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature under an inert atmosphere of N_2 in the dark for 12 h. Then KPF_6 (70 mg, 0.38 mmol) was added and the mixture was stirred for 1 h. The solvent was removed by rotary evaporation. The red crude product was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (10:1, v/v). Subsequent recrystallization of the product from CH_2Cl_2/diethyl ether afforded the complex as orange crystals. Yield: 45 mg (80%). ^{1}H NMR (400 MHz, methanol-d_4, 298 K, relative to TMS): δ = 8.43–8.37 (m, 4H, H3 of phenyl ring of pq and H3 of quinoline of pq), 8.30 (s, 1H, H3 of bpy), 8.20–8.16 (m, 4H, H3', H5 of bpy and H4 of quinoline of pq), 8.10 (d, J = 5.6 Hz, 1H, H6 of bpy), 7.84 (d, J = 8.4 Hz, 2H, H8 of quinoline of pq), 7.52 (d, J = 5.6 Hz, 1H, H6' of bpy), 7.44–7.37 (m, 5H, H5 and H7 of quinoline of pq, H5' of bpy), 7.19–7.15 (m, 2H, H6 of quinoline of pq), 7.07–7.03 (m, 2H, H4 of phenyl ring of pq),

6.82–6.79 (m, 2H, H5 of phenyl ring of pq), 6.53–6.49 (m, 2H, H6 of phenyl ring of pq), 3.92 (s, 2H, bpy-CH_2), 2.46 ppm (s, 3H, CH_3 on bpy). IR (KBr): ν = 3422 (br, N–H), 1605 (m, N–H), 845 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion clusters at *m*/*z* 800 $[M - PF_6^-]^+$. Anal. Calc. for $C_{42}H_{33}N_5PF_6Ir \cdot CH_3CN \cdot (CH_3CH_2)_2O$: C, 54.38; H, 4.37; N, 7.93. Found: C, 54.09; H, 4.41; N, 8.17%.

Fig. S1 Comparison of the ^{1}H NMR spectra of [Ir(pq)$_2$(bpy-NB-C≡CH)](PF$_6$) (black), complexes **1** (green), **2** (magenta), and **3** (red).

