Electronic Supplementary Information

A Novel Azopyridine-based Ru(II) Complex with GSH-responsive DNA Photobinding Ability

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Experimental section

Materials. 4-(4-Diethylaminophenylazo)pyridine (L2) was purchased from TCI, 4-aminopyridine, 2,2'-bipyridine, NH_4PF_6, glutathione were purchased from Sigma-Aldrich. Supercoiled pBR322 plasmid DNA was purchased from TaKaRa Biotechology Company.

Synthesis. $Ru(bpy)_2Cl_2$[1] and 4,4'-azopyridine (L1)[2] were synthesized according to the reported methods.

Synthesis of $[Ru(bpy)_2(L)_2](PF_6)_2$: 0.20 g $Ru(bpy)_2Cl_2$ and 0.21 g (1.5 equiv.) $AgNO_3$ were refluxed in 25 ml of methanol/water (1:1) for 3 h under N_2 atmosphere. Then the filtrate was refluxed again with L1 or L2 (1.5 equiv.) for 4 h under N_2 atmosphere. After removal of solvent and purification on silica gel using $CH_3CN/H_2O/KNO_3$ (40:4:1) as eluent, the solid obtained was dissolved in CH_3OH and precipitated by NH_4PF_6. The dark red solid was filtered, washed with water and vacuum dried. The yields are about 40% for the two complexes.

$[Ru(bpy)_2(L1)_2](PF_6)_2$ (complex **1**): ^{1}H NMR (400 MHz, in CD_3CN): δ = 7.34-7.37 (t, 2H, *J* = 6.3 Hz), 7.61-7.62 (d, 4H, *J* = 5.6 Hz), 7.69-7.71(d, 4H, *J* = 4.4 Hz), 7.75-7.78 (t, 2H, *J* = 5.6 Hz), 7.88-7.93 (m, 4H), 8.10-8.14 (t, 2H, *J* = 7.6 Hz), 8.24-8.26 (d, 2H, *J* = 8.0 Hz), 8.33-8.35 (d, 2H, *J* = 8.0 Hz), 8.51-8.53 (d, 4H, *J* = 5.6 Hz), 8.79-8.80 (d, 4H, *J* = 4.2 Hz), 8.92-8.94 (d, 2H, *J* = 5.2 Hz). HR ESI-MS: m/z = 391.0949 (calcd value = 391.0958 for $(M-2PF_6)^{2+}$).

$[Ru(bpy)_2(L2)_2](PF_6)_2$ (complex **2**): ^{1}H NMR (400 MHz, in CD_3CN): δ = 1.18-1.22 (t, 12H, *J* = 7.6 Hz), 3.48-3.53 (q, 8 H, *J* = 7.2 Hz), 6.80-6.82 (d, 4H, *J* = 9.2 Hz), 7.36-7.40 (t, 2H, *J* = 7.2 Hz), 7.49-7.51 (d, 4H, *J* = 6.8 Hz), 7.77-7.82 (m, 6H), 7.92-7.95 (m, 4H), 8.12-8.16 (t, 2H, *J* = 8.8 Hz), 8.28-8.30 (d, 2H, *J* = 8.4 Hz), 8.34-8.38 (m, 6H), 8.99-9.01 (d, 2H, *J* = 5.6 Hz). ESI-MS: m/z = 461.1735 (calcd value = 461.1741 for $(M-2PF_6)^{2+}$).

Computational Details

All calculations were performed with the Gaussian 03 (G03) program package[3] employing the density functional theory (DFT) method with Becke's three-parameter hybrid functional[4] and Lee-Yang-Parr's gradient corrected correlation functional (B3LYP).[5] The LanL2DZ basis set and effective core potential were used for the Ru atom, and the 6-31 G* basis set was applied for H, C, and N. The ground-state geometry of **1** was optimized in CH_3CN using the conductive polarizable continuum model (CPCM), and frequency calculation was also performed to verify the optimized

structure to be at an energy minimum. Time-dependent density functional theory (TDDFT) calculation was used to characterize the properties of singlet and triplet excited states, and the CPCM model with CH_3CN as solvent was applied to the solvent effect.

