Electronic Supplementary Material (ESI) for Chemical Communications

Cyclometalated iridium(III) polypyridine dibenzocyclooctyne complexes as the first phosphorescent bioorthogonal probes

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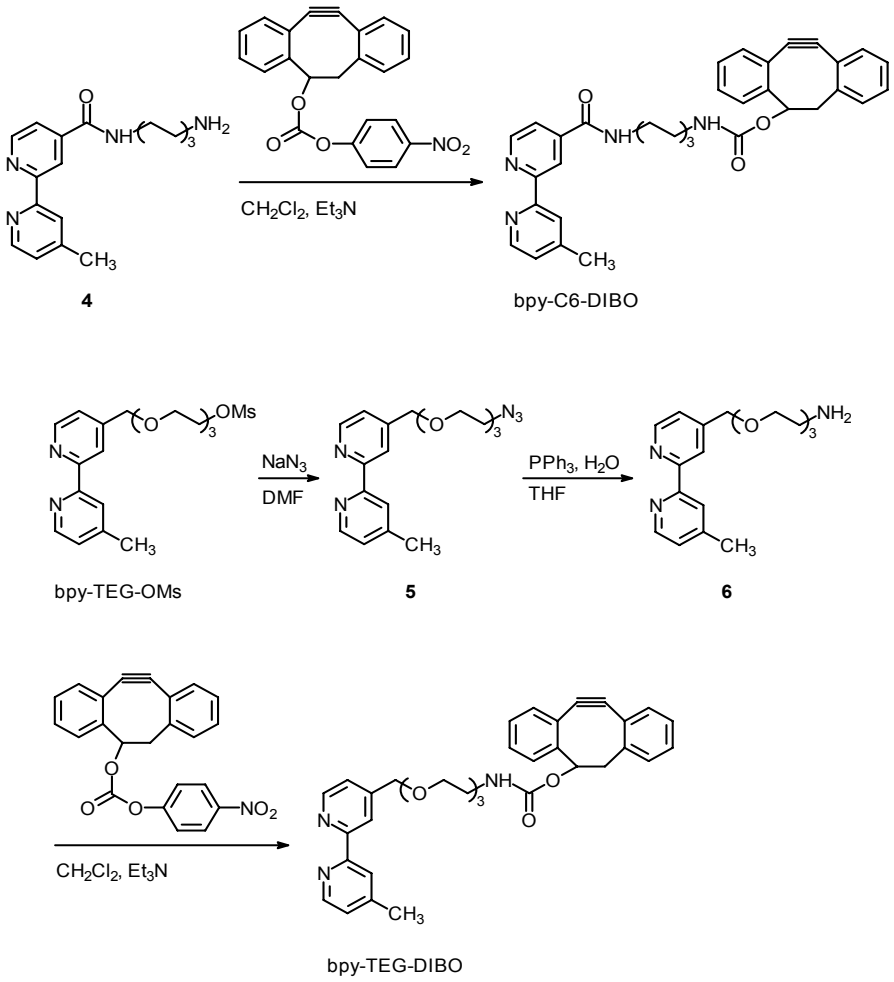
Electronic Supplementary Information

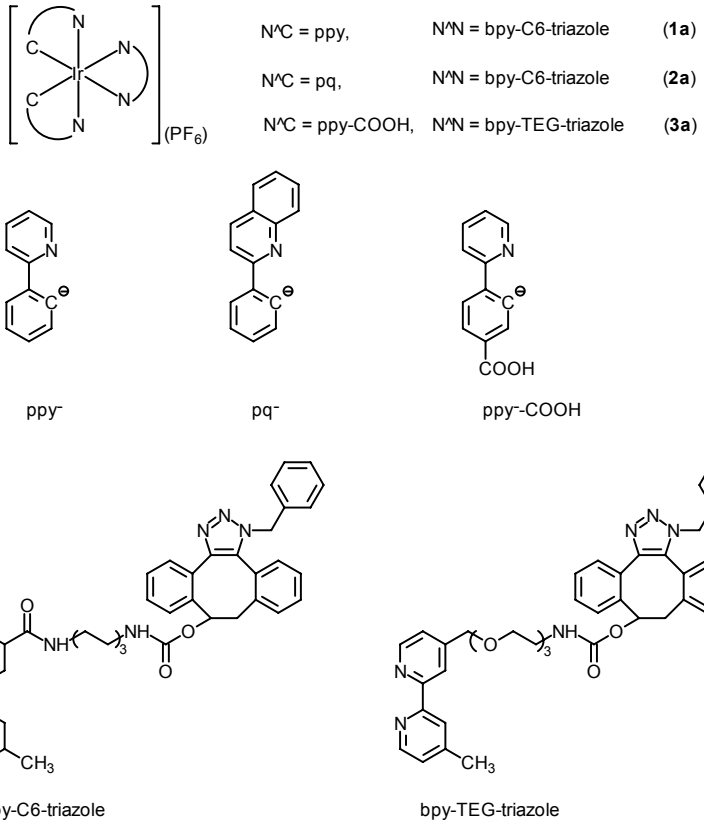
General Information

All solvents were of analytical reagent grade and purified according to standard procedures.[1] Bromine, lithium diisopropylamide, iodotrimethylsilane, methanesulfonyl chloride, *n*-butyllithium, 4-nitrophenyl chloroformate, phenylacetaldehyde, potassium hexafluorophosphate, pyridine, selenium dioxide, triethylene glycol (TEG), silver nitrate, sodium borohydride, sodium hydroxide, magnesium sulfate, potassium nitrate, colchicine, iodoacetic acid, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Acros. 4,4'-Dimethyl-2,2'-bipyridine, $IrCl_3 \cdot H_2O$, sodium azide, *tert*-butyl dimethylsilyl chloride, triphenylphosphine, and *N*-hydroxybenzotriazole (HOBt) were obtained from Sigma-Aldrich. Benzyl azide was supplied by Alfa Aesar. Bpy-C6-NH_2,[2] bpy-TEG-OMs[3] and 5-dibenzocyclooctynyl 4-nitrophenyl carbonate[4] were synthesized according to reported procedures. All buffer components were of biological grade and used without purification. Bovine serum albumin (BSA), human serum albumin (HSA) fraction V, and apotransferrin (aTf) were from Calbiochem and were used as received. Coomassie brilliant blue R250 was purchased from Serva. Bio-Rad DC protein Assay was supplied by Bio-Rad. Amicon Ultra 0.5 mL centrifugal filter and PD-10 size exclusion column were received from Millipore and GE Healthcare, respectively. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), *cis*-diamminedichloroplatinum (cis-platin) were purchased from Sigma-Aldrich. CHO cells were obtained from American Type Culture Collection. F12-nutrition mixture, fetal bovine serum (FBS), penicillin/streptomycin, phosphate-buffered saline at pH 7.2 (PBS), MitoTracker Deep Red FM, 1,3,4,6-tetra-*O*-acetyl-*N*-azidoacetyl-D-mannosamine (Ac_4ManNAz), and trypsin-EDTA were purchased from Invitrogen. The instruments for characterization and photophysical studies have been described previously.[5] Details about the measurements of emission quantum yields, lipophilicity, and cellular uptake, and experimental procedures about confocal imaging and MTT assays were undertaken have also been reported.[2,3,6]

Scheme S1 Synthesis of bpy-C6-DIBO and bpy-TEG-DIBO.