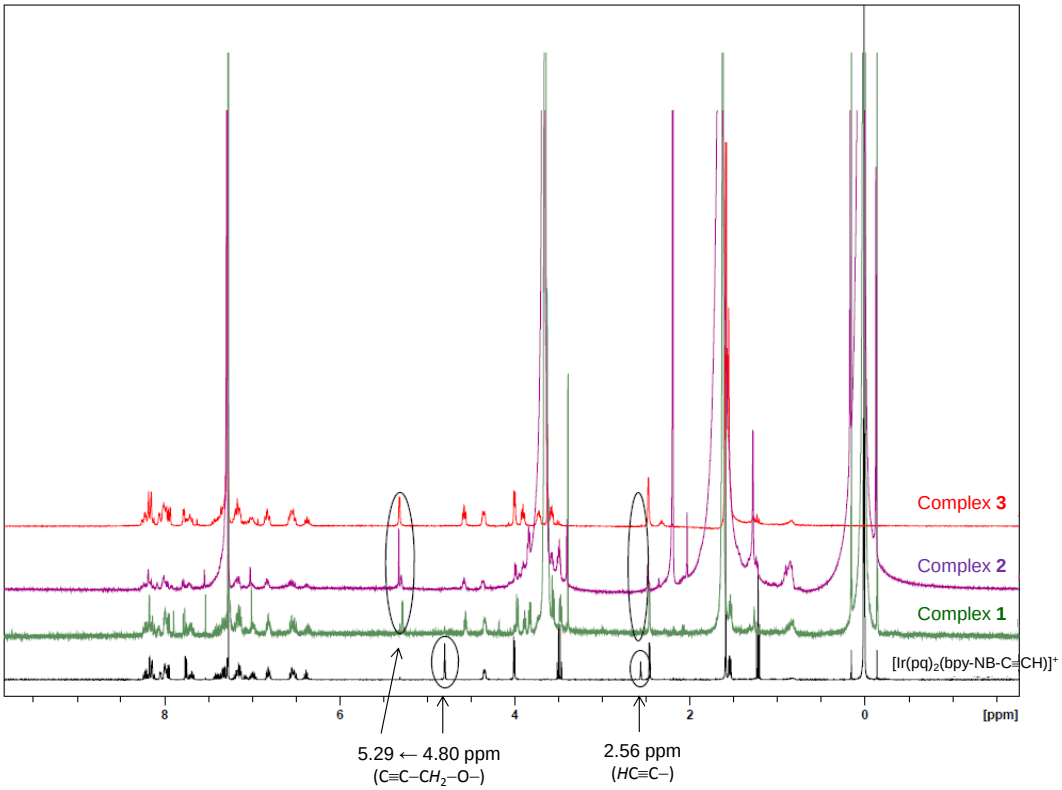


Fig. S2 Electronic absorption spectra of complex **1** in CH_2Cl_2 (solid) and CH_3CN (dashed) at 298 K.

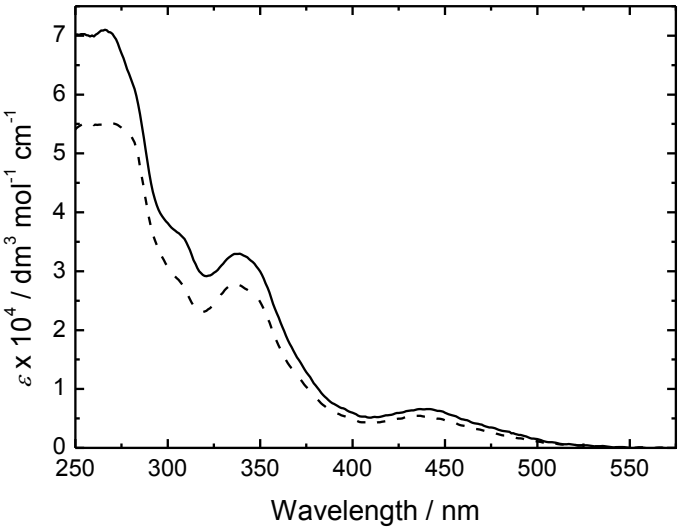


Fig. S3 Electronic absorption spectra of complex **2** in CH_2Cl_2 (solid) and CH_3CN (dashed) at 298 K.

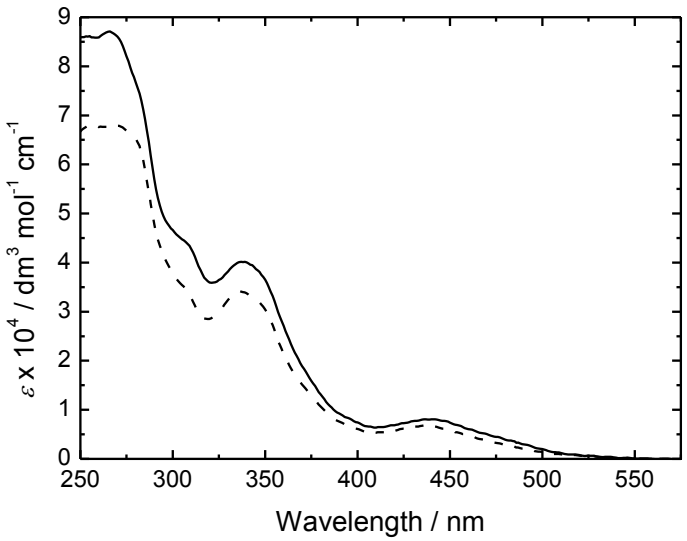


Fig. S4 Electronic absorption spectra of complex **3** in CH_2Cl_2 (solid) and CH_3CN (dashed) at 298 K.

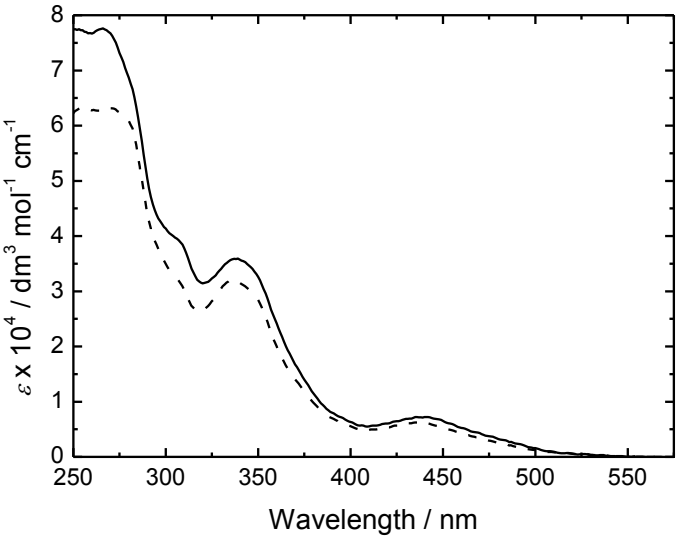


Fig. S5 Electronic absorption spectra of complex **4** in CH_2Cl_2 (solid) and CH_3CN (dashed) at 298 K.