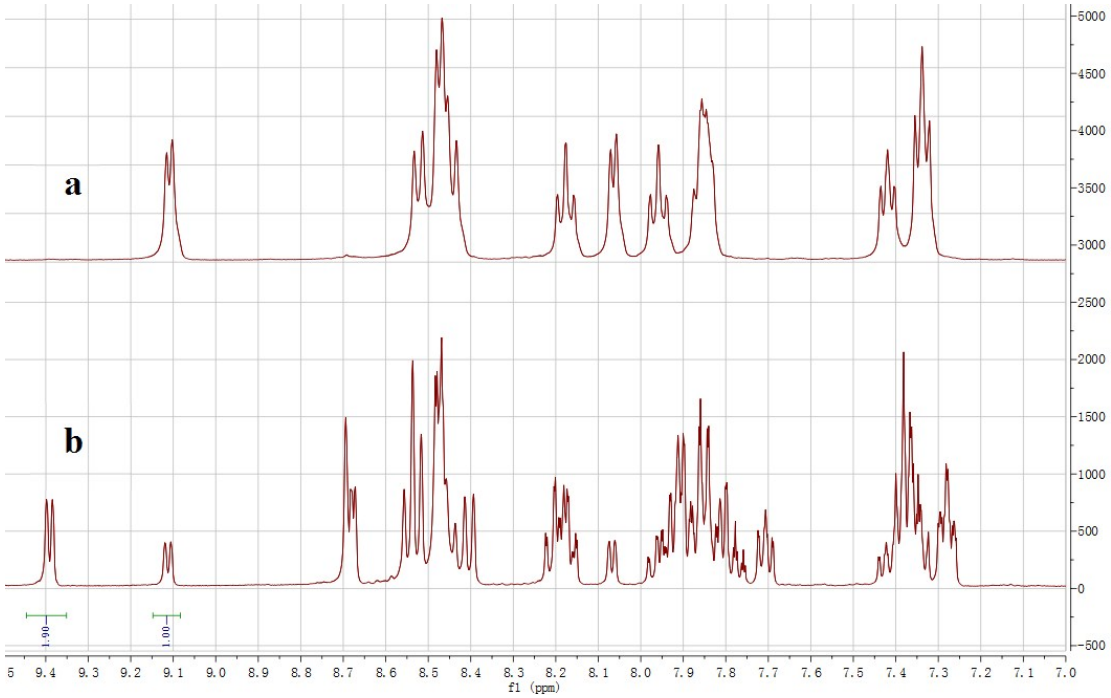


Figure S1. ^1H NMR spectra of $[Ru(bpy)_2(py)_2]^{2+}$ in CD_3COCD_3/D_2O (4/1) before (a) and after (b) irradiation ($\lambda > 470$ nm) for 45 min.

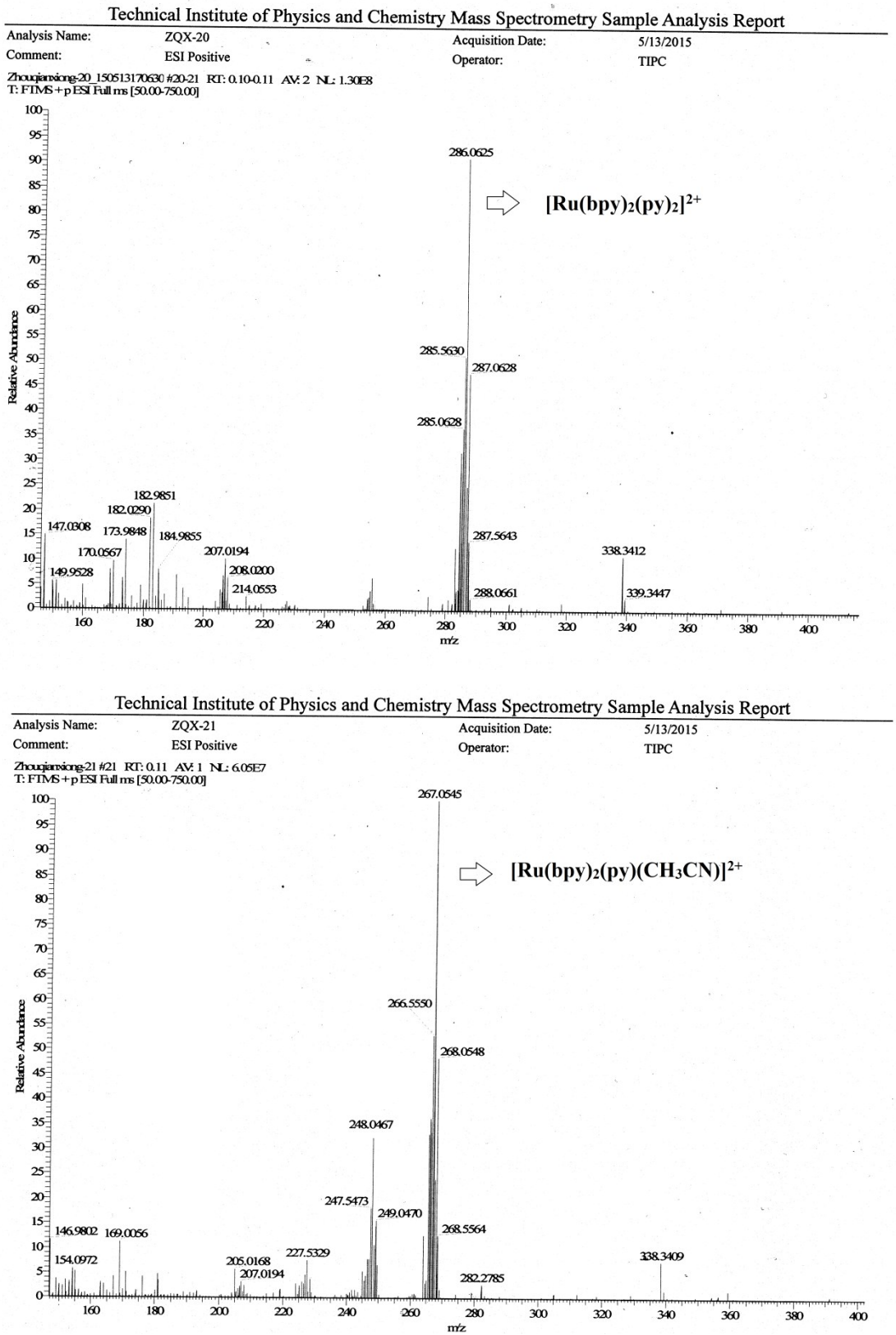


Figure S2. ESI-MS spectrum of $[Ru(bpy)_2(py)_2]^{2+}$ in CH_3CN before (top) and after (bottom) irradiation ($\lambda > 470$ nm) for 10 min.