Scheme S2 Structures of complexes **1a** – **3a**.



4-(*N*-(6-(3,4:7,8-Dibenzocyclooctyne-5-oxycarbonylamino)hexyl)aminocarbonyl)-4'-methyl-2,2'-bipyridine (bpy-C6-DIBO)

A mixture of bpy-C6-NH_2[2] (90 mg, 0.29 mmol), 5-dibenzocyclooctynyl 4-nitrophenyl carbonate[4] (111 mg, 0.29 mmol), and triethylamine (0.2 mL, 1.45 mmol) in CH_2Cl_2 (10 mL) was stirred under an inert atmosphere of nitrogen at room temperature for 12 h. The solution was evaporated to dryness. The crude product was purified by flash column chromatography on silica gel using hexane/ethyl acetate (5:1, v/v) as an eluent to afford bpy-C6-DIBO (108 mg, 67%) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-*d*, 298K, TMS): δ 8.76 (d, 1H, J = 4.8 Hz, H6 of bpy), 8.60 (s, 1H, H3 of bpy), 8.49 (d, 1H, J = 4.8 Hz, H6' of bpy), 8.25 (s, 1H, H3' of bpy), 7.74 (d, 1H, J = 4.8 Hz, H5' of bpy), 7.61 (d, 1H, J = 6.4 Hz, phenyl ring of DIBO), 7.49 – 7.25 (m, 7H, phenyl ring of DIBO), 7.14 (d, 1H, J = 4.8 Hz, H5' of bpy), 6.70 (br s, 1H, CONH), 5.49 (br, 1H, OCH of DIBO), 5.11 (t, 1H, J = 6.0 Hz, CONH), 3.42 – 3.39 (m, 2H, CONHCH_2), 3.24 – 3.23 (m, 2H, CONHCH_2), 3.15 (d, 1H, J = 15.0 Hz, CH_2 of DIBO), 2.88 (dd, 1H, J = 3.6, 15.0 Hz, CH_2 of DIBO), 2.44 (s, 3H, CH_3 on C4' of bpy), 1.63 – 1.41 (m, 8H, $CH_2(CH_2)_4CH_2$). Positive-ion ESI-MS ion cluster at m/z 559 $[M + H^+]^+$.

4-(10-Azido-2,5,8-trioxa-decyl)-4'-methyl-2,2'-bipyridine (5)

To a solution of bpy-TEG-OMs[3] (240 mg, 0.59 mmol) in DMF (5 mL) was added sodium azide (78 mg, 1.20 mmol) under an inert atmosphere of nitrogen. The mixture was stirred at 90 °C for 2 h. The reaction mixture was carefully concentrated *in vacuo* and then the residue was suspended in H_2O (5 mL). The solution was extracted with CH_2Cl_2 (20 mL × 3) and the combined organic phase was washed with saturated aqueous $NaHCO_3$ (5 mL), saturated aqueous NaCl (5 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude product was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (20:1, v/v) as an eluent. Compound **5** (178 mg, 85%) was isolated as a colorless oil. ^{1}H NMR (300 MHz, chloroform-*d*, 298 K, TMS): δ 8.58 (d, 1H, J = 5.1 Hz, H6 of bpy), 8.46 (d, 1H, J = 5.1 Hz, H6' of bpy), 8.26 (s, 1H, H3 of bpy), 8.16 (s, 1H, H3' of bpy), 7.30 (d, 1H, J = 5.1 Hz, H5 of bpy), 7.07 (d, 1H, J = 5.1 Hz, H5' of bpy), 4.61 (s, 2H, CH_2 on C4 of bpy), 3.68 – 3.59 (m, 10H, OCH_2), 3.31 (t, 2H, J = 5.1 Hz, CH_2N_3), 2.36 (s, 3H, CH_3 on C4' of bpy).

4-(10-Amino-2,5,8-trioxa-decyl)-4'-methyl-2,2'-bipyridine (6)

To a solution of compound **5** (85 mg, 0.24 mmol) in THF (10 mL) was added H_2O (43 μL, 2.4 mmol) and triphenylphosphine (188 mg, 0.72 mmol) under an inert atmosphere of nitrogen. The mixture was heated to reflux for 12 h. The solution was evaporated to dryness. The crude product was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (1:1, v/v) as an eluent. Compound **6** (76 mg, 96%) was isolated as a colorless oil. ^{1}H NMR (300 MHz, chloroform-*d*, 298K, TMS): δ 8.60 (d, 1H, J = 5.1 Hz, H6 of bpy), 8.48 (d, 1H, J = 5.1 Hz, H6' of bpy), 8.27 (s, 1H, H3 of bpy), 8.18 (s, 1H, H3' of bpy), 7.32 (d, 1H, J = 5.1 Hz, H5 of bpy), 7.09 (d, 1H, J = 5.1 Hz, H5' of bpy), 4.63 (s, 2H, CH_2 on C4 of bpy), 3.69 – 3.59 (m, 8H, OCH_2), 3.46 (t, 2H, J = 5.1 Hz, OCH_2), 2.81 (t, 2H, J = 5.1 Hz, CH_2NH_2), 2.39 (s, 3H, CH_3 on C4' of bpy). Positive-ion ESI-MS ion cluster at *m*/*z* 332 $[M + H^+]^+$.

4-(10-*N*-(3,4:7,8-Dibenzocyclooctyne-5-oxycarbonyl)amino-2,5,8-trioxa-decyl)-4'-methyl-2,2'-bipyridine (bpy-TEG-DIBO)

A mixture of compound **6** (76 mg, 0.23 mmol), dibenzocyclooctynyl 4-nitrophenyl carbonate (89 mg, 0.23 mmol), and triethylamine (0.16 mL, 1.15 mmol) in CH_2Cl_2 (10 mL) was stirred under an inert atmosphere of nitrogen at room temperature for 12 h. The solution was evaporated to dryness. The crude product was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (10:1, v/v) as an eluent. The compound bpy-TEG-DIBO (96 mg, 72%) was isolated as a colorless oil. ^{1}H NMR (400 MHz, chloroform-*d*, 298K, TMS): δ 8.64 (d, 1H, J = 4.8 Hz, H6 of bpy), 8.52 (d, 1H, J = 4.8 Hz, H6' of bpy), 8.35 (s, 1H, H3 of bpy), 8.23 (s, 1H, H3' of bpy), 7.47 – 7.24 (m, 9H, phenyl ring of DIBO and H5 of bpy), 7.11 (d, 1H, J = 4.8 Hz, H5' of bpy), 5.75 (t, 1H, J = 5.2 Hz, CONH), 5.48 (br, 1H, OCH of DIBO), 4.68 (s, 2H, CH_2 on C4 of bpy), 3.74 – 3.61 (m, 8H, OCH_2), 3.57 (t, 2H, J = 4.8 Hz, OCH_2), 3.39 (q, 2H, J = 5.2 Hz, CONHCH_2), 3.12 (d, 1H, J = 15.0 Hz, CH_2 of DIBO), 2.85 (dd, 1H, J = 4.0, 15.0 Hz, CH_2 of DIBO), 2.40 (s, 3H, CH_3 on C4' of bpy). ^{13}C NMR (100 MHz, chloroform-*d*, 298 K, TMS): δ 156.3, 155.7, 155.5, 152.1, 151.0, 149.2, 148.9, 148.6, 148.1, 129.9, 128.9, 128.0, 127.9, 127.0, 126.1, 125.9, 124.8, 123.8, 122.0, 121.8, 121.2, 119.3, 112.9, 110.0, 76.7, 71.7, 70.6, 70.6, 70.2, 70.2, 70.0, 46.1, 40.9, 21.1. Positive-ion ESI-MS ion cluster at *m*/*z* 578 $[M + H^+]^+$.