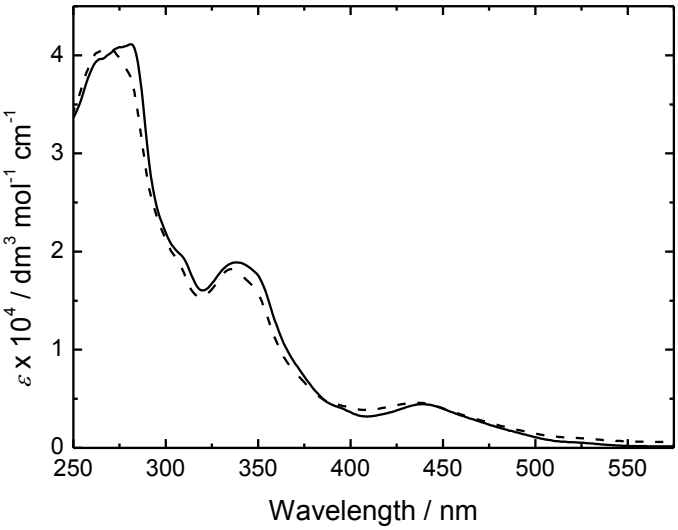


Fig. S6 Emission spectra of complex **1** in degassed CH_2Cl_2 (red), CH_3CN (blue), and buffer (green) at 298 K.

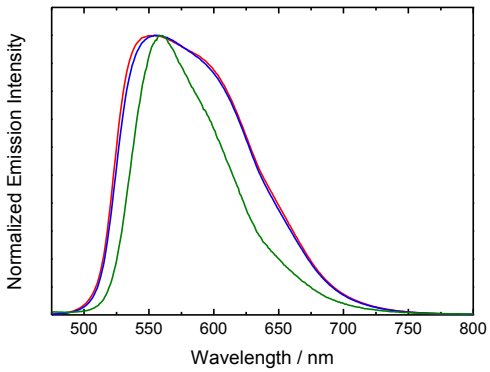


Fig. S7 Emission spectra of complex **2** in degassed CH_2Cl_2 (red), CH_3CN (blue), and buffer (green) at 298 K.

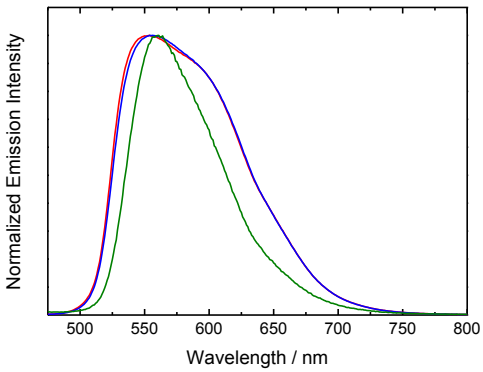


Fig. S8 Emission spectra of complex **3** in degassed CH_2Cl_2 (red) and CH_3CN (blue) at 298 K.

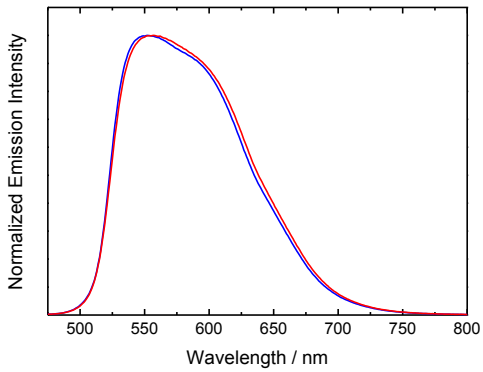


Fig. S9 Emission spectra of complex **4** in degassed CH_2Cl_2 (red) and CH_3CN (blue) at 298 K.

