**Phenanthridine-pyrene conjugates as fluorescent probes for DNA/RNA and an inactive mutant of dipeptidyl peptidase enzyme**

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**Supporting Information**

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| **Phen-Py-1** | **Phen-Py-2** |

Scheme 1. Structures of examined **Phen-Py-1-2** compounds

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| **Phen-AA** | **1-Pyrenebutyric acid (PBA)** |

Scheme 2. Structures of referent compounds

**1. Spectroscopic properties of Phen-Py-1-2 compounds**

Studied compounds were moderately soluble in DMSO (up to *c* = 1 × 10-3 mol dm-3). DMSO stock solutions of compounds were stable during few months. All measurements were recorded in Na-cacodylate buffer (*Ic* = 0.05mol dm-3) both at pH = 5.0 and pH = 7.0. Volume ratio of DMSO was less than 1% in all measurements.

UV/Vis spectra of examined compounds were recorded in Na-cacodylate buffer (*Ic* = 0.05mol dm-3) both at pH = 5 and pH = 7. Further, spectra were recorded using immersion probe with 5 cm light path length, which allowed measurements at concentration range 5 × 10-7 - 3 × 10-6 mol dm-3 to avoid self-aggregation. Absorbancies of aqueous solutions of compounds **Phen-Py-1** and **2** were proportional to their concentrations up to concentrations *c* = 3 × 10-6 mol dm-3. Thermal dependent UV/Vis spectra were recorded using quartz cuvettes (1 cm path). UV/Vis spectra of aqueous solutions of examined compounds **1-2** showed minor irreversible changes upon heating that were attributed to precipitation.

Absorption maxima and corresponding molar extinction coefficients (*ε*) were given in Table S1 and Figure S1.

**Table S1.** Electronic absorption data of **Phen-Py-1** and **2** (sodium cacodylate/HCl buffer, *Ic*= 0.05 mol dm-3,pH = 5 or pH = 7) and referent compounds Phen-AA and Pyr.

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|  | **pH = 5** | | | **pH = 7** | | |
|  | *λ*max / nm | *ε* / mmol-1 cm2 | Ha (%) | *λ*max / nm | *ε* / mmol-1 cm2 | Hb(%) |
| **1** | 276  329  343 | 19387  10994  11140 | 55% | 277  331  346 | 35310  18451  19756 | 35% |
| **2** | 276  348 | 20059  9741 | 53% | 278  277  333  350 | 27895  28154  13018  13697 | 48% |

aH(hypochromic effect, %)276 nm = 100 × {[*ε*276 nm (**Phen-AA**) + *ε*276 nm (**PBA**)]*-ε*276 nm (**Phen-Py-1** or **2**)276 nm }/ [*ε*276 nm (**Phen-AA**) + *ε*276 nm (**PBA**)]; *ε*276 nm (**Phen-AA**) = 11.35 mol-1 cm2; *ε*276 nm (**PBA**) = 31.65 mol-1 cm2; *ε*276 nm (**1**) = 19.39 mol-1 cm2; *ε*276 nm (**2**) = 20.06 mol-1 cm2;(pH = 5.0)

bH (hypochromic effect, %)277 nm = 100 × {[*ε*277 nm (**Phen-AA**) + *ε*277 nm (**PBA**)]*-ε*277 nm (**Phen-Py-1** or **2**)277 nm }/ [*ε*277 nm (**Phen-AA**) + *ε*277 nm (**PBA**)]; *ε*277 nm (**Phen-AA**) = 9.20 mol-1 cm2; *ε*277 nm (**PBA**) = 44.91 mol-1 cm2; *ε*277 nm (**1**) = 35.31 mol-1 cm2; *ε*277 nm (**2**) = 28.15 mol-1 cm2 (pH = 7.0)

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Figure S1. UV/Vis spectra of **Phen-Py-1** and **2** and referent compounds **Phen-AA** and **PBA** at pH = 5 and pH = 7

Fluorescence emission of **Phen-Py-1-2** measured at pH = 5 and pH = 7 (cacodylate buffer, *Ic* =0.05 mol dm-3) was linearly dependent on the concentration up to 3 × 10-6 mol dm-3 (Figure S2).