[Ir(ppy)$_2$(bpy-C6-DIBO)](PF$_6$) (1)

A mixture of [Ir$_2$(ppy)$_4$Cl$_2$] (56 mg, 0.052 mmol) and bpy-C6-DIBO (58 mg, 0.10 mmol) in CH_2Cl_2 (10 mL) was stirred under an inert atmosphere of nitrogen at room temperature for 12 h. Then, KPF_6 (74 mg, 0.40 mmol) was added. The mixture was stirred for 10 min and then evaporated to dryness. The yellow solid was dissolved in CH_2Cl_2 and purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (20:1, v/v). Subsequent recrystallization from CH_3CN/diethyl ether afforded complex **1** as yellow crystals. Yield: 78 mg (65%). ^{1}H NMR (400 MHz, acetone-d_6, 298K, TMS): δ 8.94 (s, 0.5H, H3 of bpy), 8.91 (s, 0.5H, H3 of bpy), 8.70 (s, 0.5H, H3' of bpy), 8.65 (s, 0.5H, H3' of bpy), 8.27 (t, 2H, J = 8.0 Hz, H3 of pyridyl ring of ppy), 8.17 (t, 1H, J = 6.0 Hz, H6 of bpy), 8.02 – 7.84 (m, 9H, H4 and H6 of pyridyl ring of ppy, H3 of phenyl ring of ppy, H5 and H6' of bpy and CONH), 7.62 – 6.66 (m, 16H, H5 of pyridyl ring of ppy, H4 and H5 of phenyl ring of ppy, H5' of bpy, phenyl ring of DIBO and CONH), 6.38 – 6.34 (m, 2H, H6 of phenyl ring of ppy), 5.50 – 5.46 (m, 1H, OCH of DIBO), 3.44 – 3.22 (m, 4H, CONHCH_2), 2.80 – 2.73 (m, 2H, CH_2 of DIBO), 2.43 (s, 1.5H, CH_3 on C4' of bpy), 2.34 (s, 1.5H, CH_3 on C4' of bpy), 1.59 – 1.44 (m, 8H, $CH_2(CH_2)_4CH_2$). IR (KBr): ν = 3432 (br, NH), 846 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 1060 [M – PF_6^-]$^+$. Anal. Calc. for $C_{57}H_{50}N_6O_3PF_6Ir{\cdot}H_2O$: C, 55.87; H, 4.15; N, 6.92. Found: C, 56.01; H, 4.29; N, 6.88%.

[Ir(pq)$_2$(bpy-C6-DIBO)](PF$_6$) (2)

The synthetic procedure was similar to that of complex **1**, except that [Ir$_2$(pq)$_4$Cl$_2$] was used instead of [Ir$_2$(ppy)$_4$Cl$_2$]. Yield: 69%. ^{1}H NMR (400 MHz, acetone-d_6, 298K, TMS): δ 8.74 (s, 0.5H, H3 of bpy), 8.68 (s, 0.5H, H3 of bpy), 8.56 – 8.39 (m, 6H, H3 and H4 of quinolinyl ring of pq, H3' and H6 of bpy), 8.27 – 8.23 (m, 2H, H3 of phenyl ring of pq), 8.18 (dd, 1H, J = 5.6, 1.6 Hz, H6' of bpy), 7.98 – 7.89 (m, 3H, H6 of quinolinyl ring of pq and H5 of bpy), 7.57 – 6.89 (m, 17H, H5, H7 and H8 of quinolinyl ring of pq, H4 of phenyl ring of pq, H5' of bpy and phenyl ring of DIBO), 6.86 – 6.82 (m, 2H, H5 of phenyl ring of pq), 6.59 – 6.54 (m, 2H, H6 of phenyl ring of pq), 5.46 – 6.45 (m, 1H, OCH of DIBO), 3.31 – 3.11 (m, 5H, CONHCH_2 and CH_2 of DIBO), 2.81 – 2.72 (m, 1H, CH_2 of DIBO), 2.39 (s, 1.5H, CH_3 on C4' of bpy),

2.29 (s, 1.5H, CH_3 on C4' of bpy), 1.50 – 1.31 (m, 8H, $CH_2(CH_2)_4CH_2$). IR (KBr): ν = 3425 (br, NH), 846 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion cluster at *m*/*z* 1160 [*M* – PF_6^-]$^+$. Anal. Calc. for $C_{65}H_{54}N_6O_3PF_6Ir{\cdot}H_2O$: C, 58.86; H, 4.28; N, 6.22. Found: C, 59.04; H, 4.27; N, 6.36%.

[Ir(ppy-COOH)$_2$(bpy-TEG-DIBO)](PF$_6$) (3)

A mixture of $[Ir_2(ppy\text{-}COOH)_4Cl_2]$ (113.5 mg, 0.09 mmol) and bpy-TEG-DIBO (105.1 mg, 0.18 mmol) in CH_2Cl_2/MeOH (50 mL, 1:10, v/v) was stirred under an inert atmosphere of nitrogen at room temperature for 12 h. Then, KPF_6 (132 mg, 0.72 mmol) was added. The mixture was stirred for 10 min and then evaporated to dryness. The yellow solid was dissolved in CH_2Cl_2 and purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (10:1, v/v). Subsequent recrystallization from MeOH/diethyl ether afforded complex **3** as yellow crystals. Yield: 166.2 mg (70%). ^{1}H NMR (400 MHz, methanol-d_4, 298K, TMS): δ 8.57 – 8.56 (m, 1H, H3 of bpy), 8.49 (s, 1H, H3' of bpy), 8.24 – 8.20 (m, 2H, H6 of pyridyl ring of ppy-COOH), 7.94 – 7.83 (m, 5H, H4 of pyridyl ring of ppy-COOH, H4 of phenyl ring of ppy-COOH and H6 of bpy), 7.73 – 7.68 (m, 5H, H3 of pyridyl ring of ppy-COOH and H6' of bpy and H3 of phenyl ring of ppy-COOH), 7.56 – 7.48 (m, 1H, phenyl ring of DIBO), 7.50 – 6.88 (m, 13H, H5 of bpy, phenyl ring of DIBO, H4 and H6 of pyridyl ring of ppy-COOH and H5' of bpy), 5.34 – 5.26 (m, 1H, OCH of DIBO), 4.60 (s, 1H, CH_2 on C4 of bpy), 4.57 (s, 1H, CH_2 on C4 of bpy), 3.70 – 3.66 (m, 8H, OCH_2), 3.56 – 3.47 (m, 2H, OCH_2), 3.33 – 3.17 (m, 3H, CONHCH_2 and CH_2 of DIBO), 2.72 – 2.69 (m, 1H, CH_2 of DIBO), 2.39 (s, 1.5H, CH_3 on C4' of bpy), 2.36 (s, 1.5H, CH_3 on C4' of bpy). IR (KBr): ν = 1709 (s, C=O), 844 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion cluster at *m*/*z* 1167 [*M* – PF_6^-]$^+$. Anal. Calc. for $C_{59}H_{51}N_5O_9PF_6Ir{\cdot}2H_2O$: C, 52.65; H, 3.81; N, 4.98. Found: C, 52.60; H, 4.11; N, 5.20%.

