Supporting Information

Highly phosphorescent N^C^N platinum(II)-peptide nucleic acid conjugates: synthesis, photophysical studies and hybridization behaviour

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I. Materials and methods

Synthesis of ligand L. Commercial reagents were used without further purification. 3-(3,5-dibromophenyl)propanoic acid (**1**) was purchased from BLD Pharmatech GmbH. 2-tributylstannyl pyridine (**3**), $PdCl_2(PPh_3)_2$ and K_2PtCl_4 were purchased from Merck Sigma-Aldrich. **Pt1** complex was synthesized according to literature.[1]

Thin-layer Chromatography (TLC) was performed with Supelco silica gel 60 matrix on Al foils with fluorescent indicator 254 nm. Column chromatography was carried out with Supelco silica gel (70-230 mesh). ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker DRX-400 MHz instrument. Chemical shifts are given as δ (ppm) relative to the residual protonated solvent resonances and coupling constant in Hz.

High-resolution mass spectra were acquired in a positive or negative polarity with Synapt G2-Si QToF instrument (Waters) interfaced through a ZsprayTM ESI-probe for electrospray ionization (Waters). Data were processed with a MassLynxTM v4.2 software (Waters), and the deconvoluted HR-MS spectra were obtained using an integrated MaxEnt (Ferrige et al., 1991 https://doi.org/10.1002/rcm.1290050810) tool.

Solid phase synthesis of PNA decamer and the corresponding Pt(II)-conjugates PNA-Pt1 and PNA-Pt2. The unconjugated PNA (**unPNA**) was prepared by Solid Phase Synthesis (SPS) according to the established Fmoc/Bhoc strategy.[2] The protected PNA monomers (Fmoc-PNA-T-OH; Fmoc-PNA-C(Bhoc)-OH; Fmoc-PNA-G(Bhoc)-OH; Fmoc-PNA-A(Bhoc)-OH) were purchased from ASM Research Chemicals GmbH (Hannover, Germany). The H-rink amide ChemMatrix was purchased from Merck. Polypropylene one-way syringes (1.5 mL or 4 mL) and corresponding PTFE frits, used as reaction vessels for the manual SPS, were purchased from SepaChrom (Milan, Italy). SPS syntheses were performed using an orbital rotator shaker at 500 rpm. Centrifugation steps were performed using a 0.6 LISA centrifuge.

The RP-HPLC purifications of **Pt1-PNA** and **Pt2-PNA** conjugates were performed on an Agilent 1200 Series system, using the semi-preparative column Luna C18 (250 × 10 mm, 5 μm) at a flow rate of 3 mL/min. Solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) were used in the following gradient: 0% B (5 min), B 0 - 50% (30 min), B 50 - 100% (1 min), B 100% (5 min), B 100-0% (1 min), B 0% (3 min). This method was used with detection by UV (220, 260, and 280 nm). The RP-HPLC analyses of the purified Pt-PNA conjugates were performed using the analytical column Luna Omega (150 × 4.6 mm, 5 μm) at a flow rate of 1 mL/min, using the same gradient reported for the semi-preparative column.

Melting temperature and circular dichroism spectroscopy studies. Melting temperatures (T_m) were determined by measuring the changes in the absorbance at 260 nm (and 280 nm as a control), in 10 mM phosphate buffer adjusted to pH 7, containing 20 mM NaCl, using dual-beam UV−vis Nicolet Evolution 300 spectrophotometer equipped with the Peltier module (Thermo Scientific). Oligonucleotides were prepared at 4 μM concentration of each strand, and for complexes they were mixed in a 1:1 ratio. The temperature was changed from 10 °C to 110 °C at a rate of 1 °C/min. The T_m were calculated as the inflection point on the two-state transition curve derived from the change in absorbance at 260 nm.[3] The presented T_m are averages from six measurements.

Circular dichroism (CD) spectra were collected using the Biokine MOS-450/AF-CD spectrometer equipped with the Xe lamp. The samples were prepared in a 0.1 cm CD cell. The acquisition duration time was 5 s with a resolution of 0.5 nm. The measurements were performed in 10 mM phosphate buffer adjusted to pH 7, containing 20 mM NaCl, in the wavelength range 190-320 nm and at room temperature. The reference CD spectrum of plain buffer was subtracted from all recorded spectra. Oligonucleotides were prepared at 4 μM concentration of each strand, and for complexes they were mixed in a 1:1 ratio. The graphs presenting the CD spectra were smoothed with the Savitzky-Golay method and are the averages of three scans. Each CD experiment was conducted twice.

Photophysical studies. Electronic absorption spectra at different concentrations were registered using a Shimadzu UV3600 spectrophotometer and quartz cuvettes with 1 cm optical path length. The employed solvents were dichloromethane in the case of **Pt1** and **Pt2**, and MilliQ water for **Pt2-PNA**.

Steady-state and time-resolved fluorescence data were obtained using an FLS980 spectrofluorimeter (Edinburgh Instrument Ltd). Emission spectra were corrected for background intensity and quantum efficiency of the photomultiplier tube; excitation spectra were corrected for the intensity fluctuation of a 450 W Xenon arc lamp.

Luminescence measurements were carried out after three Freeze-Pump-Thaw (FPT) cycles to remove dissolved oxygen. The employed solvents were dichloromethane in the case of **Pt1** and **Pt2**, and MilliQ water for **Pt2-PNA**.

Excitation and emission spectra were registered at different concentrations, using quartz cuvettes with 1 cm optical path length for diluted solutions, meanwhile quartz cuvettes of 2 mm optical path length for concentrated solutions, to avoid the inner filter effect.

Absolute photoluminescence quantum yield (Φ) for a solution or thin film was measured using a C11347 Quantaurus Hamamatsu Photonics K.K spectrometer. A description of the experimental setup and measurement method can be found in the article of K. Suzuki *et al.*[4] Φ was calculated through Equation:

$$\Phi = \frac{PN\ (Em)}{PN\ (Abs)} = \frac{\int \frac{\lambda}{hc}\left[I_{em}^{sample}(\lambda) - I_{em}^{reference}(\lambda)\right]d\lambda}{\int \frac{\lambda}{hc}\left[I_{exc}^{sample}(\lambda) - I_{exc}^{reference}(\lambda)\right]d\lambda}$$

where *PN(Em)* is the number of emitted photons, *PN(Abs)* the number of absorbed photons, λ the wavelength, h the Planck's constant, c the speed of light, I_{em}^{sample} and $I_{em}^{reference}$ the photoluminescence intensities of the sample and reference, I_{exc}^{sample} and $I_{exc}^{reference}$ the excitation light intensities of the sample and reference. *PN(E_m)* is calculated in the wavelength interval [λ_i, λ_f], where λ_i is taken at about 10 nm above the excitation wavelength, while λ_f is the upper end wavelength in the emission spectrum. The estimated error is 0.1%.

Time-resolved fluorescence measurements were performed through the time-correlated single photon counting technique with an Edinburgh Picosecond Pulsed Diode Laser (emitted wavelength 374 nm) or by multi-channel scaling (MCS) method using a pulsed Xenon arc lamp. Moreover, time-resolved fluorescence curves were fitted using a multi-exponential function:

$$I(\lambda,t) = \sum_{i=1}^{m} \alpha_i(\lambda) exp\left(\frac{-t}{\tau_i}\right)$$

where *m* is the number of exponentials, $\alpha_i\ (\lambda)$ is the amplitude at wavelength λ and τ_i is the lifetime of the component *i*. The quality of the fit was evaluated through the reduced χ^2values.

In the case of multi-exponential decay, an average lifetime is defined as:

$$\tau_{av} = \frac{\sum_{n=1}^{m} \alpha_n \tau_n^2}{\sum_{n=1}^{m} \alpha_n \tau_n}$$

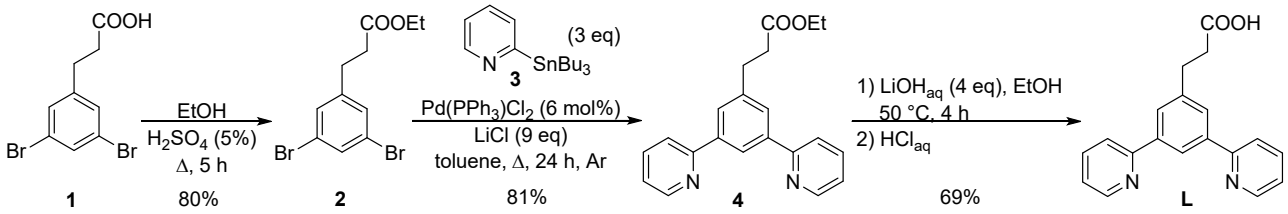
where *m* is the multi-exponential decay number of the fit.

In the case of excimers, the fit of the decay was performed according to an equation of the type:

$$I(t) = \left[A_1 exp\left(-\frac{t}{\tau_1}\right) - A_2 exp\left(-\frac{t}{\tau_2}\right)\right] + b$$

where the fitting parameters (A_1, A_2), (τ_1, τ_2), and b are pre-exponential coefficients, the lifetimes, and the background intensity, respectively.

II. Synthesis of ligand L



Scheme S1. Synthesis of ligand **L**

Ethyl 3-(3,5-dibromophenyl)propanoate (2). To a solution of 3,5-dibromophenylpropionic acid (**1**) (200 mg, 0.658 mmol) in ethanol (66 mL), a solution of H_2SO_4 in ethanol (5 %, 2 mL) was added and the resulting mixture was stirred at reflux for 5 hours. The mixture was then cooled to room temperature and EtOH was removed under reduced pressure. The residue was taken up with DCM (20 mL) and the organic phase was washed with a 1 M aqueous solution of NaOH (10 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The product **2** was obtained as a colorless oil (176 mg, 80%) and was used in the next step without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.50 (t, J =1.6 Hz, 1H, CH), 7.29 (d, J = 1.6 Hz, 2H, CH), 4.14 (q, J = 7.1 Hz, 2H, CH_2), 2.89 (t, J = 7.6, 2H, CH_2), 2.60 (t, J = 7.6, 2H, CH_2), 1.24 (t, J =7.1 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 172.0 (C=O), 144.5 (C_q), 132.0 (CH), 130.3 (2CH), 122.9 ($2C_q$), 60.6 (CH_2), 35.2 (CH_2), 30.2 (CH_2), 14.2 ppm (CH_3). HRMS (ESI$^+$) *m/z*: [M + Na] calcd for $C_{11}H_{12}O_2Br_2Na$ 356.9102, found 356.9098.