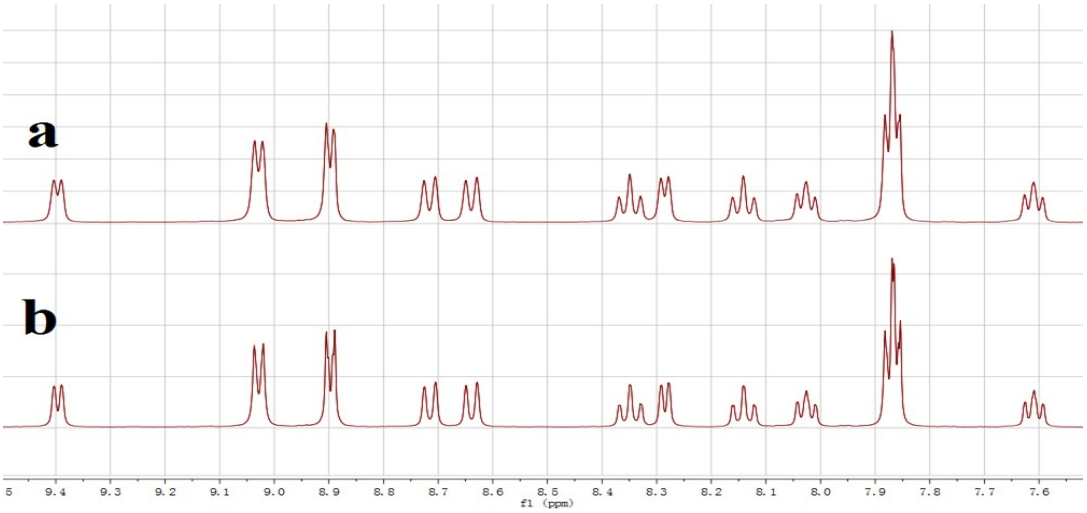


Figure S3. ^{1}H NMR spectra of **1** in CD_3COCD_3/D_2O (4/1) before (a) and after (b) irradiation ($\lambda >$ 470 nm) for 45 min.

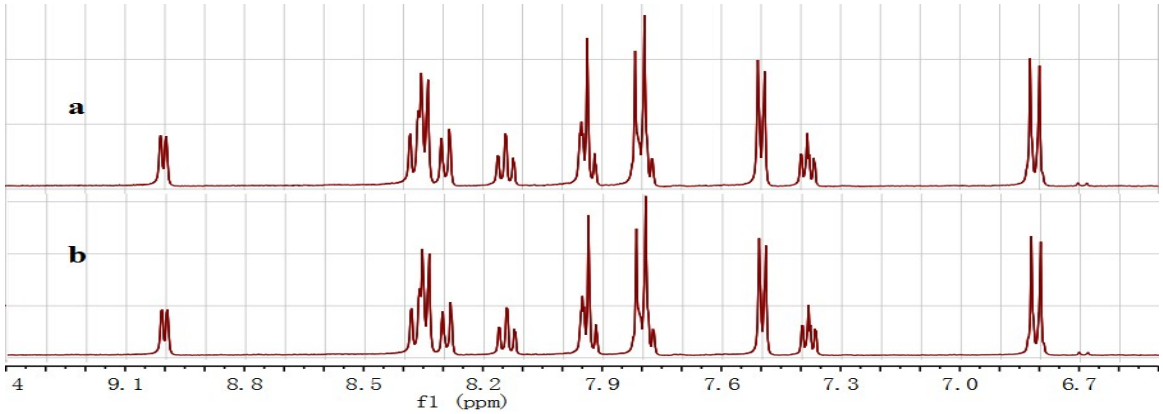


Figure S4. ^{1}H NMR spectra of **2** in CD_3COCD_3/D_2O (4/1) before (a) and after (b) light irradiation ($\lambda >$ 470 nm) for 45 min.