Fluorescence intensity was decreased upon time and also upon temperature increase without reproducibility after cooling back. That was attributed to the temperature-induced unstacking of phenanthridine and pyrene but also aggregation/precipitation of the compounds.

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Figure S2. Normalized fluorescence emission spectra of **Phen-Py-1** (*λ*exc = 350 nm), **Phen-Py-2** (*λ*exc = 350 nm), referent Phen-AA (*λ*exc = 250 nm) and referent PBA (*λ*exc = 342 nm) compounds (sodium cacodylate buffer, *Ic* = 0.05 mol dm-3, left: pH = 5 right: pH = 7)

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Figure S3. Fluorescence emission spectra of **Phen-Py-1** (*c* = 2 × 10-6 mol dm-3) measured during 60 cycles every 2 minutes at 25 °C, *λ*exc = 352 nm in methanol **A**: MeOH

**2. Study of interactions of Phen-Py-1-2 with ds-DNA and ds-RNA in aqueous media**

2.1. Thermal melting experiments

Non-covalent binding of small molecules to ds-polynucleotides usually has a certain effect on the thermal stability of helices thus giving different *T*m values (temperature of dissociation of double stranded helix into two single stranded polynucleotides)[[1]](#endnote-1). Difference between *T*m value of free polynucleotide and complex with a small molecule (Δ*T*m value) is an important factor in the characterisation of small molecule / *ds*-polynucleotide interactions. All thermal melting experiments were performed sodium cacodylate buffer, pH = 7.0, *Ic* = 0.05 mol dm-3.

At pH = 7 **Phen-Py-1** and **Phen-Py-2** didn’t thermally stabilize any *ds*-DNA/RNA (Table 2,Figure S4-Figure S6).



Figure S4. Melting curves of *ct-*DNA upon addition of **Phen-Py-1** and **Phen-Py-2** (*c*(DNA)= 2 ×10-5 mol dm-3; ratio *r*[compound] / [polynucleotide] = 0.3) at pH = 7.0 (sodium cacodylate buffer, *Ic* = 0.05 mol dm-3).



Figure S5. Melting curves of poly rA-poly rU upon addition of **Phen-Py-1** and **Phen-Py-2** (*c*(RNA)= 2 ×10-5 mol dm-3; ratio *r*[compound] / [polynucleotide] = 0.3) at pH = 7.0 (sodium cacodylate buffer, *Ic* = 0.05 mol dm-3).



Figure S6. Melting curves of poly dAdT – poly dAdT upon addition of **Phen-Py-1** and **Phen-Py-2** (*c* (DNA) = 2 ×10-5 mol dm-3; ratio *r*[compound] / [polynucleotide] = 0.3) at pH = 7.0 (sodium cacodylate buffer, *Ic* = 0.05 mol dm-3).

Table S2. The aΔ*T*m values (°C ) of studied ds-polynucleotides upon addition of **Phen-Py-1** and **Phen-Py-2** (ratio *rb* = 0.3)at pH = 7.0 (buffer sodium cacodylate, *Ic* = 0.05 mol dm-3 ), c(DNA / RNA) = 1-2 ×10-5 mol dm-3.