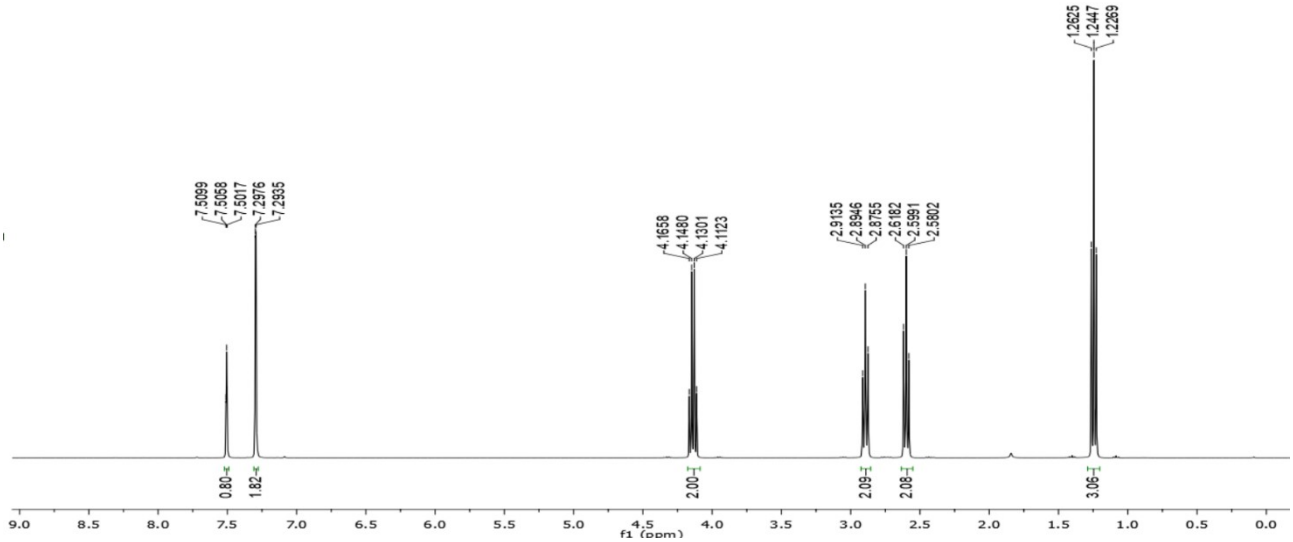
Ethyl 3-(3,5-di(pyridin-2-yl)propanoate (4). To a flame-dried Schlenk tube ester **2** (340 mg, 0.905 mmol), $PdCl_2(PPh_3)_2$ (38 mg, 0.054 mmol, 6 mol%), and LiCl (345 mg, 8.145 mmol, 9 eq) were added. The reaction vessel was fitted with a silicon septum, evacuated, and back-filled with argon, and this sequence was repeated twice. Anhydrous toluene (7 mL) and 2-tributhylstannylpyridine (**3**) (877 µL, 2.716 mmol, 3 eq) were added under a stream of argon by syringe at room temperature, and the reaction mixture was stirred at reflux for 24 hours. After being cooled to room temperature, the reaction mixture was diluted with AcOEt, poured into an aqueous solution of NaOH (1 M, 50 mL), and the resulting mixture was stirred in the open air for 20 min and then extracted with AcOEt (3 × 50 mL). The collected organic phases were dried, and concentrated under reduced pressure and the residue was purified by chromatography on silica gel with a mixture of hexane: AcOEt (from 7:3 to 5:5) to afford the

desired product **4** (244 mg, 81%) as a yellowish oil. ^{1}H-NMR (400 MHz, CD_2Cl_2) δ: 8.73 (dd, J_1 = 1.8 Hz, J_2 = 5.1 Hz, 2H, CH), 8.55 (t, J = 1.6 Hz, 1H, CH), 7.99 (d, J = 1.6 Hz, 2H, CH), 7.89 (d, J = 8.0 Hz, 2H, CH), 7.83 (ddd, J_1 = 1.8 Hz, J_2 = 7.4 Hz, J_3 = 8.0 Hz, 2H, CH), 7.30 (ddd, J_1 = 1.2 Hz, J_2 = 5.1 Hz, J_3 = 7.4 Hz, 2H, CH), 4.15 (q, J = 7.1 Hz, 2H, CH_2), 3.15 (t, J = 7.6 Hz, 2H, CH_2), 2.77 (t, J = 7.6 Hz, 2H, CH_2), 1.26 ppm (t, J = 7.1 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_2Cl_2) δ:172.7 (C=O), 158.9 (C_q), 149.6 (CH), 141.8 (C_q), 140.0 (C_q), 136.7 (CH), 127.4 (CH), 123.3 (CH), 122.3 (CH), 120.5 (CH), 60.4 (CH_2), 35.9 (CH_2), 31.1 (CH_2), 14.0 ppm (CH_3). HRMS (ESI$^+$) *m/z*: [M + H] calcd for $C_{21}H_{21}N_2O_2$ 333.1603, found 333.1603; [M + Na] calcd for $C_{21}H_{20}N_2O_2Na$ 355.1422, found 355.1422.

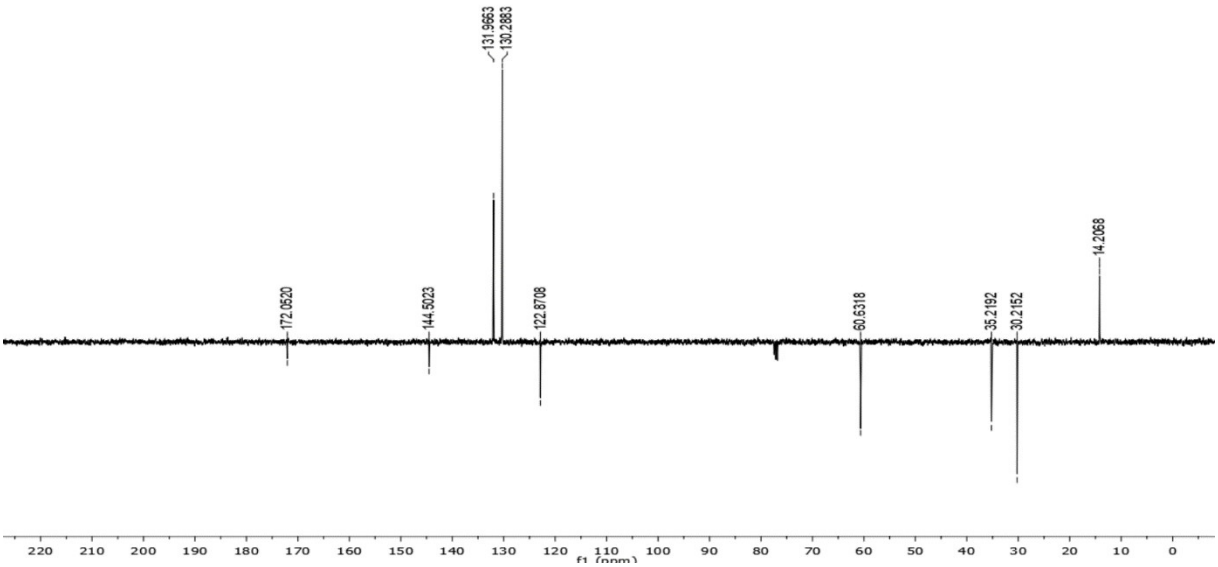
3-(3,5-di(pyridin-2-yl)propanoic acid (L). To a solution of **4** (90 mg, 0.271 mmol) in ethanol (1.6 mL) a solution of LiOH (26 mg, 1.084 mmol, 4 eq) in water (0.5 mL) was added and the final mixture was stirred at 50 °C for 4 hours. After the completion of the reaction, the mixture was cooled to room temperature and poured into a 3 M aqueous solution of HCl (10 mL). The aqueous phase was extracted with AcOEt (6 x 10 mL) and the collected organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The ligand **L** was obtained as a white solid (57 mg, 69%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.14 (s, 1H, -COOH), 8.71 (dd, J_1 = 1.8 Hz, J_2 = 5.2 Hz, 2H, CH), 8.62 (t, J = 1.6 Hz, 1H, CH), 8.07 (d, J = 8.0 Hz, 2H, CH), 8.03 (d, J = 1.6 Hz, 2H, CH), 7.92 (ddd, J_1 = 1.8 Hz, J_2 = 7.7 Hz, J_3 = 8.0 Hz, 2H, CH), 7.39 (ddd, J_1 = 0.9 Hz, J_2= 5.2 Hz, J_3 = 7.7 Hz, 2H, CH), 3.02 (t, J = 7.6 Hz, 2H, CH_2), 2.68 ppm (t, J = 7.6 Hz, 2H, CH_2). ^{13}C-NMR (100 MHz, DMSO-d_6) δ:174.2 (C=O), 158.7 (C_q), 150.0 (CH), 142.5 (C_q), 140.0 (C_q), 137.7 (CH), 127.7 (CH), 123.2 (CH), 123.1 (CH), 121.0 (CH), 35.7 (CH_2), 31.0 ppm (CH_2). HRMS (ESI$^+$) *m/z*: [M + H] calcd for $C_{19}H_{17}N_2O_2$ 305.1290, found 305.1289; [M + Na] calcd for $C_{19}H_{16}N_2O_2Na$ 327.1109, found 327.1109.

III. ^{1}H and ^{13}C NMR spectra

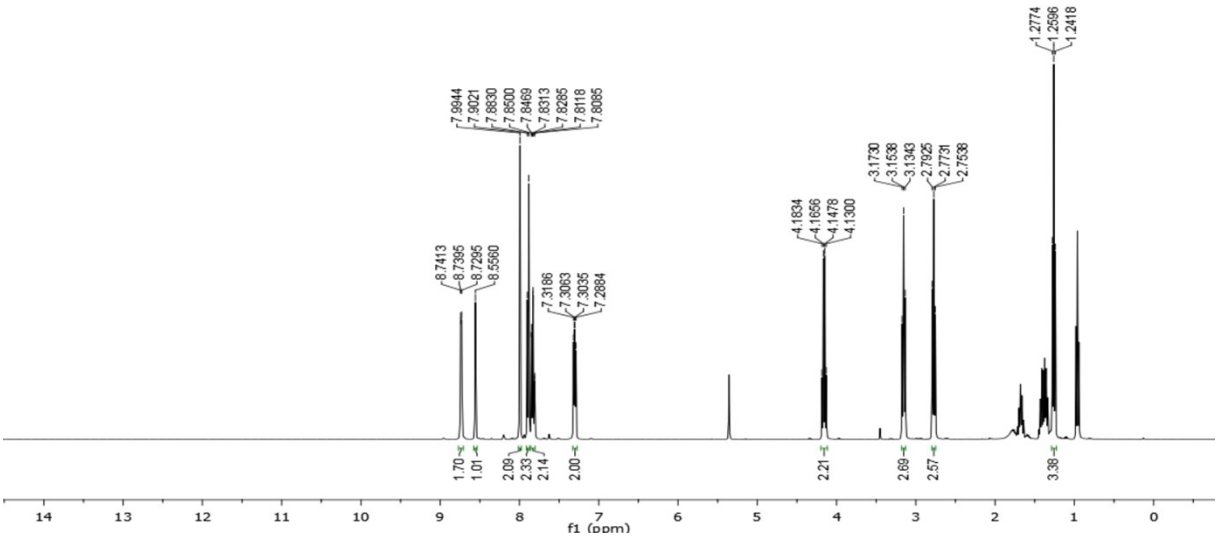
^{1}H NMR spectrum of **2** (400 MHz, $CDCl_3$)



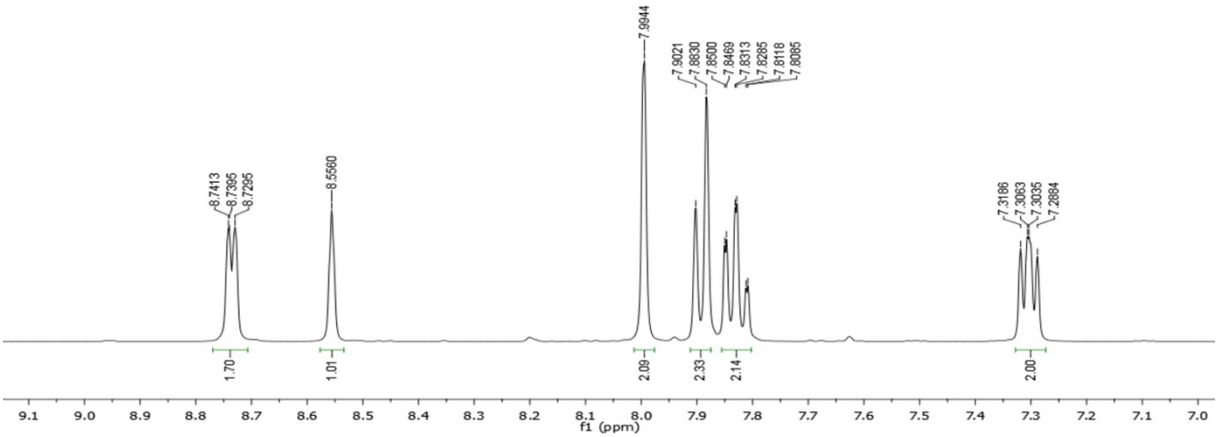
^{13}C NMR spectrum of **2** (100 MHz, $CDCl_3$)