[Ir(ppy)$_2$(bpy-C6-triazole)](PF$_6$) (1a)

A mixture of complex **1** (100 mg, 0.083 mmol) and benzyl azide (22 μL, 0.17 mmol) in CH_3CN (4 mL) was stirred under an inert atmosphere of nitrogen at room temperature for 2 h. The solution was then evaporated to dryness. Subsequent recrystallization from CH_3CN/diethyl ether afforded complex **1a** as yellow crystals.

^{1}H NMR (400 MHz, acetonitrile-d_3, 298K, TMS): δ 8.85 – 8.78 (m, 1H, H3 of bpy), 8.52 – 8.49 (m, 1H, H3' of bpy), 8.08 – 8.05 (m, 3H, H6 of bpy and H3 of pyridyl ring of ppy), 7.84 – 7.70 (m, 9H, H4 and H6 of pyridyl ring of ppy, H3 of phenyl ring of ppy, H5 and H6' of bpy and CONH), 7.60 – 6.91 (m, 21H, H5 of pyridyl ring of ppy, H4 and H5 of phenyl ring of ppy, H5' of bpy, phenyl ring of triazole and CONH), 6.30 – 6.34 (m, 2H, H6 of phenyl ring of ppy), 5.76 – 5.70 (m, 1H, OCH of triazole), 5.62 – 5.41 (m, 2H, CH_2 on phenyl ring of triazole), 3.46 – 3.37 (m, 2H, CONHCH_2), 3.19 – 3.05 (m, 3H, CONHCH_2 and CH of triazole), 2.70 – 2.62 (m, 1H, CH of triazole), 2.54 (s, 3H, CH_3 on C4' of bpy), 1.59 – 1.25 (m, 8H, CH_2(CH_2)$_4$CH_2). IR (KBr): ν = 3427 (br, NH), 845 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 1193 [M – PF_6^-]$^+$.

[Ir(pq)$_2$(bpy-C6-triazole)](PF$_6$) (2a)

The synthetic procedure was similar to that of complex **1a**, except that complex **2** was used instead of complex **1**. ^{1}H NMR (400 MHz, acetone-d_6, 298K, TMS): δ 9.20 – 9.08 (m, 1H, H3 of bpy), 8.63 – 8.42 (m, 6H, H3 and H4 of quinolinyl ring of pq, H3' and H6 of bpy), 8.26 – 8.21 (m, 2H, H3 of phenyl ring of pq), 8.17 – 8.15 (m, 1H, H6' of bpy), 8.09 – 8.03 (m, 1H, H5 of bpy), 7.92 – 7.86 (m, 2H, H6 of quinolinyl ring of pq), 7.58 – 7.07 (m, 22H, H5, H7 and H8 of quinolinyl ring of pq, H4 of phenyl ring of pq, H5' of bpy and phenyl ring of triazole), 6.84 – 6.79 (m, 2H, H5 of phenyl ring of pq), 6.56 – 6.53 (m, 2H, H6 of phenyl ring of pq), 6.09 – 5.91 (m, 1H, OCH of triazole), 5.76 – 5.53 (m, 2H, CH_2 on phenyl ring of triazole), 3.43 – 2.80 (m, 6H, CONHCH_2 and CH_2 of triazole), 2.35 (s, 3H, CH_3 on C4' of bpy), 1.46 – 1.15 (m, 8H, CH_2(CH_2)$_4$CH_2). IR (KBr): ν = 3416 (br, NH), 845 cm^{-1} (s, PF_6^-). Positive-ion ESI-MS ion cluster at m/z 1293 [M – PF_6^-]$^+$.

[Ir(ppy-COOH)$_2$(bpy-TEG-triazole)](PF$_6$) (3a)

A mixture of complex **3** (60.2 mg, 0.05 mmol) and benzyl azide (14 μL, 0.1 mmol) in MeOH (4 mL) was stirred under an inert atmosphere of nitrogen at room temperature for 2 h. The mixture was then evaporated to dryness. Subsequent recrystallization from MeOH/diethyl ether afforded complex **3a** as yellow crystals. Yield: 57.0 mg (86%). ^{1}H NMR (400 MHz, methanol-d_6, 298K, TMS): δ 8.64 – 8.54 (m, 2H, H3

and H3' of bpy), 8.24 – 8.18 (m, 2H, H6 of pyridyl ring of ppy-COOH), 7.93 – 7.66 (m, 6H, H4 of pyridyl ring of ppy-COOH, H6 and H6' of bpy and H4 of phenyl ring of ppy-COOH), 7.72 – 7.66 (m, 4H, H3 of pyridyl ring of ppy-COOH and H3 of phenyl ring of ppy-COOH), 7.41 – 6.94 (m, 19H, H5 of bpy, H4 of pyridyl ring of ppy-COOH, H5' of bpy and phenyl ring of triazole), 6.05 – 5.98 (m, 0.5H, OCH of triazole), 5.76 – 5.52 (m, 2.5H, OCH of triazole and CH_2 on phenyl ring of triazole), 4.77 – 4.63 (m, 2H, CH_2 on C4 of bpy), 3.72 – 3.58 (m, 8H, OCH_2), 3.52 – 3.42 (m, 2H, OCH_2), 3.22 – 3.14 (m, 3H, CONHCH_2 and CH_2 of triazole), 3.07 – 3.00 (m, 1H, CH_2 of triazole), 2.52 – 2.50 (m, 3H, CH_3 on C4' of bpy). Positive-ion ESI-MS ion cluster at *m*/*z* 1300 $[M - PF_6^-]^+$. IR (KBr): ν = 1701 (s, C=O), 845 cm^{-1} (s, PF_6^-). Anal. Calc. for $C_{66}H_{58}N_8O_9PF_6Ir{\cdot}2H_2O{\cdot}MeOH$: C, 53.38; H, 4.24; N, 7.23. Found: C, 53.21; H, 4.40; N, 7.41%.