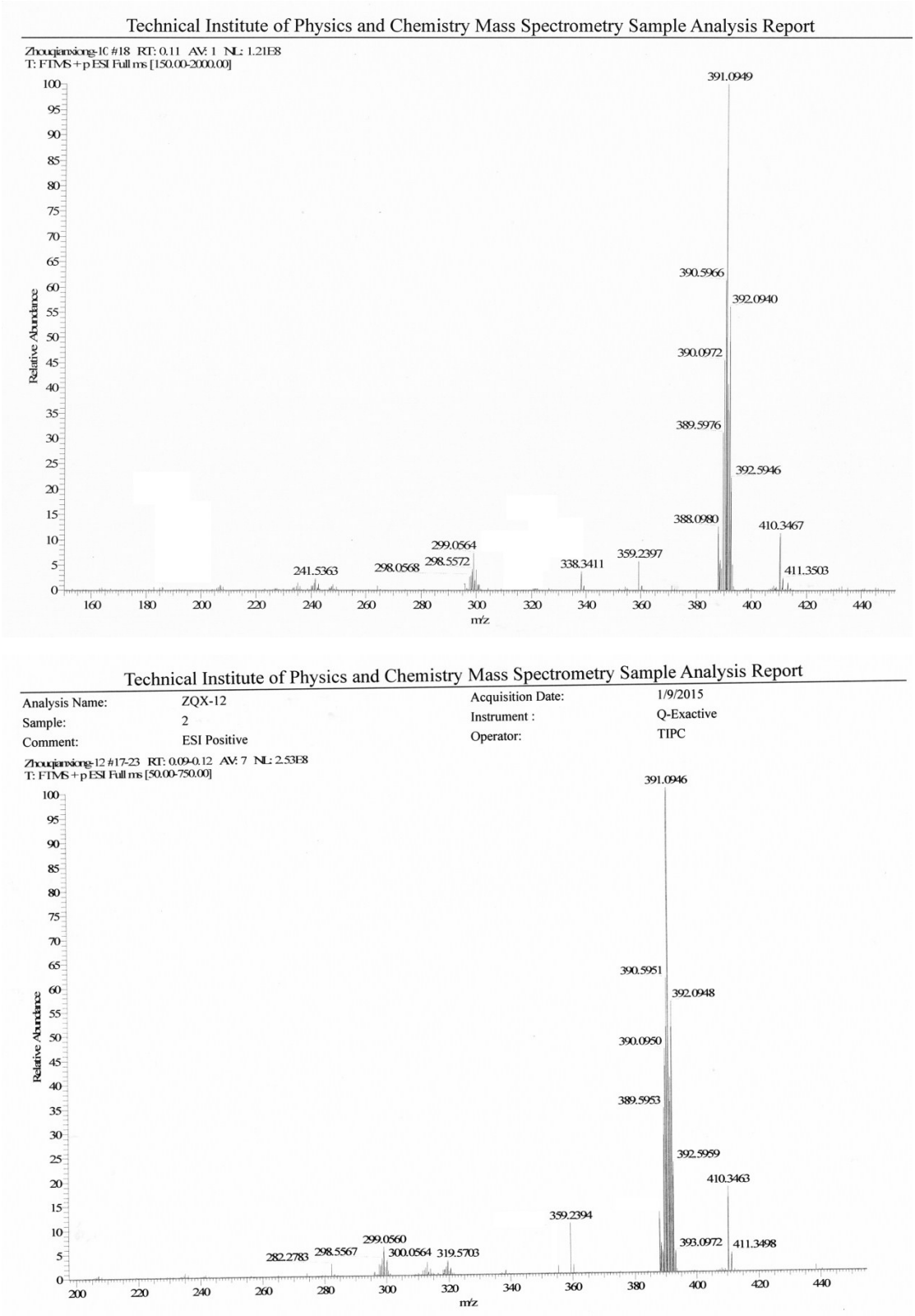


Figure S5. ESI-MS spectra of **1** in CH_3COCH_3/H_2O (4:1) before (top) and after (bottom) light irradiation ($\lambda > 470$ nm) for 45 min.