|  | **Δ*Tm* / °C** | | |
| --- | --- | --- | --- |
| **Compound** | ***ct*-DNA** | **poly dAdT** – **poly dAdT** | **poly rA** – **poly rU** |
| **Phen-Py-1** | 0 | 0 | 0 |
| **Phen-Py-2** | precipitation | 0 | precipitation |

a Error in Δ*Tm* : ± 0.5°C;

b *r* ***=*** [compound] / [polynucleotide];

d not determined due to precipitation

***2.1. Spectrophotometric titrations***

Table S3. Stability constants (log *Ks*)a of complexes of **Phen-Py-1-2** with ds-polynucleotides and protein calculated according to fluorimetric titrations (Na-cacodylate buffer, *Ic* = 0.05 mol dm-3,pH = 7.0; *λ*exc = 352 nm; *λ*em= 370-600, *c*(**Phen-Py-1-2**) = 1-2 × 10-6 mol dm-3)

|  | log *Ks*a | |
| --- | --- | --- |
| polynucleotide | **Phen-Py-1** | **Phen-Py-2** |
| *ct*-DNA | 6.97 | <4c |
| poly dAdT–poly dAdT | 5.97 | d |
| poly dGdC–poly dGdC | 6.87 | d |
| poly rA–poly rU | 7.07 | c |
| protein | **Phen-Py-1** | **Phen-Py-2** |
| E451A | 7.54 (*n* = 1)b | d |

a Processing of titration data by means of Scatchard equation[[2]](#endnote-2) and Global Fit procedure[[3]](#endnote-3) gave stability constants and values of ratio n = [bound compounds] / [polynucleotide or protein]; for easier comparison values of log Ks for polynucleotide complexes were recalculated for fixed n=0.2; correlation coefficients were >0.9 for all calculated *Ks*

b *n* = [bound compound] / [protein];

c small /linear fluorescence change / no fluorescence change disabled calculation of stability constant

d not determined

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Figure S7. Left: Fluorimetric titration of **Phen-Py-1** with *ct-*DNA, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3, Right: Experimental (●) fluorescence intensities of **Phen-Py-1** at *λ*em = 400 nm upon addition of *ct-*DNA (**pH = 5.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

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Figure S8. Left: Fluorimetric titration of **Phen-Py-1**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with poly rA-poly rU, Right: Experimental (●) fluorescence intensities of **Phen-Py-1** at *λ*em = 400 nm upon addition of poly rA-poly rU (**pH = 5.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

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Figure S9. Left: Fluorimetric titration of **Phen-Py-2**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with *ct*-DNA, Right: Experimental (●) fluorescence intensities of **Phen-Py-2** at *λ*em = 400 nm upon addition of *ct*-DNA (**pH = 5.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

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Figure S10. Left: Fluorimetric titration of **Phen-Py-2**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with poly rA-poly rU, Right: Experimental (●) fluorescence intensities of **Phen-Py-2** at *λ*em = 400 nm upon addition of poly rA-poly rU (**pH = 5.0**, Na cacodylate buffer, *Ic*= 0.05 mol dm-3).

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Figure S11. Left: Fluorimetric titration of **Phen-Py-2**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with *ct-*DNA, Right: Experimental (●) fluorescence intensities of **Phen-Py-2** at *λ*em = 403 nm upon addition of *ct-*DNA (**pH = 7.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

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Figure S12. Left: Fluorimetric titration of **Phen-Py-2**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with poly rA-poly rU, Right: Experimental (●) fluorescence intensities of **Phen-Py-2** at *λ*em = 400 nm upon addition of poly rA-poly rU (**pH = 7.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3

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Figure S13. Left: Fluorimetric titration of **Phen-Py-1**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with *ct-*DNA, Right: Experimental (●) fluorescence intensities of **Phen-Py-1** at *λ*em = 471 nm upon addition of *ct-*DNA (**pH = 7.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

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Figure S14. Left: Fluorimetric titration of **Phen-Py-1**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with poly rA-poly rU, Right: Experimental (●) fluorescence intensities of **Phen-Py-1** at *λ*em = 467 nm upon addition of poly rA-poly rU (**pH = 7.0**, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

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Figure S15. Left: Fluorimetric titration of **Phen-Py-1**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with poly dAdT - poly dAdT, Right: Experimental (●) fluorescence intensities of **Phen-Py-1** at *λ*em = 471 nm upon addition of poly dAdT - poly dAdT (pH = 7.0, Na cacodylate buffer, *Ic*= 0.05 mol dm-3).