Azidoacetic acid

A mixture of iodoacetic acid (2.09 g, 10 mmol) and sodium azide (1.40 g, 22 mmol) in H_2O (25 mL) was stirred slowly in the dark at room temperature for 24 h. The reaction mixture was then acidified with 1 M HCl solution and the solution was extracted with diethyl ether. The organic phase was washed with saturated $NaHSO_3$ and NaCl solutions and then dried over $MgSO_4$. The solvent was evaporated *in vacuo* to afford azidoacetic acid as a yellow oil. Yield: 455 mg (45%). ^{1}H NMR (300 MHz, chloroform-*d*, 298 K, TMS): δ 3.96 (s, 2H, CH_2).

Modification of BSA, HSA, and aTf with azidoacetic acid

A mixture of the protein (BSA, HSA, or aTf) (0.09 μmol), azidoacetic acid (450.0 μg, 4.5 μmol), EDC (1.4 mg, 9.0 μmol), and HOBt (1.2 mg, 9.0 μmol) in a 50 mM potassium phosphate buffer at pH 7.4 (500 μL) was stirred in the dark at room temperature for 24 h. The solution was loaded onto a PD-10 size exclusion column. Volume fractions between 2.5 and 5.0 mL were collected and the solution was concentrated with an Amicon Ultra 0.5 mL centrifugal filter (MWCO = 3.0 kDa). Quantities of the azide-modified proteins were determined with the Bio-Rad DC Protein Assay.

Labeling of azide-modified proteins with complexes 1 – 3

In a typical reaction, complex **1** (0.24 mg, 0.20 μmol) in anhydrous DMSO (20 μL) was added to the azide-modified BSA, HSA, or aTf (0.015 μmol) dissolved in 50 mM potassium phosphate buffer at pH 7.4 (300 μL). The solution was stirred in the dark at room temperature for 24 h. Any precipitate was removed by centrifugation and the supernatant was loaded onto a PD-10 size exclusion column. The first colored band was collected and concentrated with an Amicon Ultra 0.5 mL centrifugal filter (MWCO = 3.0 kDa).

SDS-PAGE

SDS-PAGE results of the 9 protein conjugates are shown in Fig. S7. The emission intensities of the bands for the protein conjugates of complexes **1** and **2** (especially the aTf conjugates) were weaker than those of complex **3** (top). However, Coomassie blue staining revealed that the amounts of proteins present in the lanes were similar (ca. 100 μg) (bottom). This reflects the higher emission quantum yields of complex **3** and also suggests that the labeling efficiency of complex **3** was higher than those of complexes **1** and **2**.

Kinetics studies for the reactions of complexes 2 and 3 with benzyl azide

The chemical kinetics of the cycloaddition reaction was studied by ^{1}H NMR spectroscopy. The complex and benzyl azide was dissolved separately in deuterated solvents and mixed together in a mole ratio of 1:1.2 at t = 0 s. The shift of the signal of methyl group on the bpy ligand at 20 °C was monitored. The second-order rate plot was constructed according to the following equation:

$$kt = \frac{1}{[B]_0 - [A]_0} \times \ln \frac{[A]_0([B]_0 - [P])}{([A]_0 - [P])[B]_0}$$

where k = second-order rate constant in M^{-1} s^{-1}, t = reaction time in s, $[A]_0$ = the initial concentration of substrate A in M, $[B]_0$ = the initial concentration of substrate B in M, and $[P]$ = the concentration of the product P in M.

Fig. S1 Kinetics for the reaction of complex **2** (8 mM) and benzyl azide (9.6 mM) in $(CD_3)_2CO$ at 293 K.

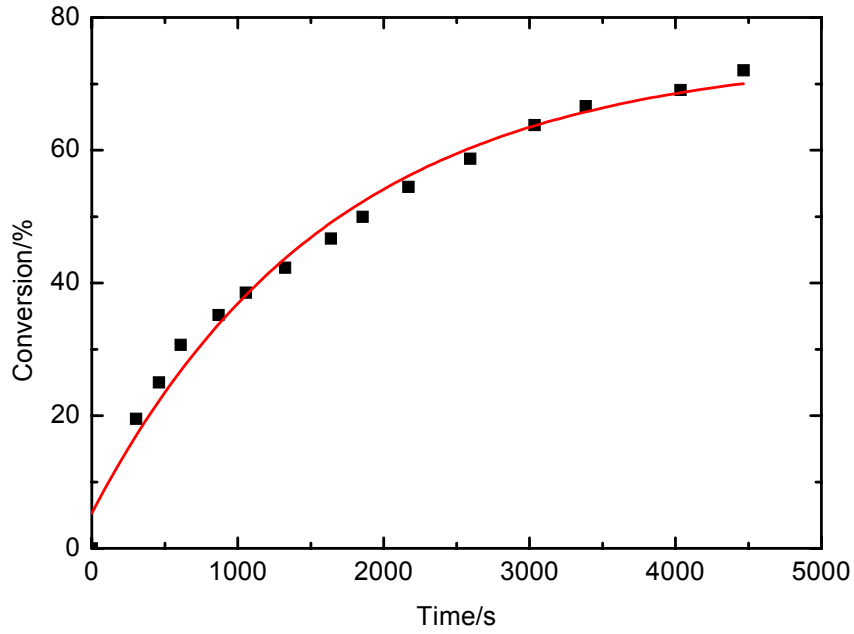


Fig. S2 The rate plot for the reaction of complex **2** (8 mM) and benzyl azide (9.6 mM) in $(CD_3)_2CO$ at 293 K.

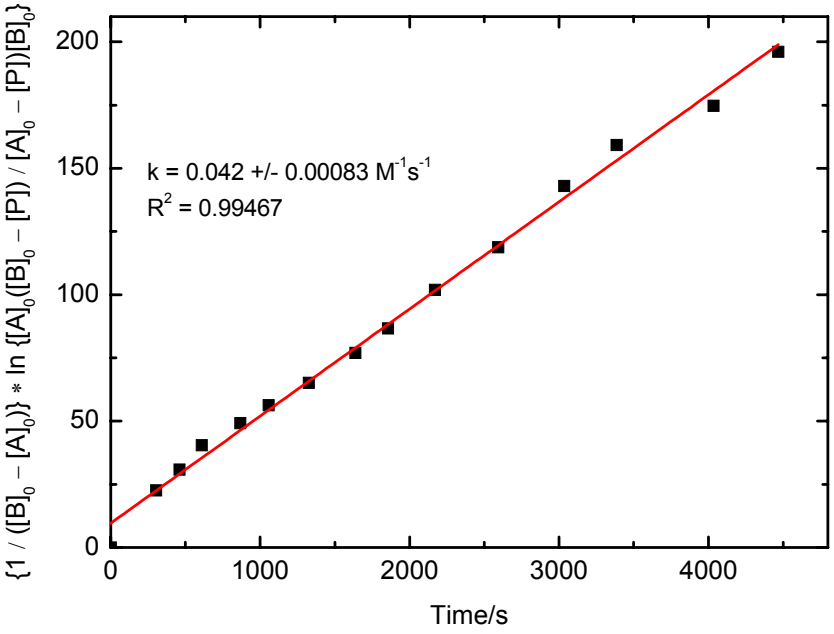


Fig. S3 Kinetics for the reaction of complex **3** (15 mM) and benzyl azide (18 mM) in CD_3OD at 293 K.