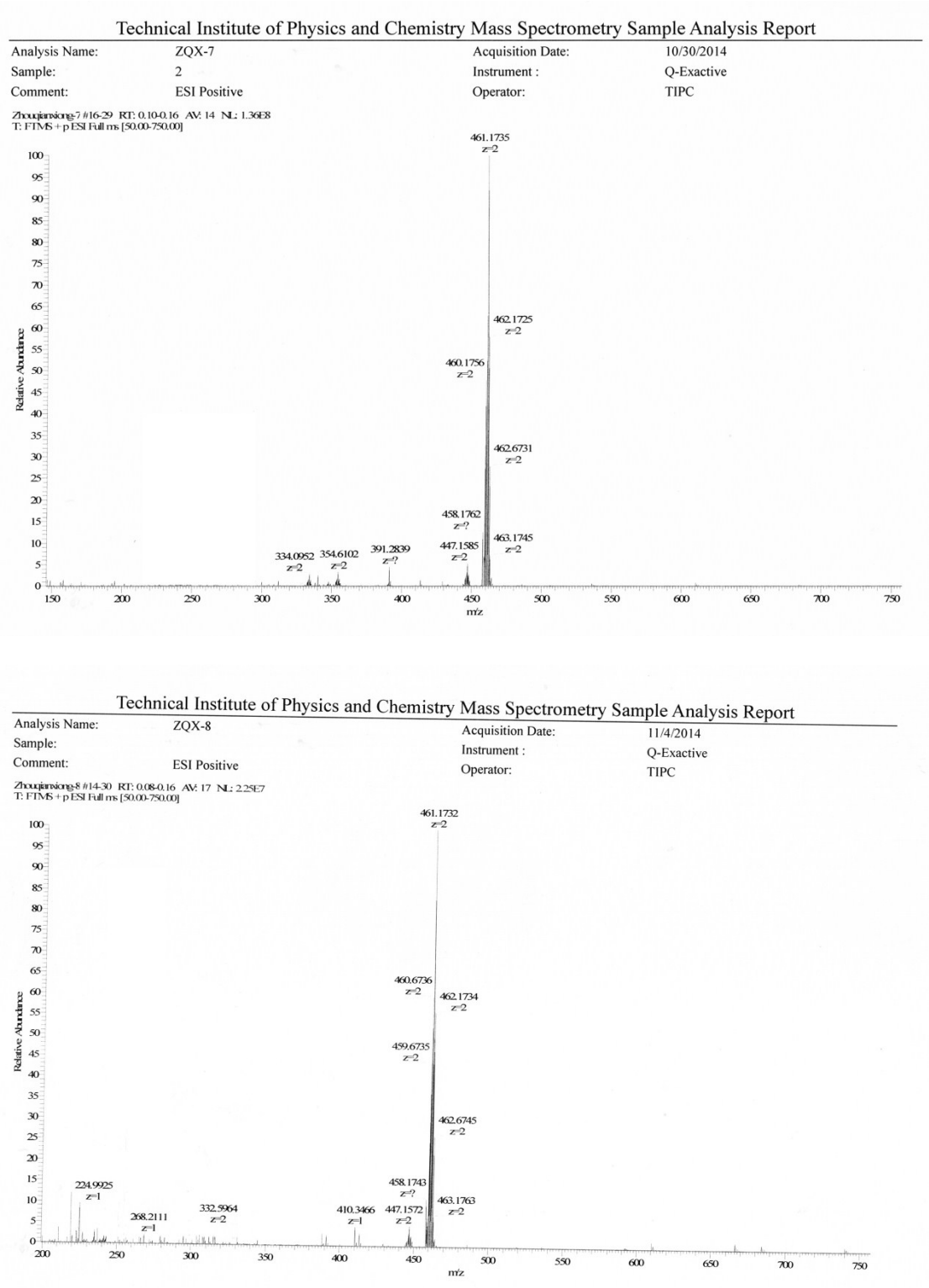


Figure S6. ESI-MS spectra of **2** in CH_3COCH_3/H_2O (4:1) before (top) and after (bottom) light irradiation ($\lambda > 470$ nm) for 45 min.

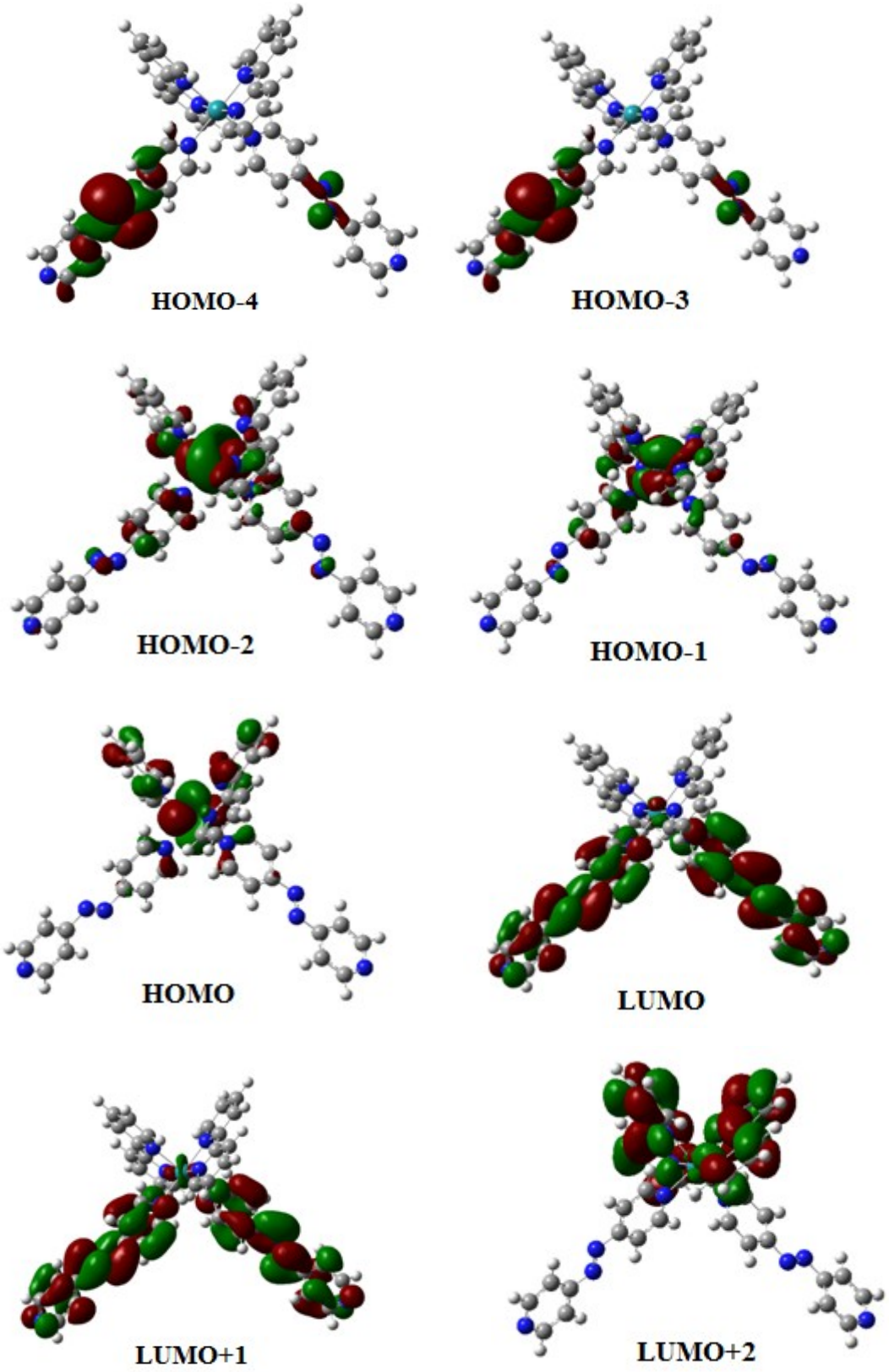


Figure S7. Selected molecular orbitals of **1** (*trans* configuration for azo-group, isovalues = 0.02).