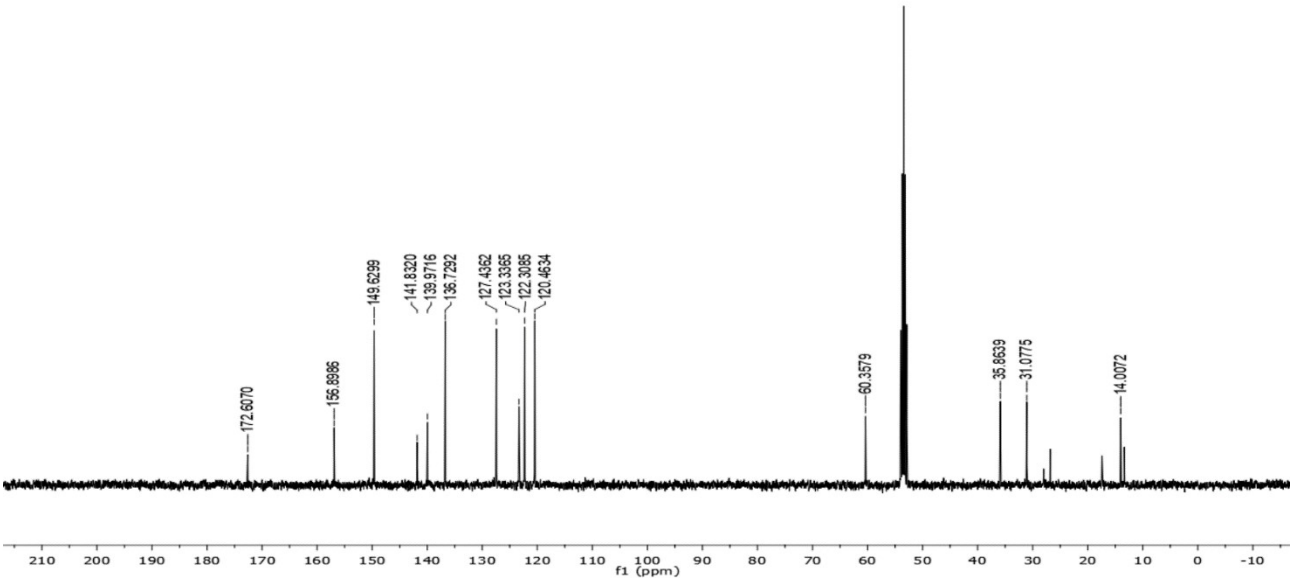
^{1}H NMR spectrum of **4** (400 MHz, CD_2Cl_2) (full spectrum)



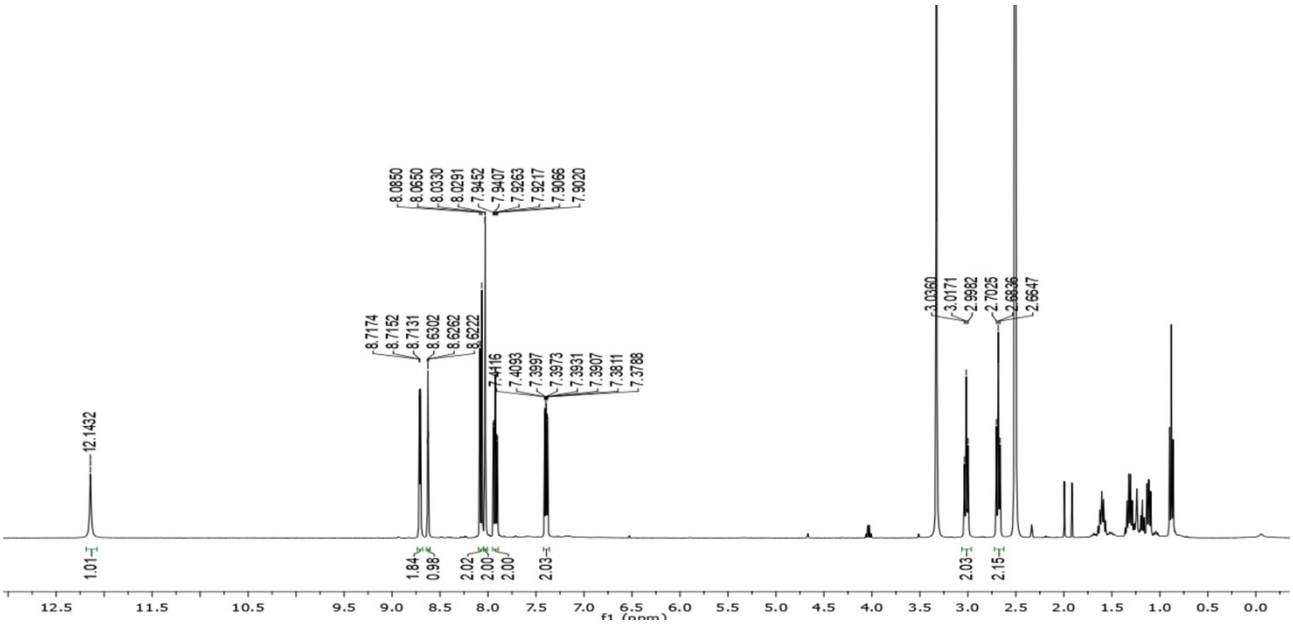
^{1}H NMR spectrum of **4** (400 MHz, CD_2Cl_2) (aromatic region)



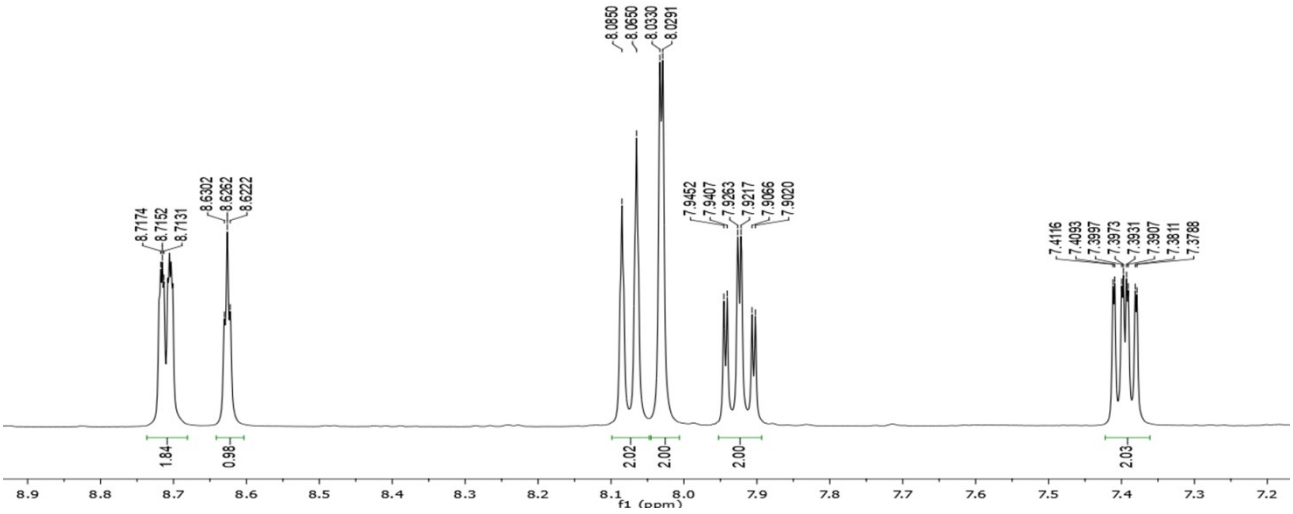
^{13}C NMR spectrum of **4** (100 MHz, CD_2Cl_2)



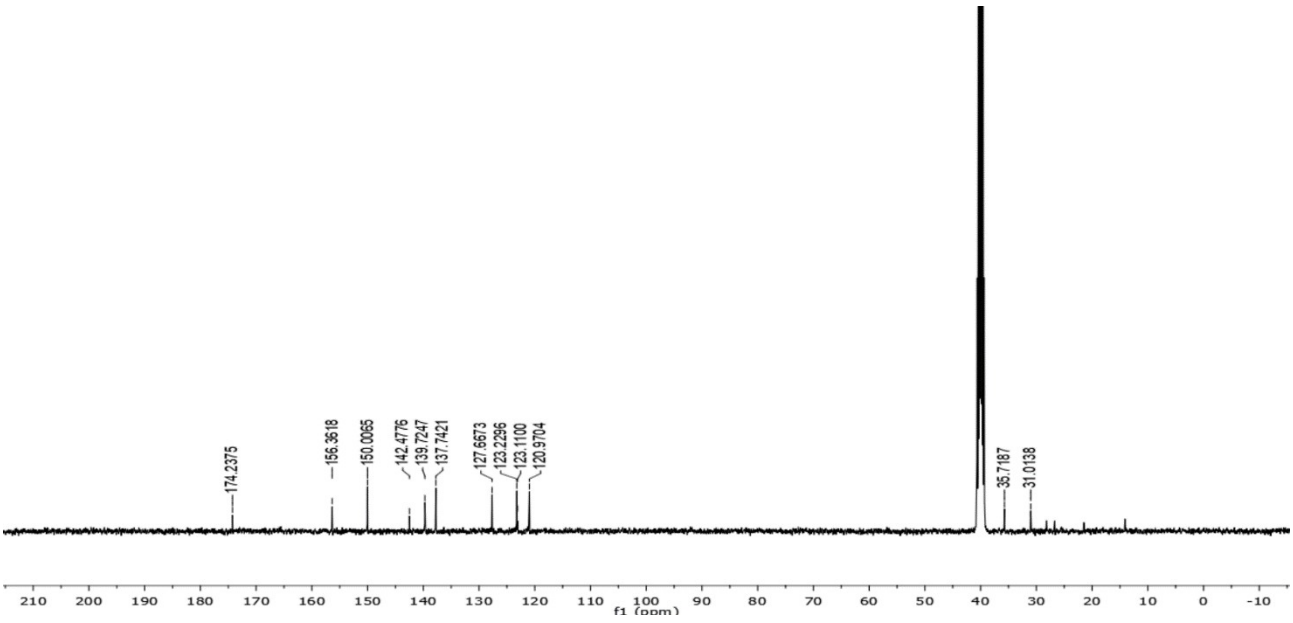
^{1}H NMR spectrum of **L** (400 MHz, DMSO-d_6) (full spectrum)