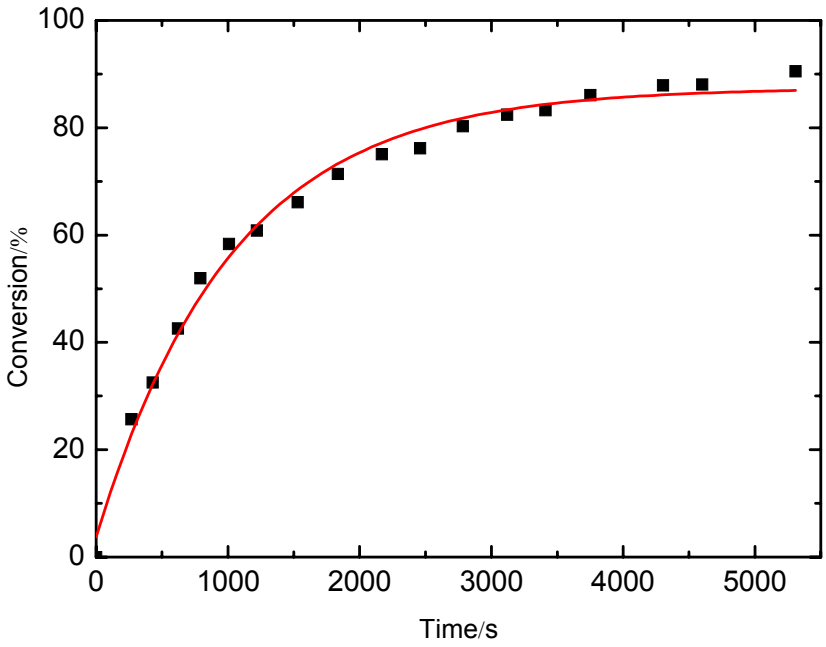


Fig. S4 The rate plot for the reaction of complex **3** (15 mM) and benzyl azide (18 mM) in CD_3OD at 293 K.

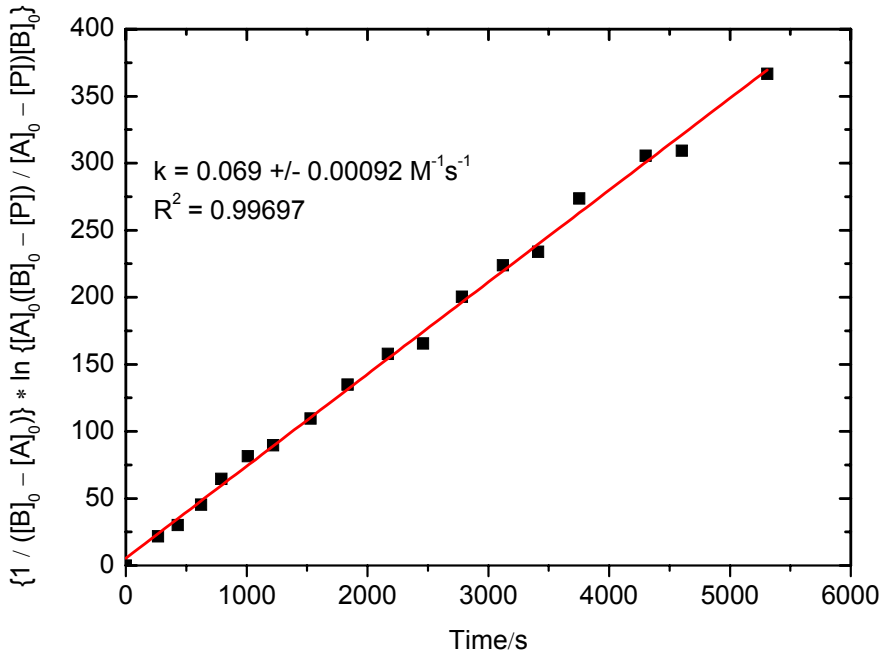


Fig. S5 Kinetics for the reaction of complex **3** (12 mM) and benzyl azide (14.4 mM) in $(CD_3)_2CO$ at 293 K.

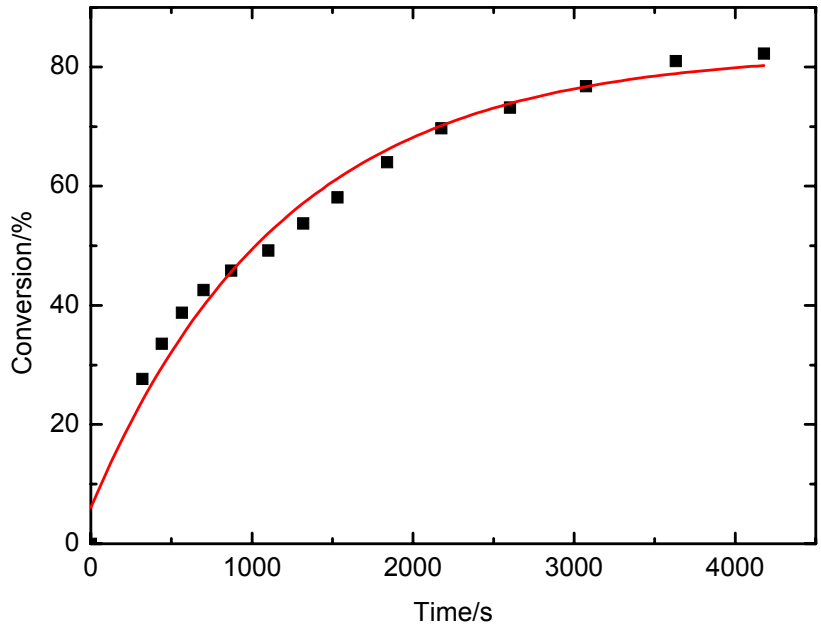


Fig. S6 The rate plot for the reaction of complex **3** (12 mM) and benzyl azide (14.4 mM) in $(CD_3)_2CO$ at 293 K.

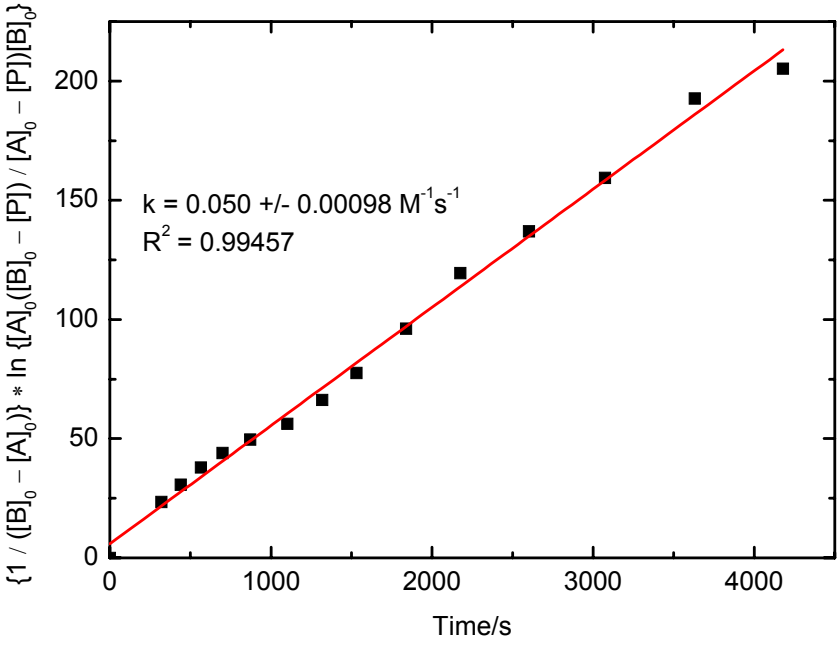


Fig. S7 SDS-PAGE analysis of the conjugates **1-aTf** – **3-aTf**, **1-BSA** – **3-BSA**, and **1-HSA** – **3-HSA** (top: upon UV transillumination; bottom: white light after Coomassie blue staining).