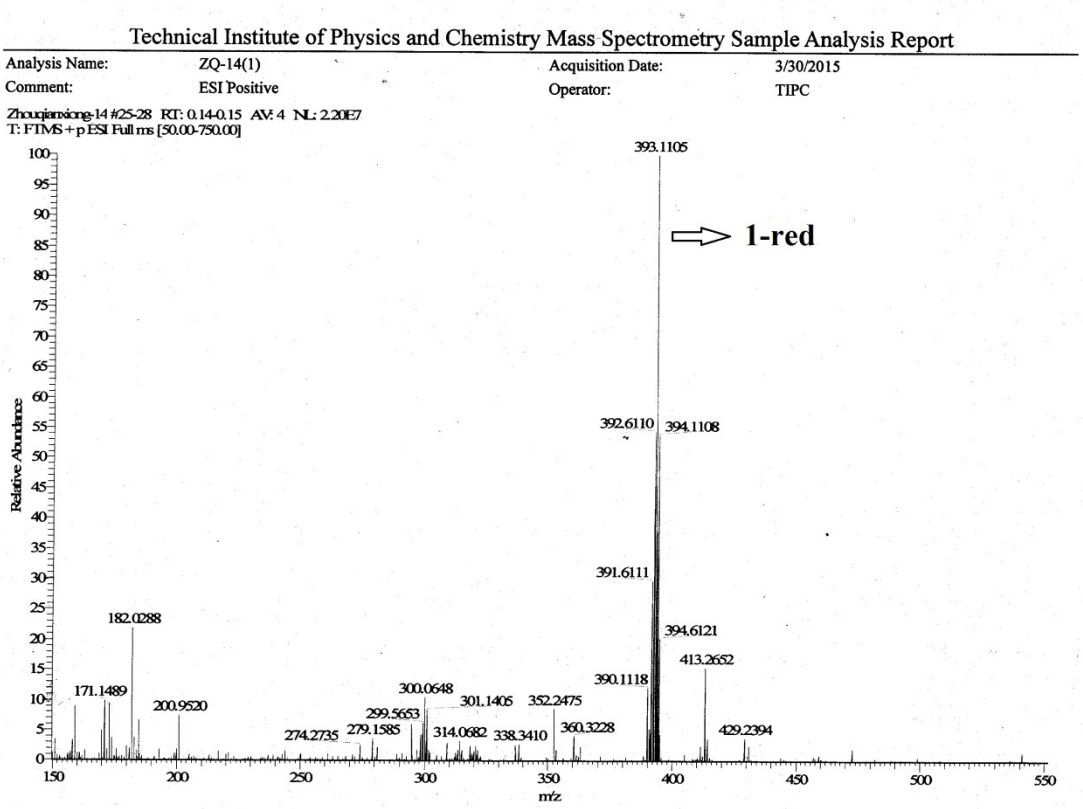


Figure S8. ESI-MS spectrum of **1** (10 μM) in H_2O after addition of GSH (1 mM) for 20 min. **1**-red = $[Ru(bpy)_2(py\text{-}NH\text{-}NH\text{-}py)_2]^{2+}$, py-NH-NH-py = 1,2-di(pyridine-4-yl)hydrazine.

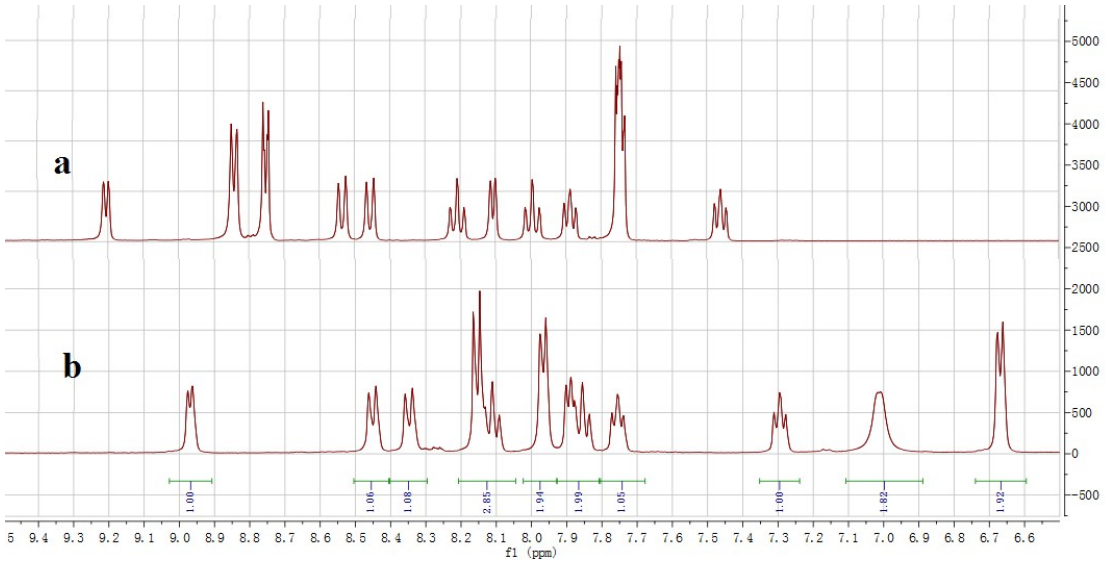


Figure S9. ^1H NMR spectra of **1** in CD_3COCD_3/D_2O (3/1) before (a) and after (b) GSH reduction.