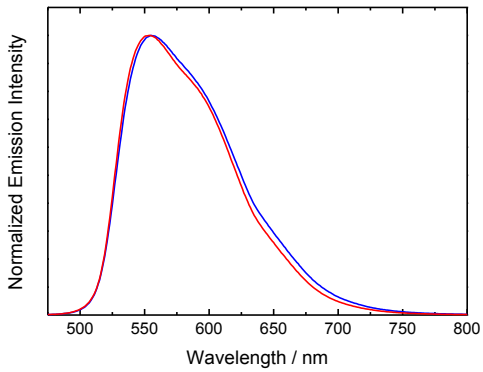


Fig. S10 HPLC chromatograms showing the conversion of complex **1** into complex **4** upon irradiation for different time periods (*t*).

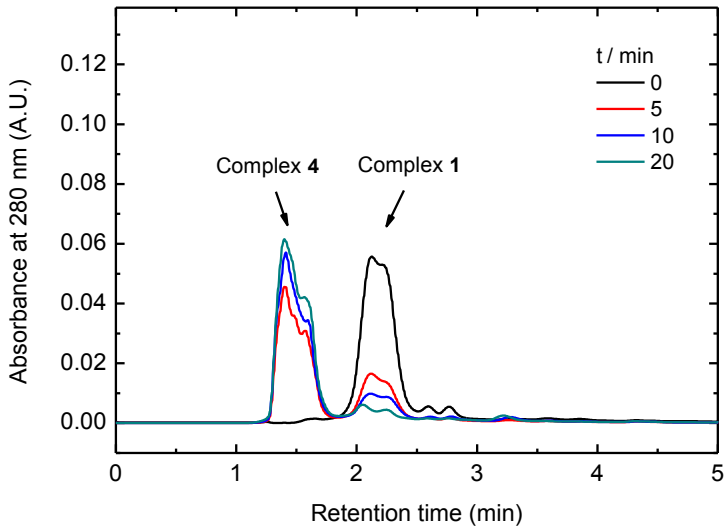


Fig. S11 Viability of HeLa cells upon treatment with complex **4** in the dark for 1 h, followed by irradiation at 365 nm for 0 (black), 5 (blue), 10 (yellow), and 20 min (red).

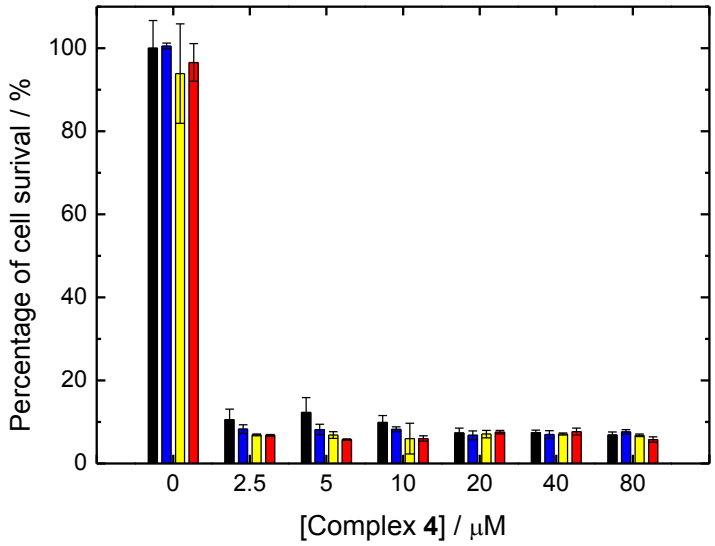


Fig. S12 Viability of HeLa cells upon treatment with complex **5** in the dark for 1 h, followed by irradiation at 365 nm for 0 (black), 5 (blue), 10 (yellow), and 20 min (red).