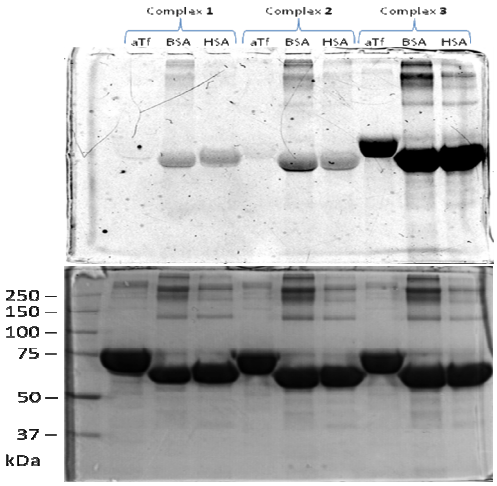


Fig. S8 Laser-scanning confocal microscopy images of CHO cells incubated with complexes **1** (top) and **2** (bottom), respectively, (30 μM, 37 °C, 15 min). Conditions used from left to right: no inhibitors added; with the cytoskeletal inhibitor colchicine (10 μM, 30 min); with the ATPase inhibitor KNO_3 (50 mM, 1 h); incubation temperature = 4 °C.

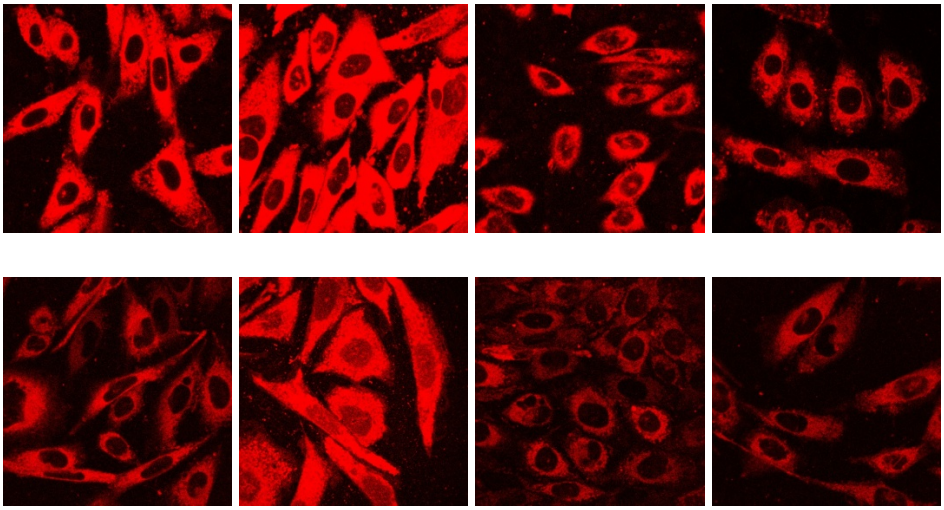


Fig. S9 Laser-scanning confocal microscopy images of Ac_4ManNAz-pretreated (50 μM, 37 °C, 72 h) CHO cells incubated with complex **1** (30 μM, 37 °C, 20 min) (left) and MitoTracker (100 nM, 37 °C, 15 min) (middle). The overlaid image is shown on the right.

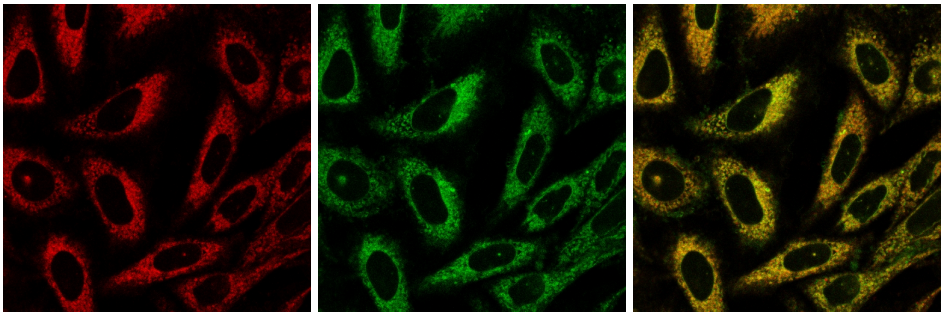


Fig. S10 Laser-scanning confocal microscopy images of Ac_4ManNAz-pretreated (50 μM, 37 °C, 72 h) CHO cells incubated with complex **2** (30 μM, 37 °C, 20 min) (left) and MitoTracker (100 nM, 37 °C, 15 min) (middle). The overlaid image is shown on the right.

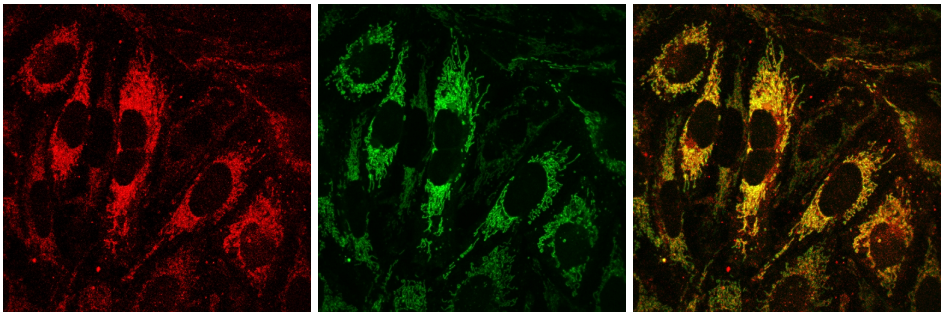


Fig. S11 Flow cytometric results of CHO cells incubated with blank medium (black) and Ac_4ManNAz-pretreated (red) and -untreated (blue) CHO cells incubated with complex **1** (50 μM) at 37 °C for 5 h.

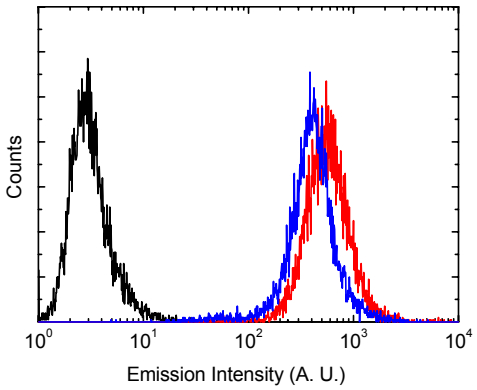


Fig. S12 Flow cytometric results of CHO cells incubated with blank medium (black) and Ac_4ManNAz-pretreated (red) and -untreated (blue) CHO cells incubated with complex **2** (50 μM) at 37 °C for 5 h.