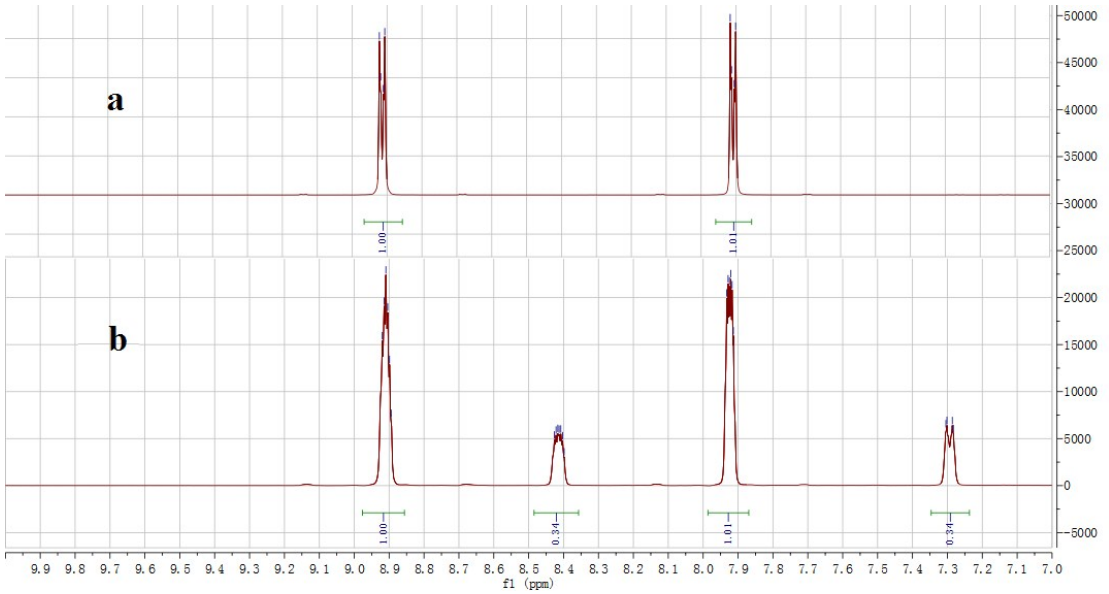


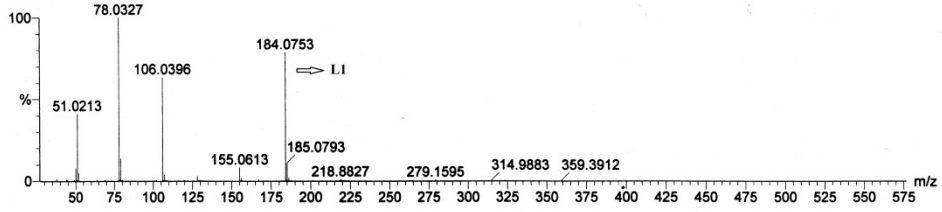
Figure S10. ^{1}H NMR spectra of L1 in CD_3COCD_3/D_2O (3/1) before (a) and after (b) GSH reduction.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 6 mass(es) processed - displaying only valid results
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Odd and Even Electron Ions
53 formula(e) evaluated with 2 results within limits (up to 56 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-20 N: 0-4
1
ZQX6 403 (6.718) Cm (403-(284+643))

TOF MS EI+
2.42e+003

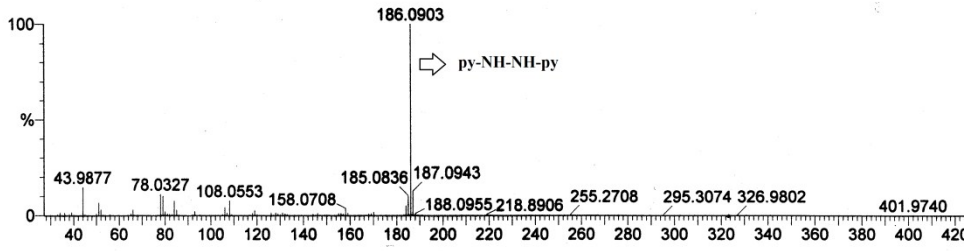


Minimum:	10.00				-1.5		
Maximum:	100.00		5.0	10.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
106.0396	63.44	106.0405	-0.9	-8.5	5.5	6.3	C5 H4 N3
184.0753	78.76	184.0749	0.4	2.2	9.0	11.1	C10 H8 N4

Elemental Composition Report

Multiple Mass Analysis: 6 mass(es) processed - displaying only valid results
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Odd and Even Electron Ions
51 formula(e) evaluated with 2 results within limits (up to 56 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-20 N: 0-4
2
ZQX7 537 (8.951) Cm (537-(441+822))



Minimum:	10.00				-1.5		
Maximum:	100.00		5.0	10.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
185.0836	10.09	185.0827	0.9	4.9	8.5	8002.4	C10 H9 N4
186.0903	100.00	186.0905	-0.2	-1.1	8.0	0.7	C10 H10 N4

Figure S11. EI-MS spectrum of **L1** before (top) and after (bottom) GSH reduction.