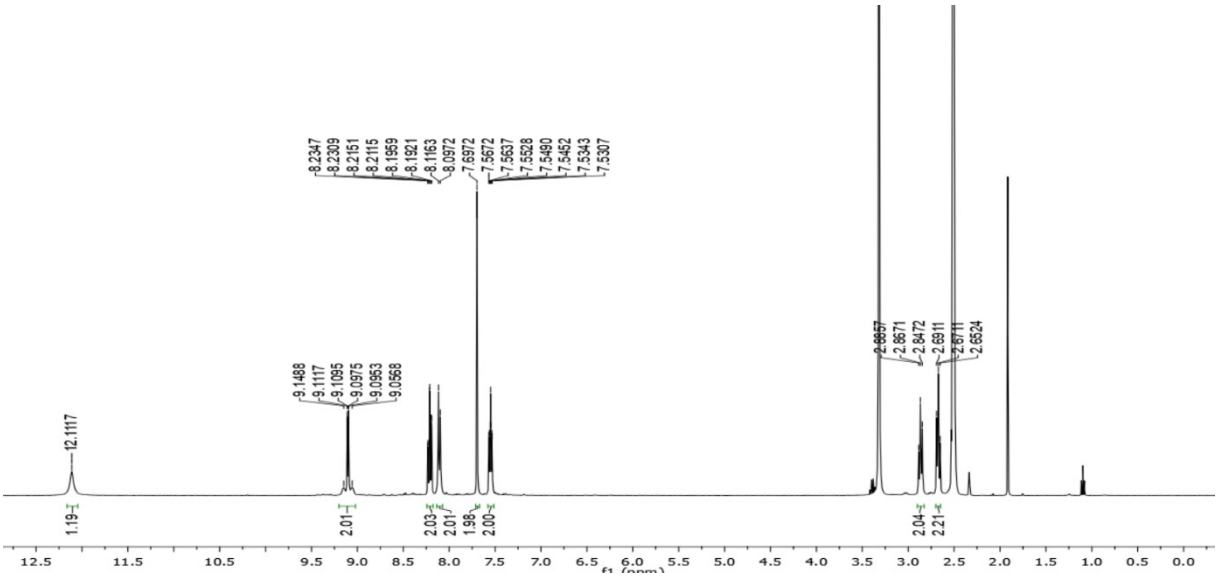
^{1}H NMR spectrum of **L** (400 MHz, DMSO-d_6) (aromatic region)



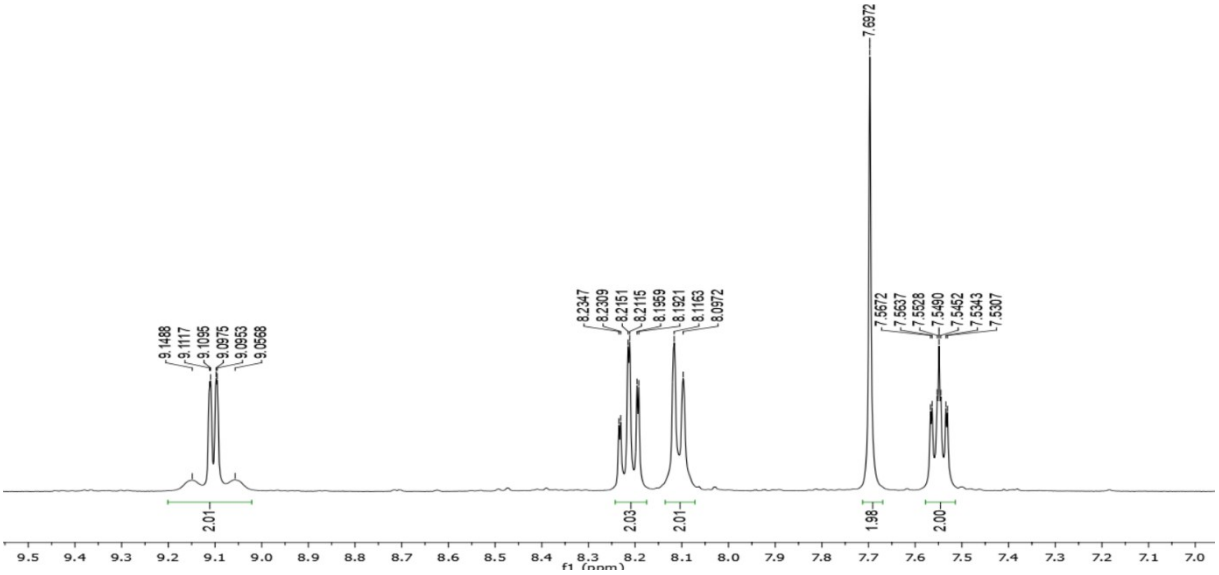
^{13}C NMR spectrum of **L** (100 MHz, DMSO-d_6)



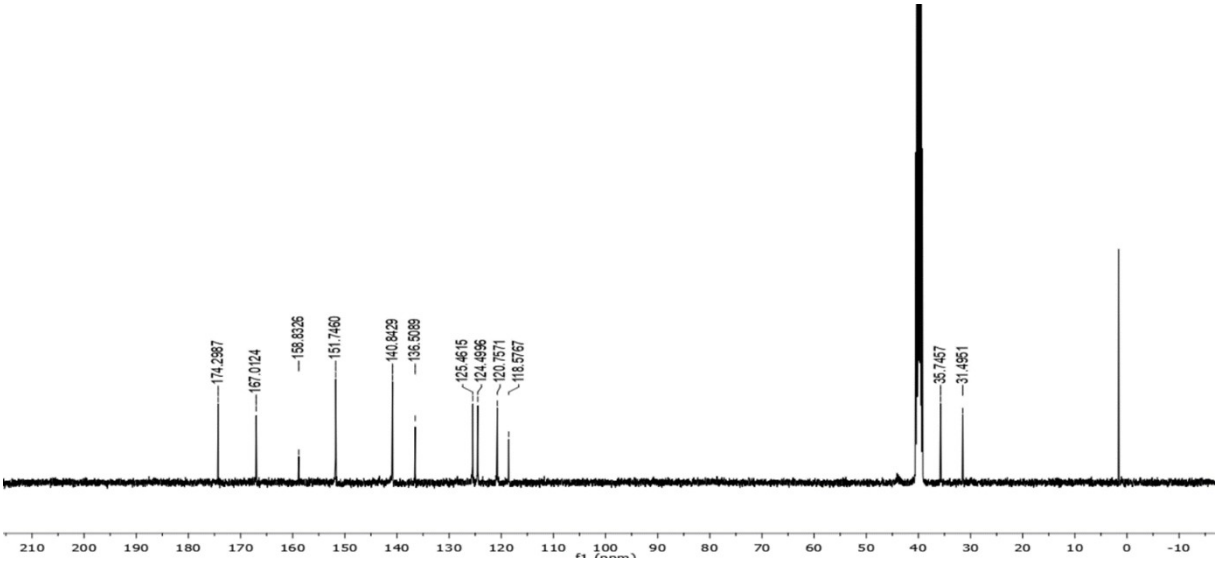
^{1}H NMR spectrum of **Pt2** (400 MHz, DMSO-d_6) (full spectrum)



^{1}H NMR spectrum of **Pt2** (400 MHz, DMSO-d_6) (aromatic region)

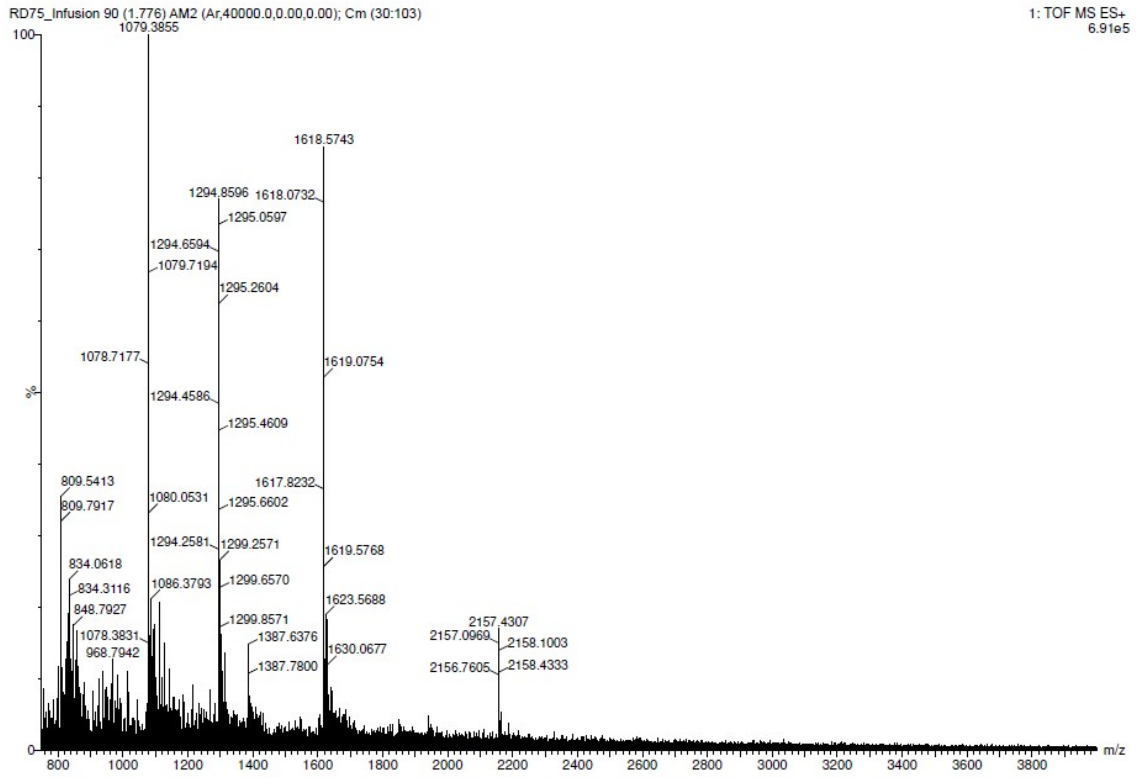


^{13}C NMR spectrum of **Pt2** (100 MHz, DMSO-d_6)