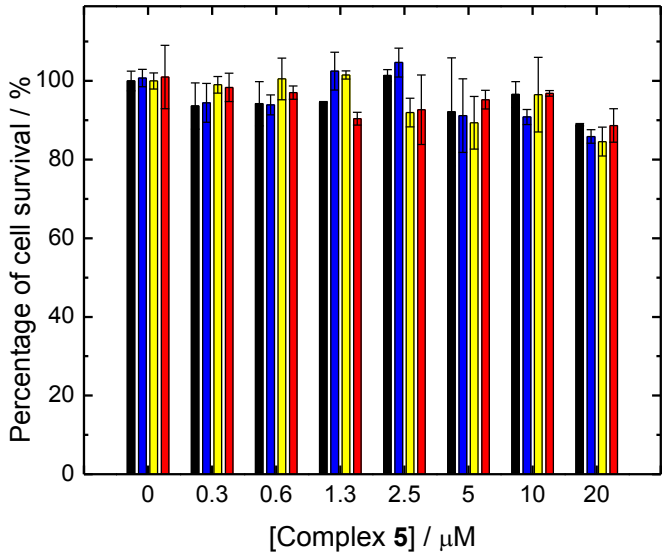
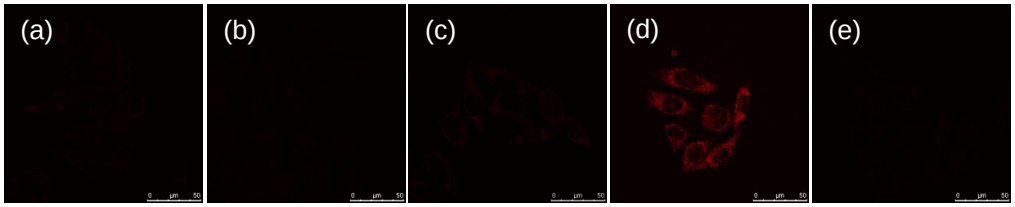


Fig. S13 Detection of oxidative stress using CellROX® Deep Red. HeLa cells were treated with (a) complex **1** (20 μM, 1 h, 37°C) and (b) complex **5** (20 μM, 1 h, 37°C), and the cells were irradiated for 10 min. (c) HeLa cells were irradiated for 10 min in the absence of any complex. (d) HeLa cells were treated with H_2O_2 (positive control; 100 μM, 1 h, 37°C). (e) Untreated HeLa cells were used as a negative control for basal level of fluorescence.



	(a)	(b)	(c)	(d)	(e)
Irradiation	+	+	+	−	−
Complex **1**	+	−	−	−	−
Complex **5**	−	+	−	−	−
H_2O_2	−	−	−	+	−

Fig. S14 Laser-scanning confocal microscopy images of HeLa cells upon incubation with complexes **1**, **2**, and **4** (1 h, λ_{ex} = 405 nm), and then by MitoTracker Deep Red FM (100 nM, 20 min, λ_{ex} = 635 nm) at 37°C.