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Figure S16. Left: Fluorimetric titration of **Phen-Py-1**, *λ*exc = 352 nm, *c* = 2 × 10-6 mol dm-3 with poly dGdC - poly dGdC, Right: Experimental (●) fluorescence intensities of **Phen-Py-1** at *λ*em = 471 nm upon addition of poly dGdC - poly dGdC (pH = 7.0, Na cacodylate buffer, *Ic* = 0.05 mol dm-3).

*2.3.* Circular dichroism (CD) experiments

CD spectroscopy was chosen to monitor conformational changes of polynucleotide secondary structure induced by small molecule binding[[4]](#endnote-4). Compounds **Phen-Py-1** and **Phen-Py-2** were built using chiral amino acid building blocks and consequently have intrinsic CD spectrum.

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Figure S17. Changes in the CD spectrum of *ct-*DNA (*c*(DNA) = 2 × 10-5 mol dm-3) upon addition of **Phen-Py-1** (left) and **Phen-Py-2** (right) at different molar ratios *r* = [compound] / [polynucleotide], pH = 7.0, sodium cacodylate buffer, *Ic* = 0.05 mol dm-3

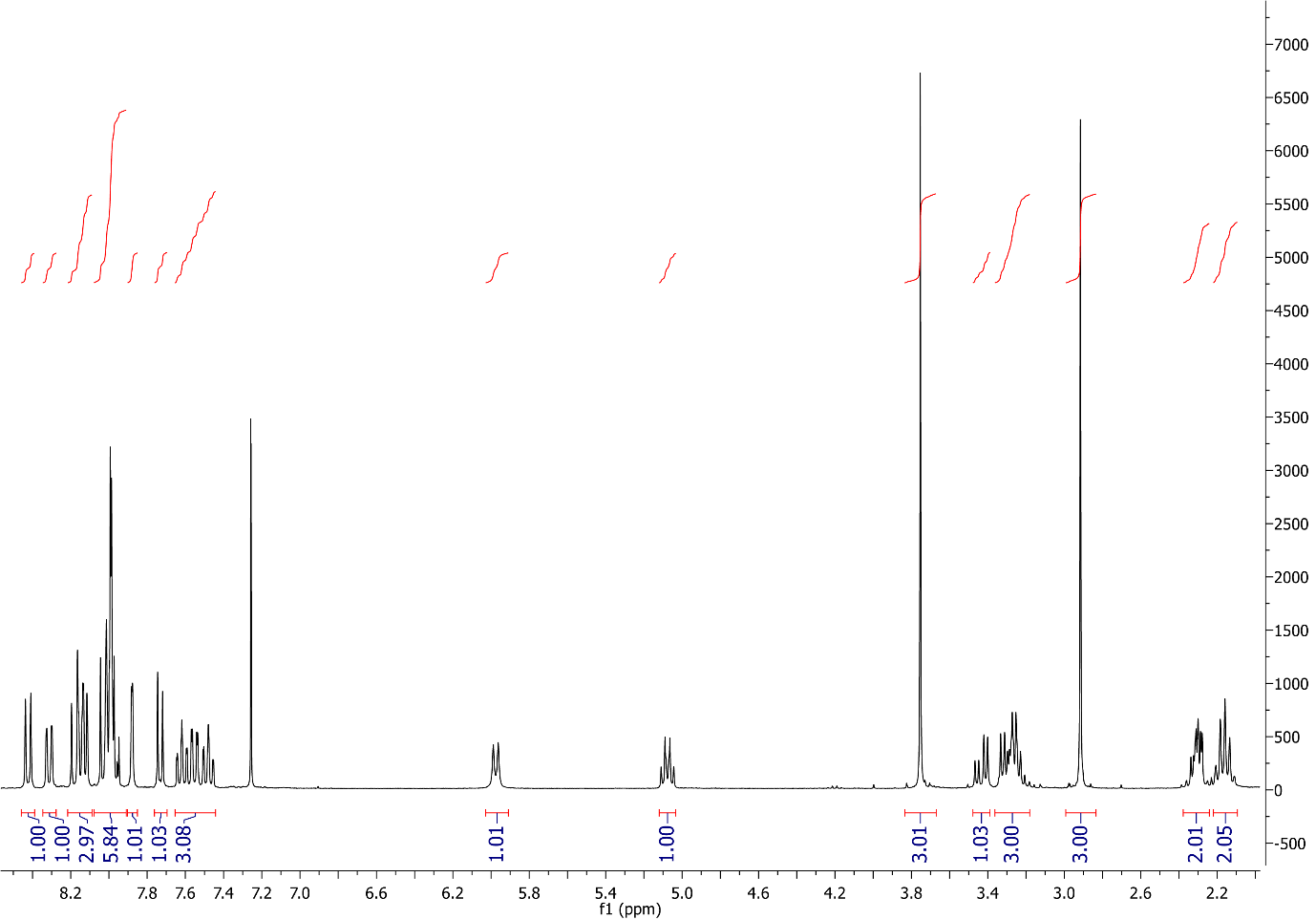
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Figure S18. Changes in the CD spectrum of poly rA-poly rU upon addition of **Phen-Py-1** (*c*(RNA) =1 × 10-5 mol dm-3) (left) and **Phen-Py-2;** (*c*(RNA) = 1 × 10-5 mol dm-3) (right) at different molar ratios *r* = [compound] / [polynucleotide], pH = 7.0, sodium cacodylate buffer, *Ic* = 0.05 mol dm-3