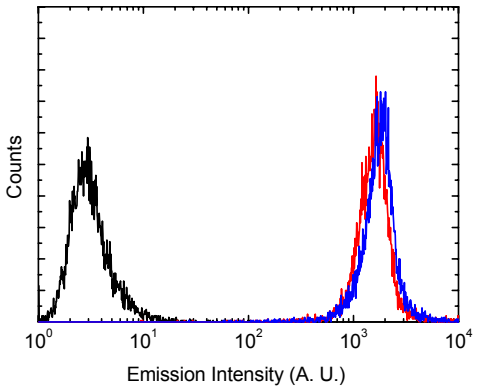


Fig. S13 Flow cytometric results of CHO cells incubated with blank medium (black) and Ac_4ManNAz-pretreated (red) and -untreated (blue) CHO cells incubated with complex **3** (200 μM) at 37 °C for 2 h.

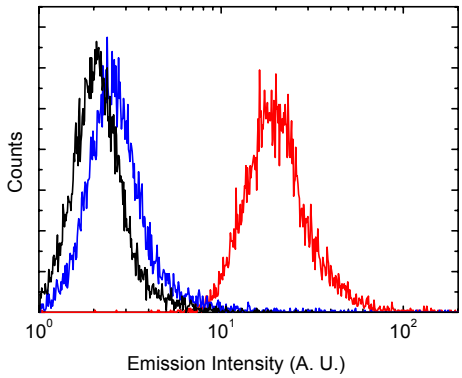


Table S1 Electronic absorption spectral data of complexes **1** – **3** and **1a** – **3a** at 298 K.

Complex	Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1	CH_2Cl_2	253 sh (53 490), 271 (58 590), 290 sh (49 215), 306 (38 555), 323 sh (16 415), 340 sh (10 180), 364 sh (8475), 386 sh (7655), 420 sh (3295), 469 sh (1010)
	CH_3CN	255 sh (53 560), 270 (54 715), 288 sh (46 995), 304 (38 640), 321 sh (15 955), 340 sh (9685), 356 sh (8515), 381 sh (7140), 420 sh (2740), 476 sh (885)
1a	CH_2Cl_2	253 (55 365), 271 sh (48 555), 316 sh (19 525), 331 sh (11 660), 348 sh (9480), 372 sh (8855), 401 sh (6320), 458 sh (1930)
	CH_3CN	250 (50 645), 267 sh (45 480), 312 sh (17 290), 340 sh (8245), 369 sh (6470), 401 sh (3670), 460 sh (830)
2	CH_2Cl_2	259 sh (62 400), 282 (71 185), 287 sh (67 000), 306 (41 720), 336 (26 580), 350 sh (24 820), 397 sh (6560), 439 (6040), 524 sh (830)
	CH_3CN	254 sh (58 795), 280 (69 105), 285 sh (64 905), 304 (41 645), 336 (25 510), 352 sh (22 130), 393 sh (6420), 433 (6060), 524 sh (540)
2a	CH_2Cl_2	257 (67 330), 274 sh (64 970), 306 sh (29 935), 332 sh (27 605), 382 sh (9140), 438 (6985)
	CH_3CN	255 (69 425), 274 sh (65 250), 302 sh (29 935), 326 sh (26 275), 382 sh (8120), 435 (6435)
3	CH_2Cl_2	273 (61 675), 288 (55 800), 308 sh (47 680), 364 sh (6955), 409 (4505), 447 sh (2955), 482 sh (370)
	CH_3CN	270 (56 680), 287 (51 585), 307 sh (41 575), 363 sh (6340), 405 (4325), 442 sh (2795), 469 sh (560)
3a	CH_2Cl_2	262 (67 330), 281 sh (54 335), 301 sh (40 610), 313 sh (31 965), 368 sh (7495), 409 (5295), 442 sh (4055), 486 sh (345)
	CH_3CN	260 (59 205), 278 sh (47 055), 298 sh (36 005), 310 sh (29 360), 361 sh (7110), 405 (4620), 441 sh (3085), 478 sh (350)

Table S2 Photophysical data of complexes **1a** – **3a**.

Complex	Medium (T/K)	λ_{em}/nm	τ_o/μs	Φ_{em}
1a	CH_2Cl_2 (298)	603	0.41	0.10
	CH_3CN (298)	610	0.24	0.042
	MeOH (298)	619	0.084	0.018
	Glass[a] (77)	537, 573 sh	4.94	
2a	CH_2Cl_2 (298)	557, 593 sh	0.74	0.23
	CH_3CN (298)	562, 597 sh	0.56	0.099
	MeOH (298)	563 sh, 603	0.14	0.029
	Glass[a] (77)	540 (max), 582, 636 sh	4.73	
3a	CH_2Cl_2 (298)	513 (max), 543, 596 sh	3.02	0.60
	CH_3CN (298)	511 (max), 541, 591 sh	3.28	0.43
	MeOH (298)	512 sh, 556	0.44 (51%), 1.51 (49%)	0.17
	Glass[a] (77)	506 (max), 545, 594 sh	6.55	

[a] EtOH/MeOH (4:1, v/v).

Table S3 Photophysical data of conjugates **1-BSA** – **3-BSA**, **1-HSA** – **3-HSA**, and **1-aTf** – **3-aTf** in potassium phosphate buffer (50 mM, pH 7.4).

Conjugate	λ_{em}/nm	τ_o/μs
1-BSA	580	0.18 (58%), 0.57 (42%)
2-BSA	571, 613 sh	1.02 (30%), 2.42 (70%)
3-BSA	509 sh, 549	0.25 (65%), 1.01 (35%)
1-HSA	572	0.13 (60%), 0.53 (40%)
2-HSA	571, 617 sh	1.10 (45%), 2.57 (55%)
3-HSA	509 sh, 542	0.21 (75%), 1.17 (25%)
1-aTf	589	0.12 (39%), 0.43 (60%)
2-aTf	568, 609 sh	1.17 (35%), 2.83 (65%)
3-aTf	509 sh, 557	0.13 (84%), 0.89 (16%)

Table S4 Lipophilicity (log $P_{o/w}$), cellular uptake efficiency, and cytotoxicity toward CHO cells of complexes **1** – **3**.

Complex	log $P_{o/w}$	Amount of Ir[a]/fmol	Cell viability[b]/%
1	5.69	0.57 (0.62)	75.6 ± 2.3
2	7.14	0.43 (0.50)	84.9 ± 3.2
3	5.20	0.019 (0.069)	98.9 ± 2.4

[a] Amounts of iridium associated with an average CHO cell upon incubation with the complexes (50 μM, 37 °C, 5 h); data in parentheses: Ac_4ManNAz-treated CHO cells. [b] Percentage of surviving CHO cells after incubation with the complexes (50 μM, 37 °C, 6 h).

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