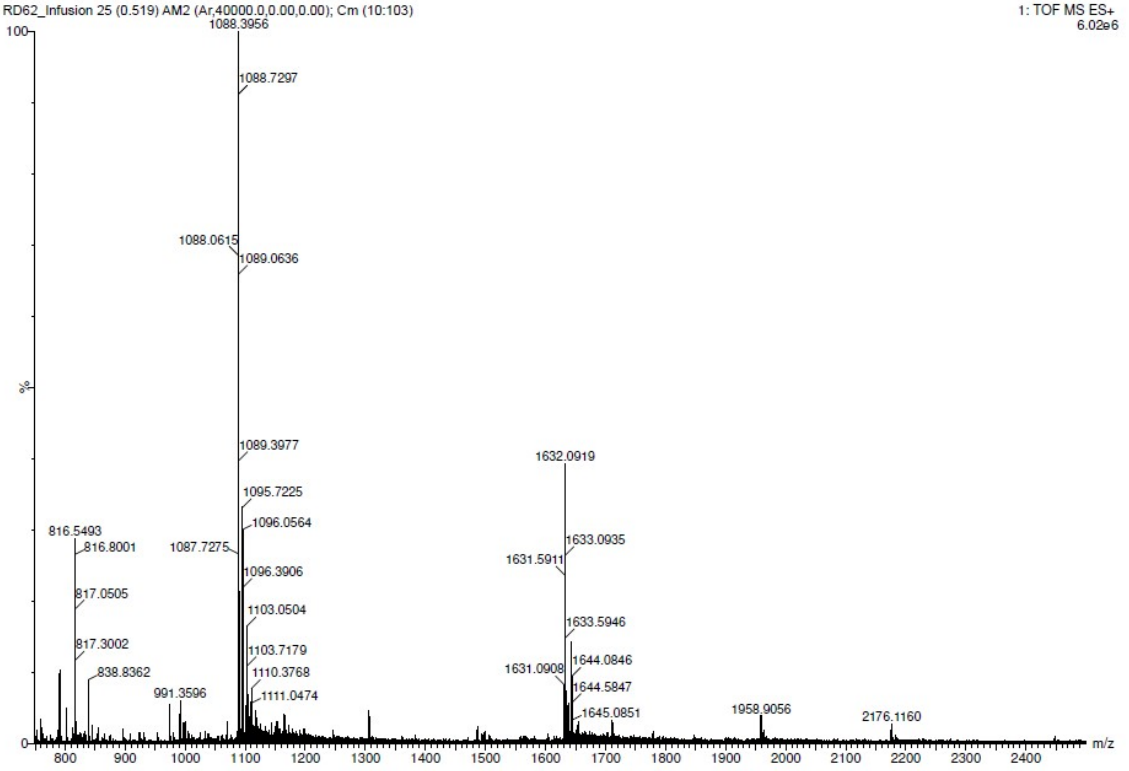


IV. HRMS spectra and RP-HPLC of Pt-PNA conjugates

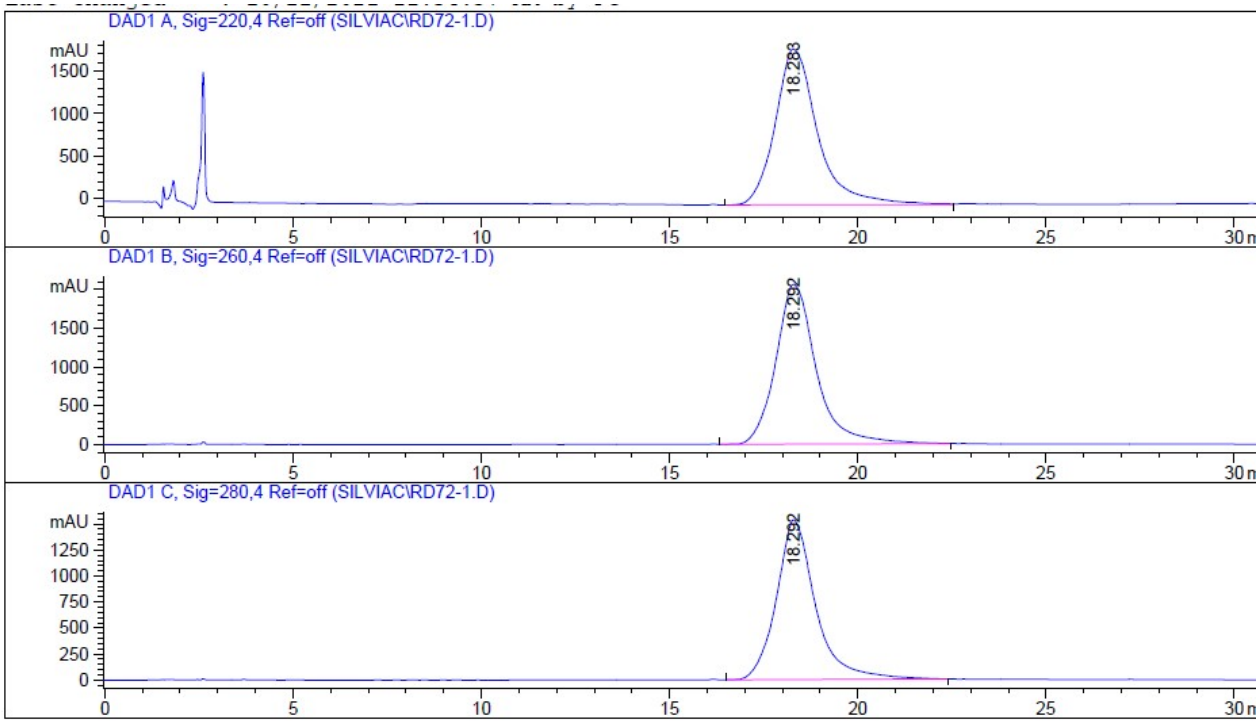
HRMS ESI$^+$ spectrum of **Pt1-PNA**



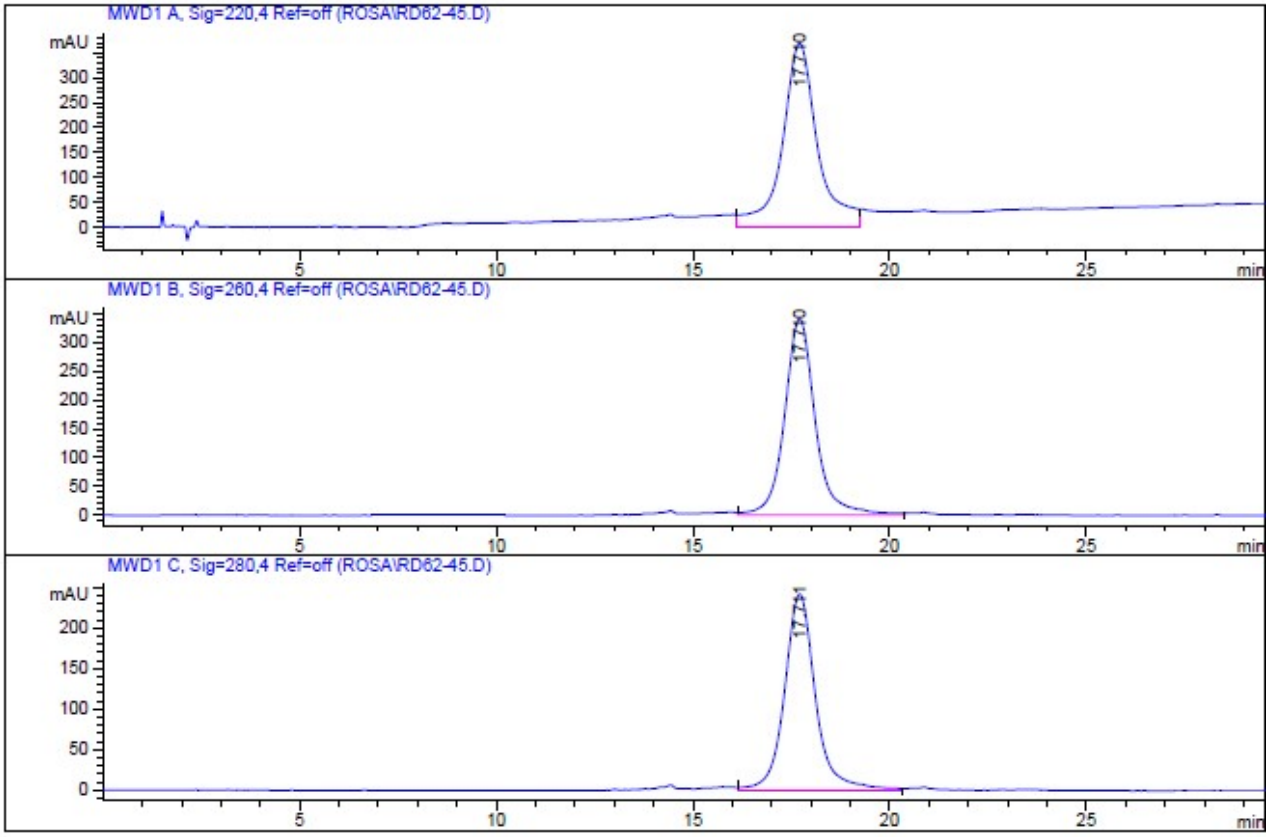
HRMS ESI$^+$ spectrum of Pt2-PNA



RP-HPLC trace of **Pt1-PNA**



RP-HPLC trace of **Pt2-PNA**



V. Melting temperatures

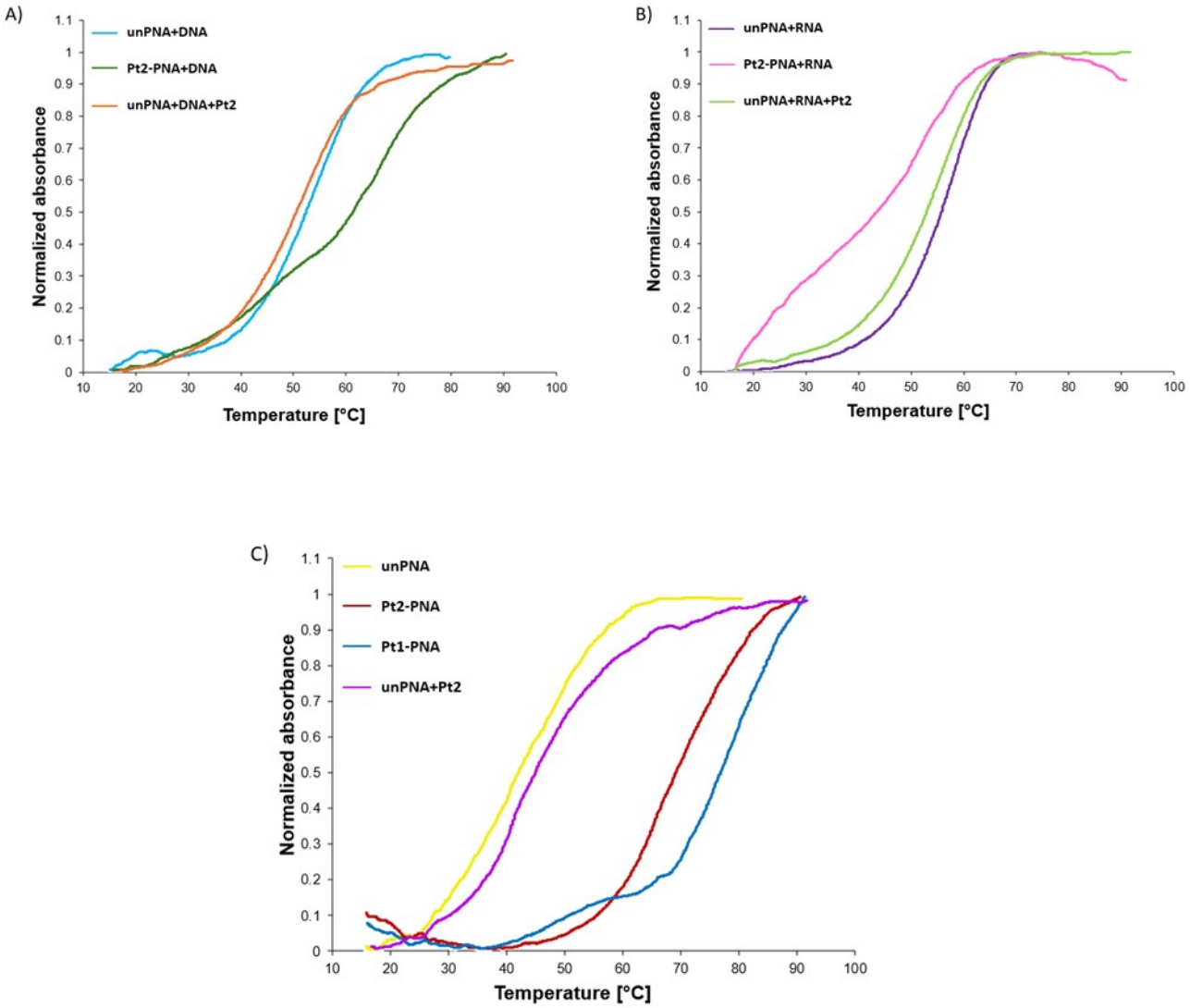


Figure S1. Normalized absorbance obtained from UV-monitored melting of the samples as a function of temperature

VI. Photophysical data in solution

Photophysical characterization of Pt2

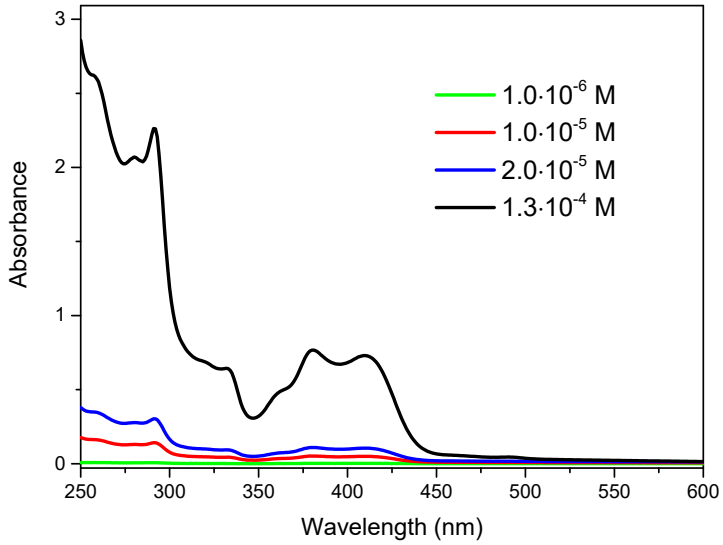


Figure S2. Absorption spectra of **Pt2** in CH_2Cl_2 at different concentrations.