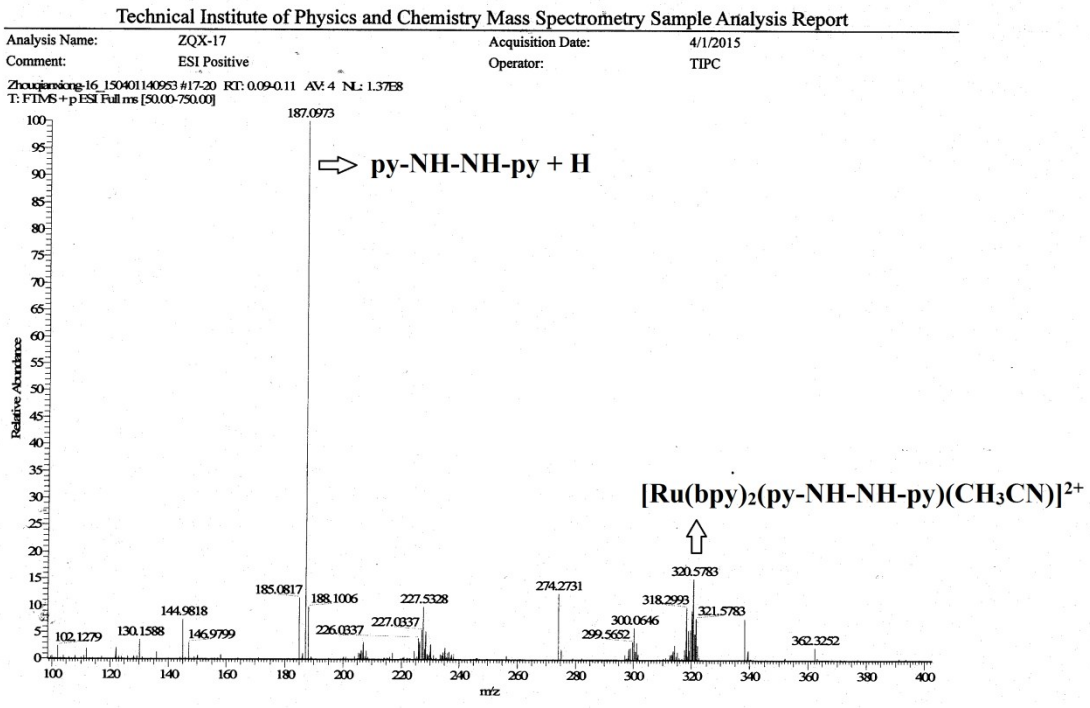


Figure S12. ESI-MS spectrum of **1**-red in CH_3CN after light irradiation ($\lambda > 470$ nm) for 10 min.

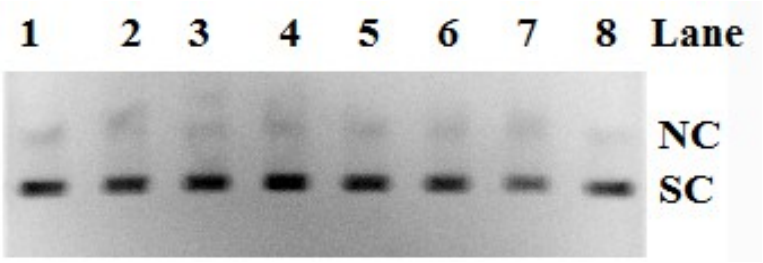


Figure S13. Agarose gel electrophoresis pattern of supercoiled pUC19 DNA (40 μg/ml) in Tris-CH_3COOH-EDTA buffer (pH = 7.4) in the presence of varied concentrations of **1** in the dark (Lane 1-4) or under light irradiation (λ > 470 nm) for 25 min (Lane 5-8). Lane 1: DNA alone; Lane 2: DNA + 60 μM **1**; Lane 3: DNA + 100 μM **1**; Lane 4: DNA + 160 μM **1**; Lane 5: DNA + 60 μM **1**; Lane 6: DNA + 100 μM **1**; Lane 7: DNA + 160 μM **1**; Lane 8: DNA alone. SC and NC denote supercoiled circular and nicked circular forms, respectively.

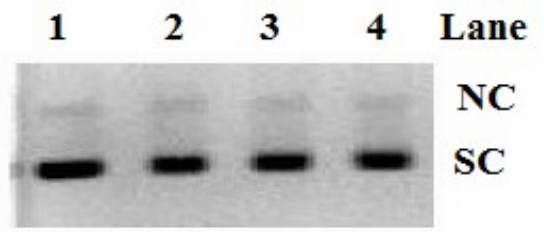


Figure S14. Agarose gel electrophoresis pattern of supercoiled pUC19 DNA (40 μg/ml) in Tris-CH_3COOH-EDTA buffer (pH = 7.4) in the presence of GSH (1 mM) and varied concentrations of **1**. All samples were kept in the dark for 20 min. Lane 1: DNA alone; Lane 2-4: the concentration of 1 are, respectively, 60, 100, and 160 μM. SC and NC denote supercoiled circular and nicked circular forms, respectively.

Table S1. Selected TDDFT triplet transitions of **1** in the ground-state optimized geometry (*trans* configuration for azo-group).

	Energy (eV)	**Wavelength (nm)**	**Oscillator Strength**	**Major Contributions**
1	1.658	747.9	0.0	H-4->LUMO (31%), H-4->LUMO+1 (-28%), H-3->LUMO (47%), H-3->LUMO+1 (-44%), H-3->L+19 (15%)
2	1.659	747.2	0.0	H-4->LUMO (45%), H-4->LUMO+1 (46%), H-4->L+20 (15%), H-3->LUMO (-29%), H-3->LUMO+1 (-30%)
3	2.060	601.8	0.0	H-12->LUMO (-27%), H-11->LUMO+1 (-28%), H-2->LUMO (43%), H-1->LUMO (-16%), H-1->LUMO+1 (37%), H->LUMO (-23%)
4	2.075	597.4	0.0	H-12->LUMO+1 (-27%), H-11->LUMO (-28%), H-2->LUMO (13%), H-2->LUMO+1 (40%), H-1->LUMO (36%), H->LUMO+1 (-28%)

References

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