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Figure S19. Comparation of spectra of RNA-dye complex (*r*= 0.5, **―**) and sum of poly rA-poly rU and dye spectra (―) of appropriate concentrations

3. NMR spectra of



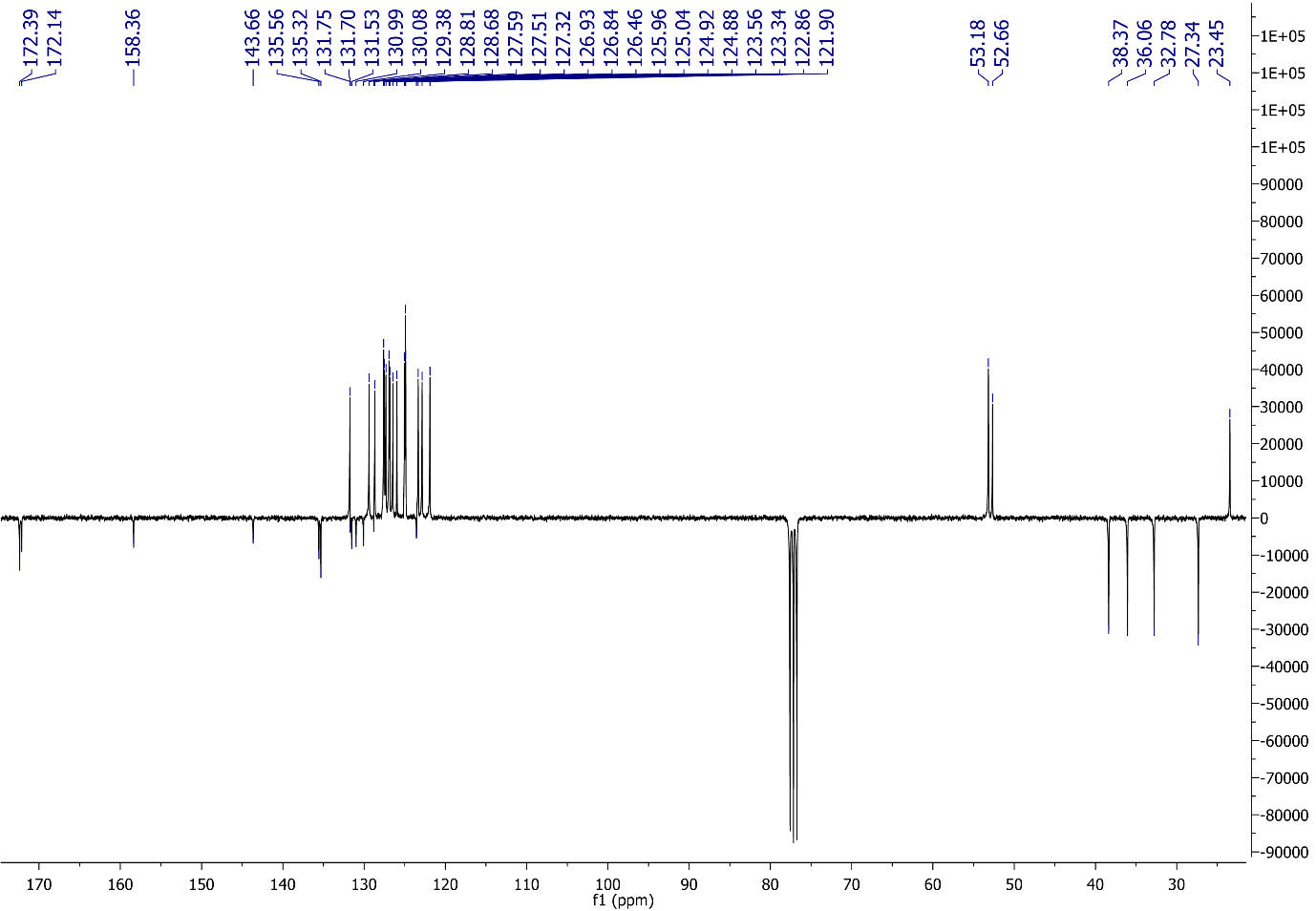
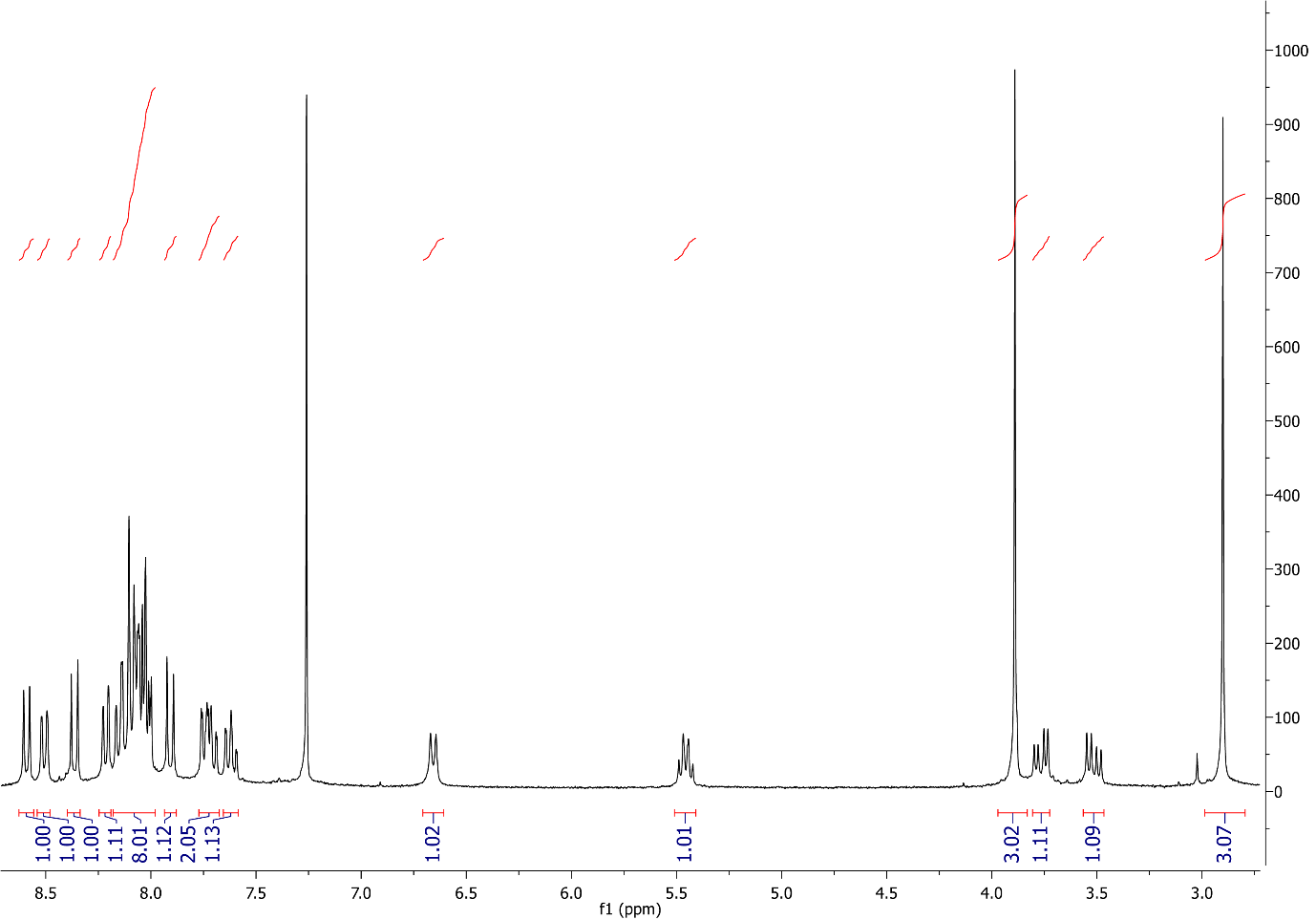