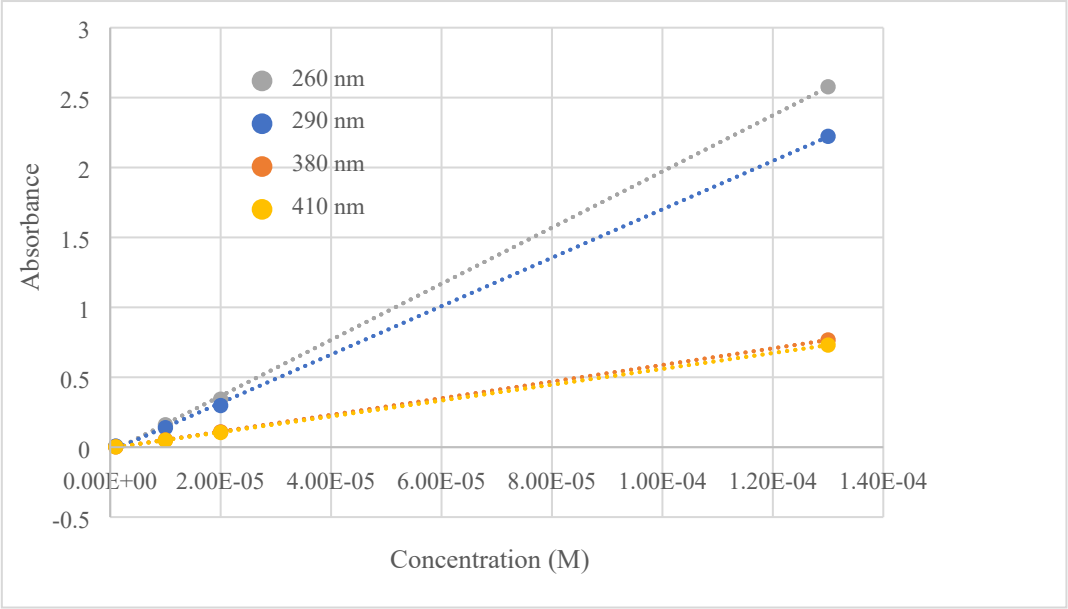


Figure S3. Absorbance vs Concentration for **Pt2** at 260 nm, 290 nm, 380 nm and 410 nm.

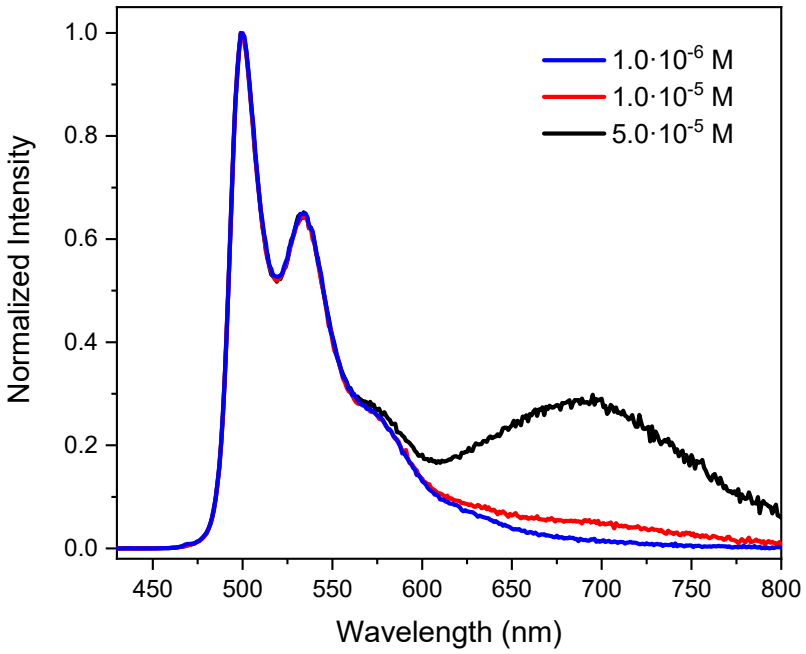


Figure S4. Emission spectra of **Pt2** in CH_2Cl_2 at different concentrations after FPT. Excitation wavelength 410 nm.

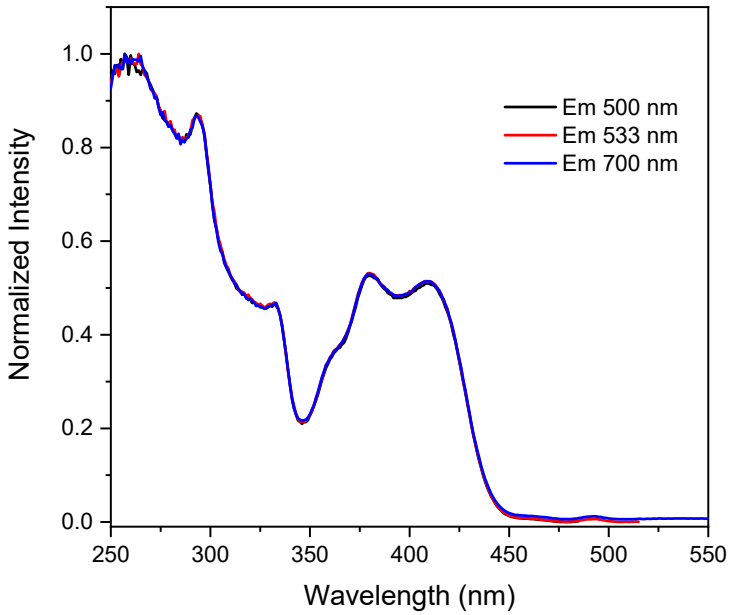


Figure S5. Excitation spectra of **Pt2** $5.0 \cdot 10^{-5}$ M in CH_2Cl_2 at different emission wavelengths after FPT.

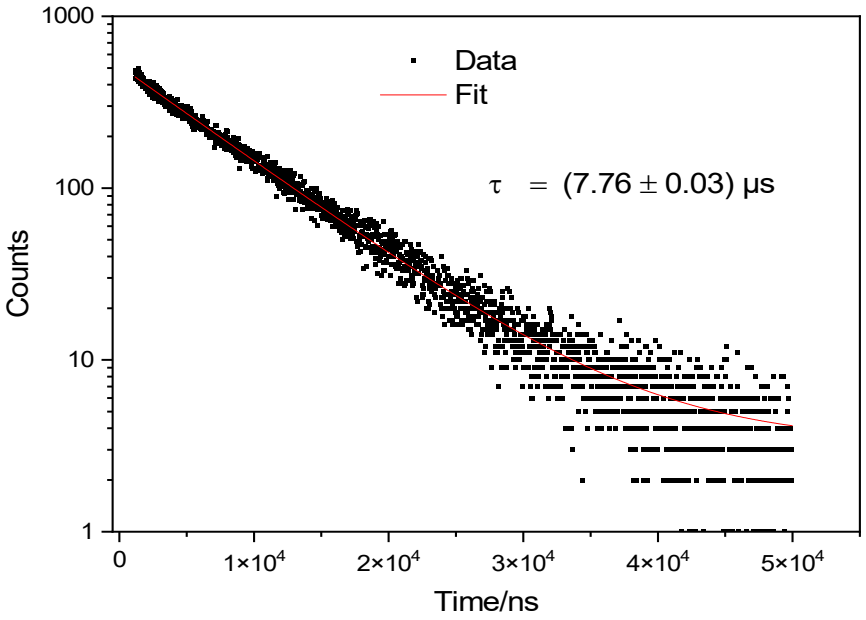


Figure S6. Lifetime measurement of **Pt2** in CH_2Cl_2 at $1 \cdot 10^{-6}$ M after FPT, excitation wavelength 374 nm, emission wavelength 499 nm.

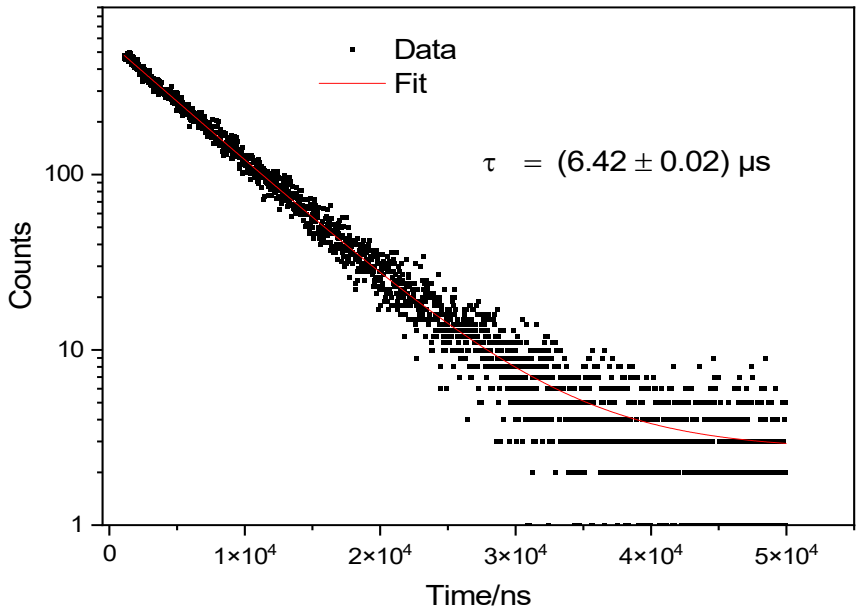


Figure S7. Lifetime measurement of **Pt2** in CH_2Cl_2 at $1 \cdot 10^{-5}$ M after FPT, excitation wavelength 374 nm, emission wavelength 499 nm.