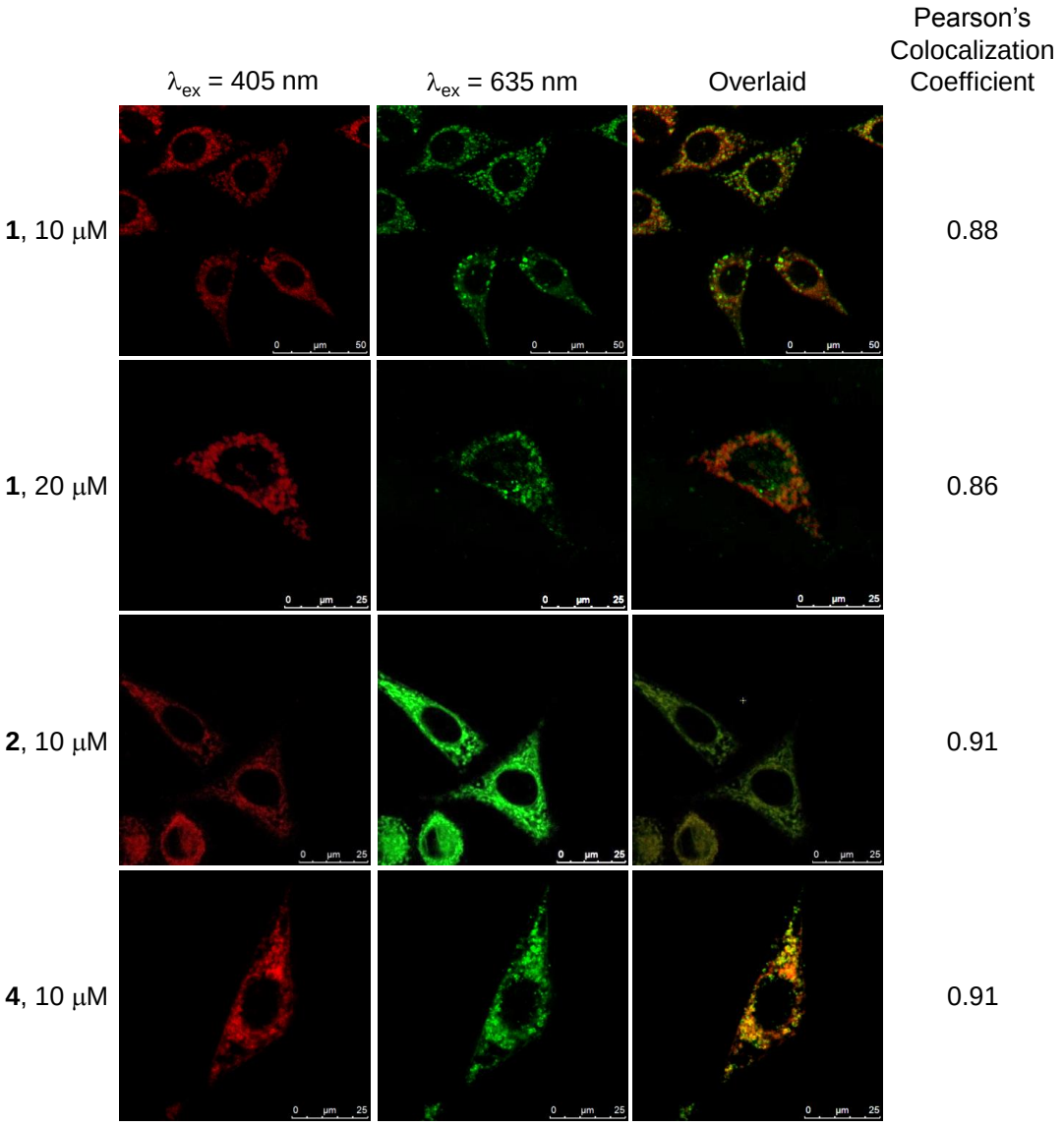


Table S1 Electronic absorption spectral data of complexes **1** – **4** at 298 K.

Complex	Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1	CH_2Cl_2	255 (70 265), 266 (70 925), 283 sh (59 780), 307 sh (35 840), 338 (32 980), 349 sh (30 405), 440 (6600)
	CH_3CN	258 (54 570), 271 (54 635), 280 sh (52 880), 306 sh (28 355), 335 (27 635), 348 sh (25 275), 437 (5435)
2	CH_2Cl_2	255 (86 160), 266 (86 775), 283 sh (72 960), 307 sh (43 780), 338 (40 160), 349 sh (37 040), 440 (8045)
	CH_3CN	258 (67 730), 271 (67 850), 280 sh (65 315), 306 sh (35 085), 335 (34 080), 348 sh (31 450), 437 (5740)
3	CH_2Cl_2	255 (77 280), 266 (77 420), 283 sh (65 165), 307 sh (33 460), 338 (30 930), 349 sh (28 500), 440 (6210)
	CH_3CN	258 (62 930), 271 (63 160), 280 sh (60 840), 306 sh (32 440) , 335 (31 970), 348 sh (29 400), 437 (6250)
4	CH_2Cl_2	263 sh (39 250), 273 (40 680), 281 (41 140), 306 sh (19 850), 337 (18 680), 350 sh (17 560), 438 (4330)
	CH_3CN	259 sh (39 265), 269 (40 340), 280 sh (38 200), 305 sh (19 490) , 335 (17 850), 350 sh (15 380), 438 (4120)

Table S2 Photophysical data of complexes **1** – **4** at 298 K.

Complex	Medium	λ_{em}/nm	τ_o/μs	Φ_{em}
1	CH_2Cl_2	551, 602 sh	2.42	0.29
	CH_3CN	555, 603 sh	2.44	0.28
	Buffer[a]	560, 605 sh	0.84	0.01
2	CH_2Cl_2	551, 602 sh	2.15	0.25
	CH_3CN	555, 603 sh	2.40	0.27
	Buffer[a]	560, 605 sh	0.82	0.01
3	CH_2Cl_2	555, 588 sh	2.41	0.30
	CH_3CN	551, 601 sh	2.33	0.28
4	CH_2Cl_2	552, 594 sh	2.19	0.29
	CH_3CN	555, 603 sh	1.06	0.22

[a] 50 mM potassium phosphate buffer pH 7.4.

Table S3 Cellular uptake of complexes **1** – **5** towards HeLa cells.

Complex	Amount of complex/fmol[a]
1	1.58 ± 0.11
2	1.12 ± 0.45
3	1.57 ± 0.01
4	10.65 ± 1.15
5	1.35 ± 0.24

[a] Amounts of iridium associated with an average HeLa cell upon incubation with the complexes for 1 h at 37°C, as determined by ICP-MS.

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