Figure S20. 1H and 13C NMR spectra of **Phen-Py-1**.



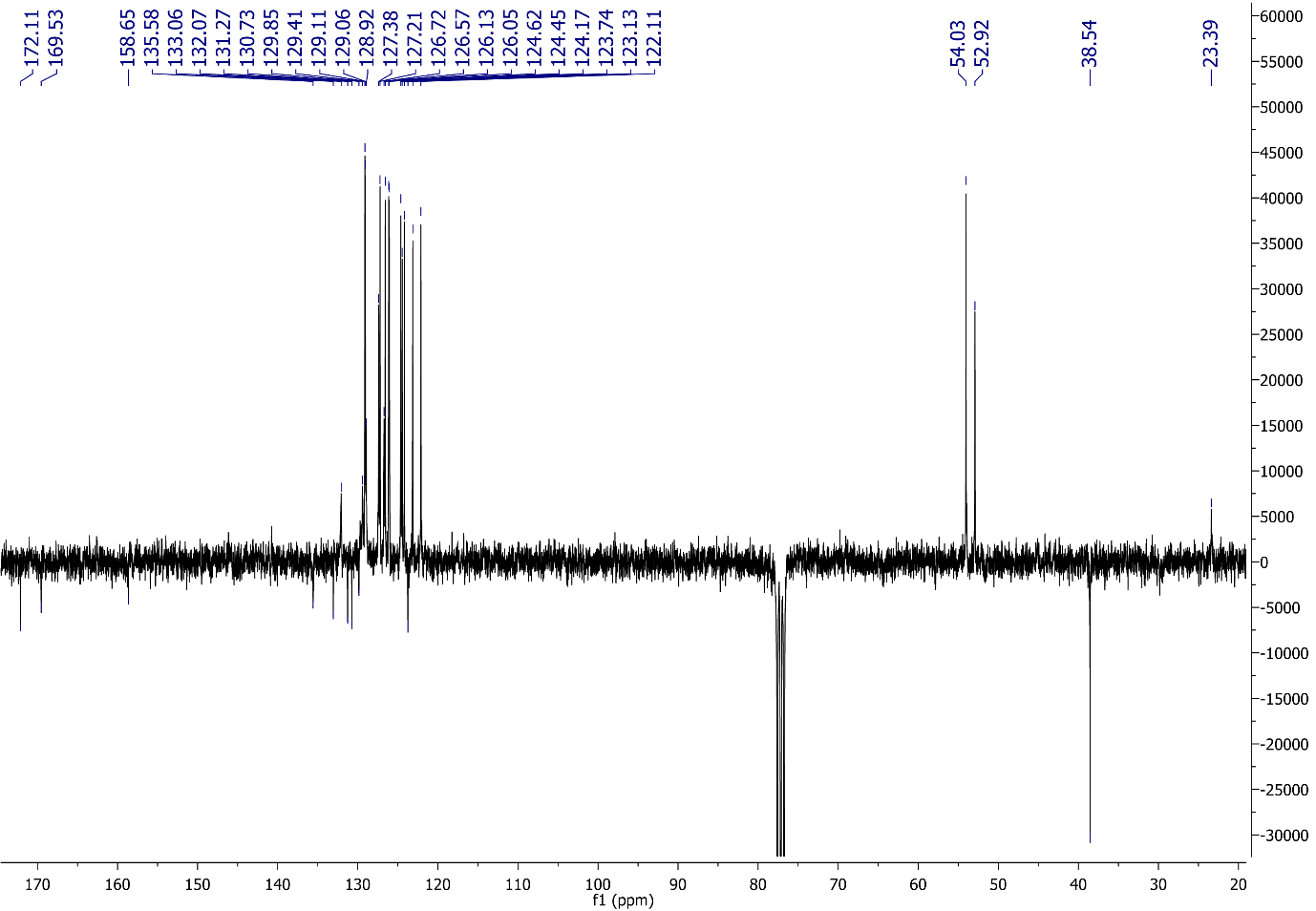


Figure S21. H and 13C NMR spectra of **Phen-Py-2**.

4. Computational analysis

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| **Phen-Py-1** at pH = 7 | **Phen-Py-2** at pH = 7 |
| C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\pi_pi_JM_163_7.jpg | C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\pi_pi_JM_210_7.jpg |
| Average value = 8.3 Å | Average value = 6.4 Å |
|  |  |
|  |  |
| **Phen-Py-1+** at pH = 5 | **Phen-Py-2+** at pH = 5 |
| C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\pi_pi_JM_163_5.jpg | C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\pi_pi_JM_210_5.jpg |
| Average value = 5.5 Å | Average value = 5.4 Å |

Figure S22. Evolution of distances between the centers of mass among pyrene and phenanthridine aromatic units during 300 ns of MD simulations in **Phen-Py-1-2** conjugates under neutral (pH = 7) and acidic (pH = 5) conditions.

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| **Phen-Py-1** at pH = 7 | **Phen-Py-2** at pH = 7 |
| C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\td_JM_163_7_uvvis.jpg | C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\td_JM_210_7_uvvis.jpg |
| *λ*max = 264 nm, 355 nm | *λ*max = 264 nm, 370 nm |
|  |  |
|  |  |
| **Phen-Py-1+** at pH = 5 | **Phen-Py-2+** at pH = 5 |
| C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\td_JM_163_5_uvvis.jpg | C:\Documents and Settings\Administrator\Desktop\TUMIR\SLIKE\td_JM_210_5_uvvis.jpg |
| *λ*max = 264 nm, 356 nm | *λ*max = 254 nm, 352 nm |

Figure S23. Calculated UV-Vis absorption spectra for **Phen-Py-1-2** conjugates under neutral (pH = 7) and acidic (pH = 5) conditions using the TD–DFT approach and the (IEF-PCM)/M06–2X/6–31+G(d) level of theory.

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