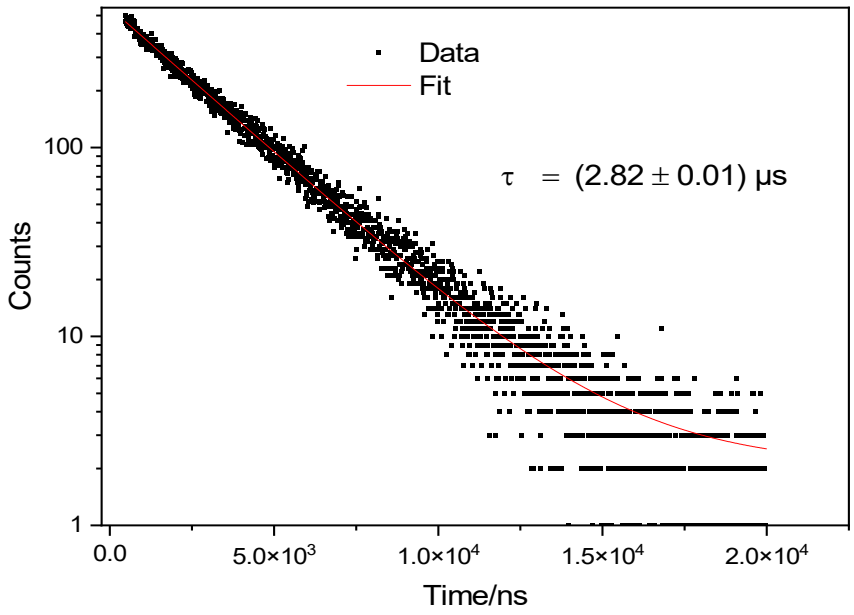


Figure S8. Lifetime measurement of **Pt2** in CH_2Cl_2 at $5 \cdot 10^{-5}$ M after FPT, excitation wavelength 374 nm, emission wavelength 499 nm.

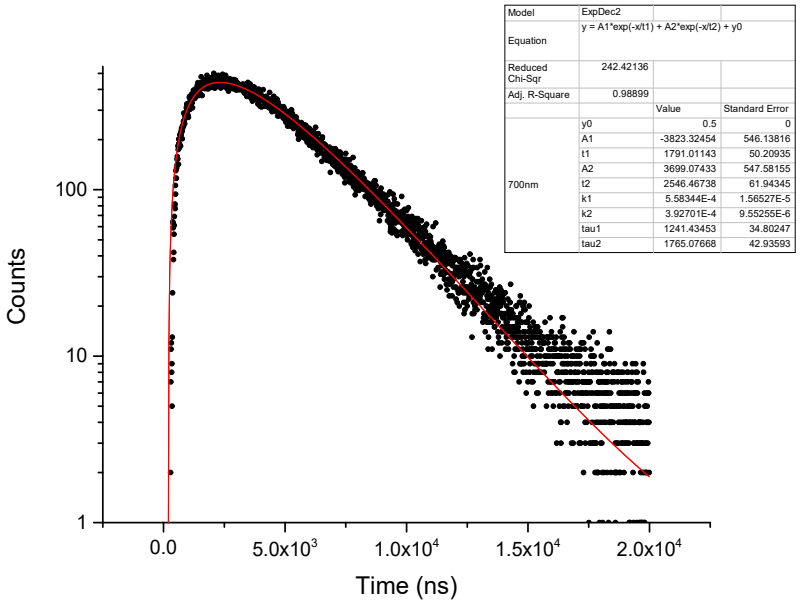


Figure S9. Lifetime measurement of **Pt2** in CH_2Cl_2 at $5 \cdot 10^{-5}$ M after FPT, excitation wavelength 374 nm, emission wavelength 700 nm.

Following the article of J.B. Birks *at al.*[5], the experimental results are consistent with an excimer formation time τ_1 of 1.79 ± 0.05 µs and an excimer decay τ_2 of 2.55 ± 0.06 µs.

Table S1. Photophysical data for complex **Pt2**.

$\lambda_{max,\ abs}$ [nm]	ε [10^3 M^{-1} cm^{-1}]	Concentration [M]	$\lambda_{max,\ em}$ [nm]	QY before FPT	QY after FPT	τ_{av} [µs]
260 290 380 410	20.7 17.3 6.0 5.7	$1.0 \cdot 10^{-6}$	499, 534	3.9%	76.5%	7.76 ± 0.03
		$1.0 \cdot 10^{-5}$	499, 534	3.0%	55.0%	6.42 ± 0.02
		$5.0 \cdot 10^{-5}$	499, 534, 695	3.0%	51.0%	2.82 ± 0.01

Photophysical characterization of Pt2-PNA

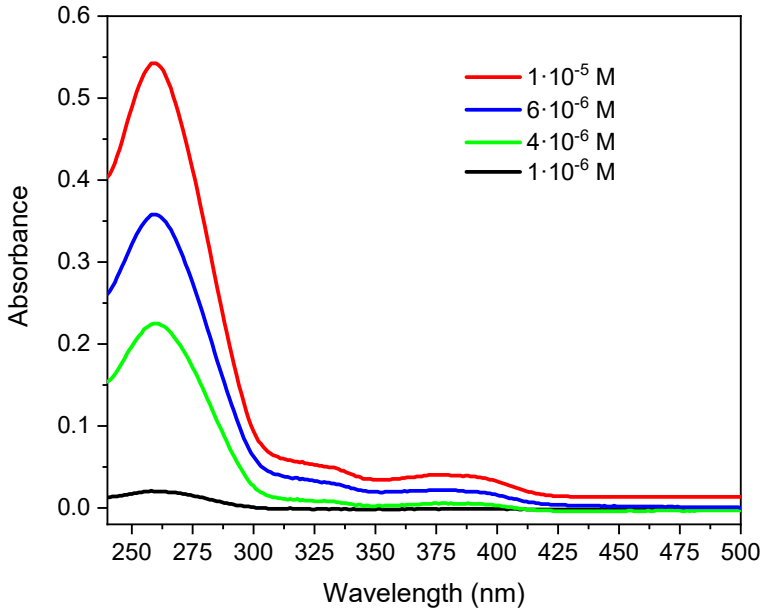


Figure S10. Absorption spectra of **Pt2-PNA** in H_2O at different concentrations.

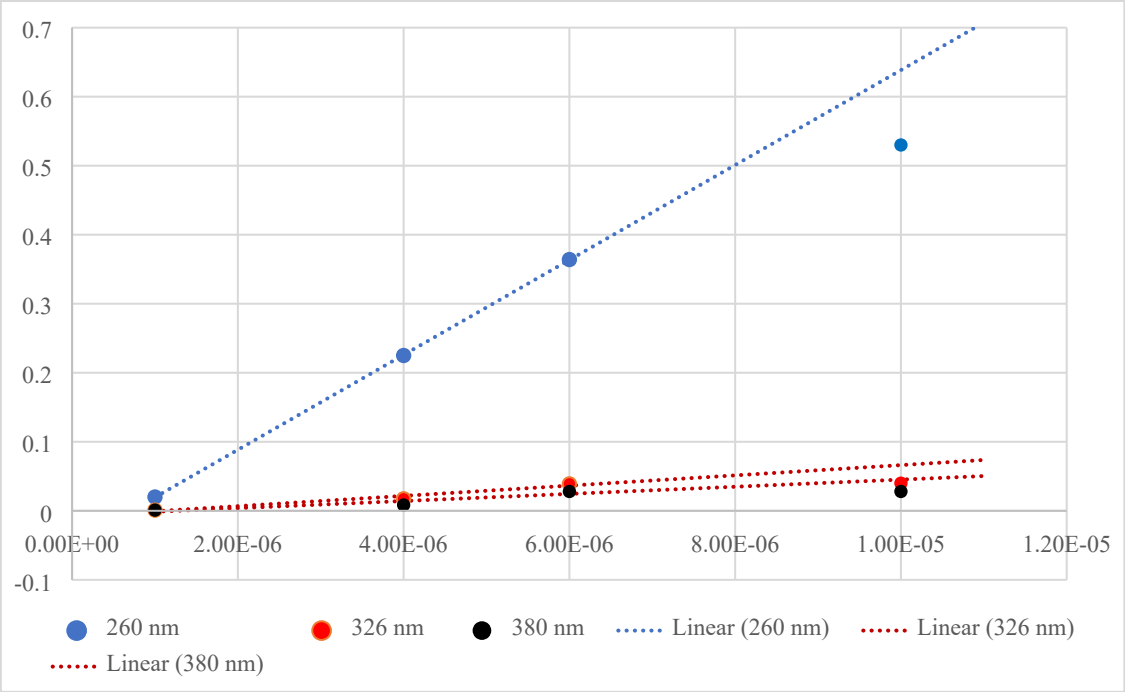


Figure S11. Absorbance vs Concentration for **Pt2-PNA** at 260 nm, 326 nm and 380 nm.

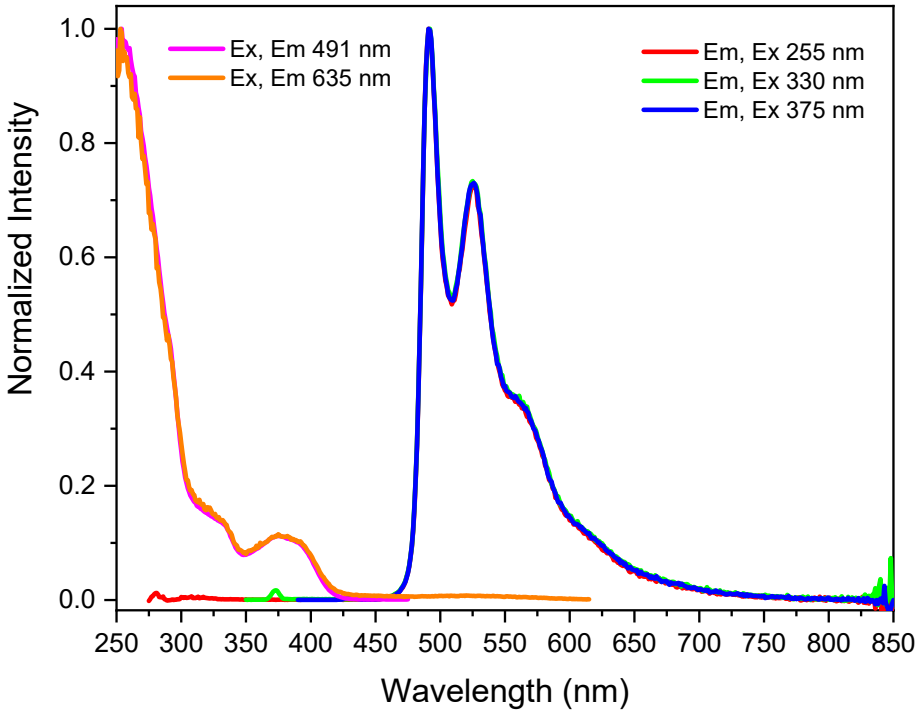


Figure S12. Emission and excitation spectra of **Pt2-PNA** $6.0 \cdot 10^{-6}$ M in H_2O after FPT.

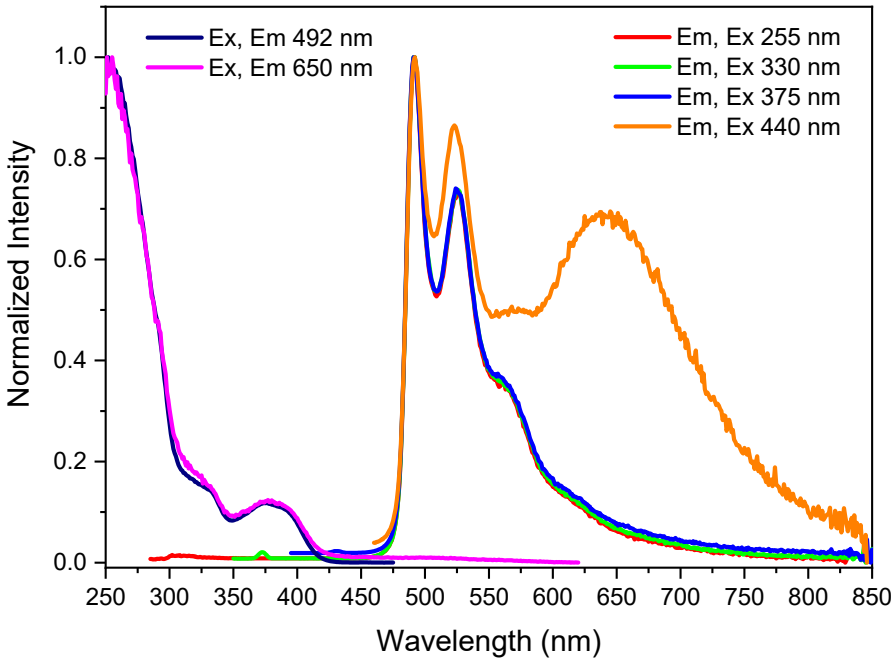


Figure S13. Emission and excitation spectra of **Pt2-PNA** $1.0 \cdot 10^{-5}$ M in H_2O after FPT.

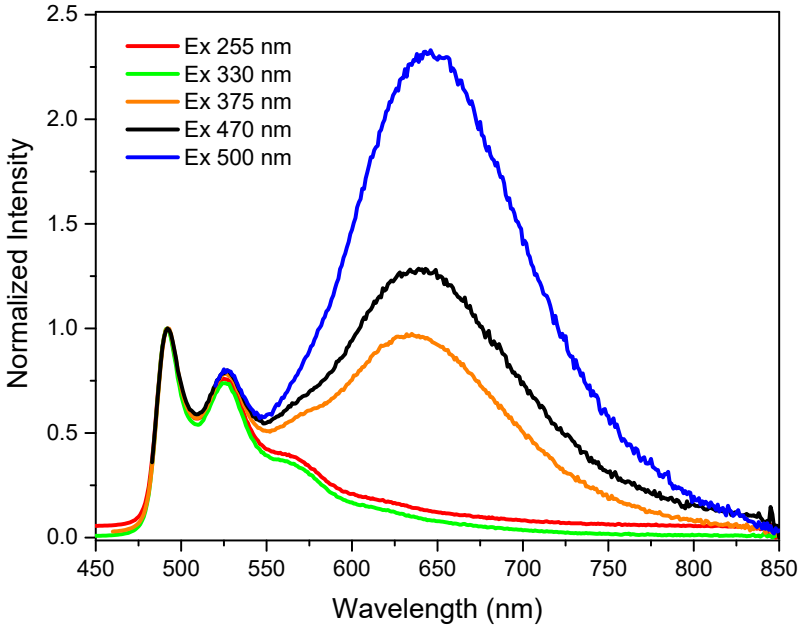


Figure S14. Emission spectra of **Pt2-PNA** $8.2 \cdot 10^{-5}$ M in H_2O at different excitation wavelengths after FPT.

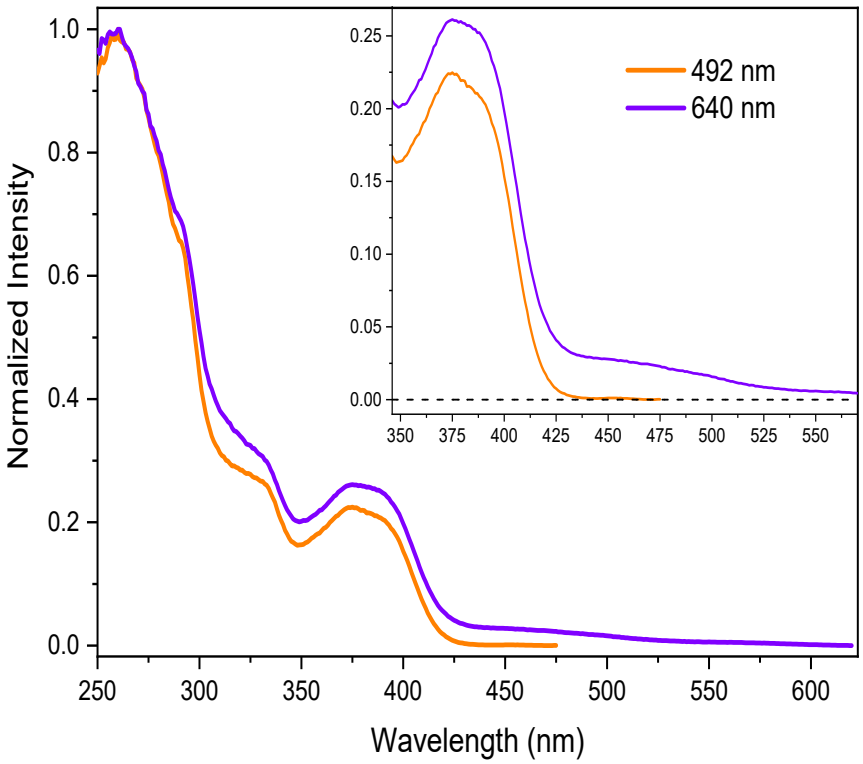


Figure S15. Excitation spectra of **Pt2-PNA** $8.2 \cdot 10^{-5}$ M in H_2O at different emission wavelengths after FPT. In the inset: expansion of the MLCT region.

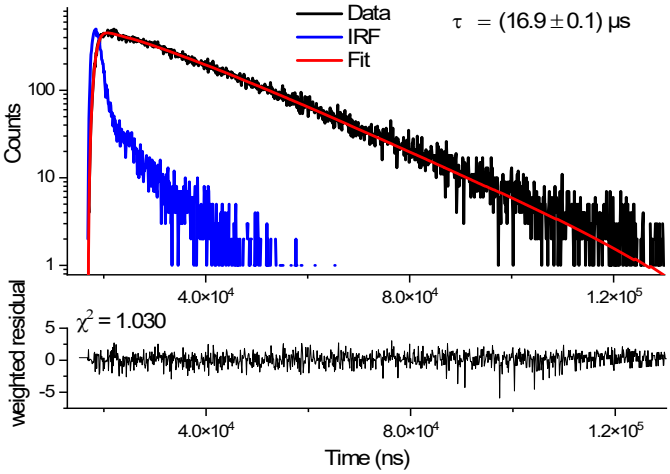


Figure S16. Excited state decay measurement of **Pt2-PNA** $6.0 \cdot 10^{-6}$ M in H_2O after FPT, excitation wavelength 374 nm, emission wavelength 492 nm.

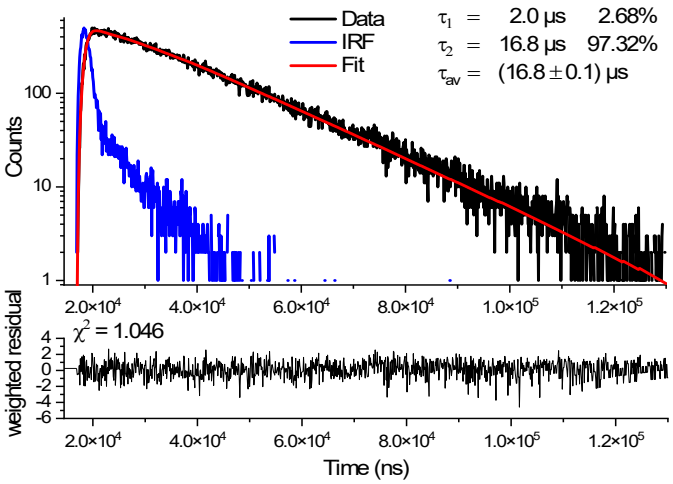


Figure S17. Excited state decay measurement of **Pt2-PNA** $1.0 \cdot 10^{-5}$ M in H_2O after FPT, excitation wavelength 374 nm, emission wavelength 492 nm.

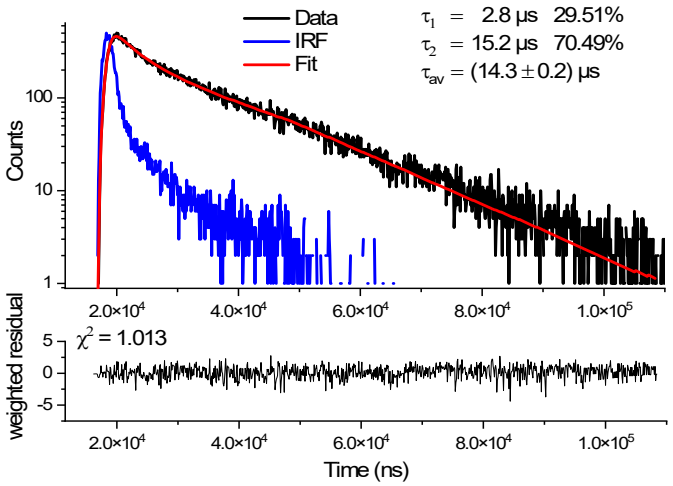


Figure S18. Excited state decay measurement of **Pt2-PNA** $1.0 \cdot 10^{-5}$ M in H_2O after FPT, excitation wavelength 374 nm, emission wavelength 644 nm.

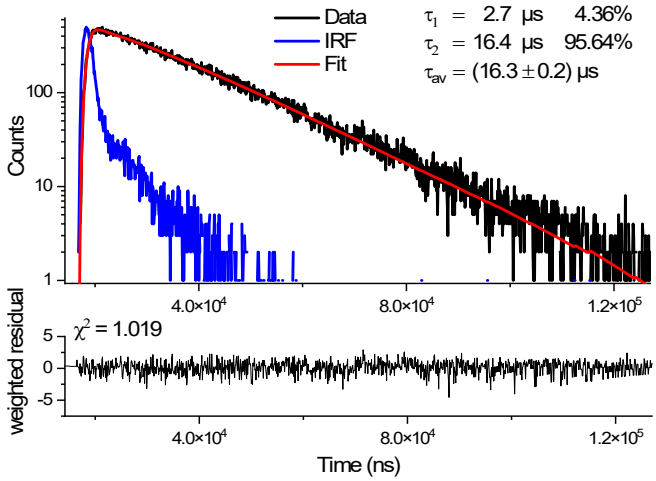


Figure S19. Excited state decay measurement of **Pt2-PNA** $8.2 \cdot 10^{-5}$ M in H_2O after FPT, excitation wavelength 374 nm, emission wavelength 492 nm.

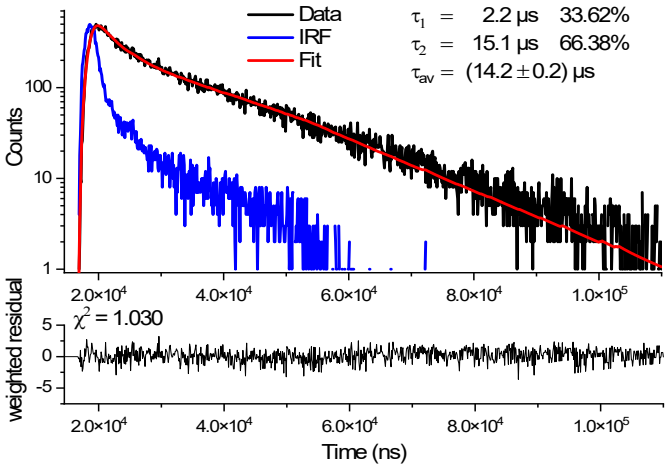


Figure S20. Excited state decay measurement of **Pt2-PNA** $8.2 \cdot 10^{-5}$ M in H_2O after FPT, excitation wavelength 374 nm, emission wavelength 640 nm.

VII. References

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