Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.


# CycloPeptidic Photosensitizer Prodrugs as Proteolytically Triggered Drug Delivery Systems of Pheophorbide a With Defined Structures for Selective PhotoDiagnosis and PhotoDynamic Therapy: Part II - Co-loading of Pheophorbide a and Black Hole Quencher – Electronic Supplementary Information

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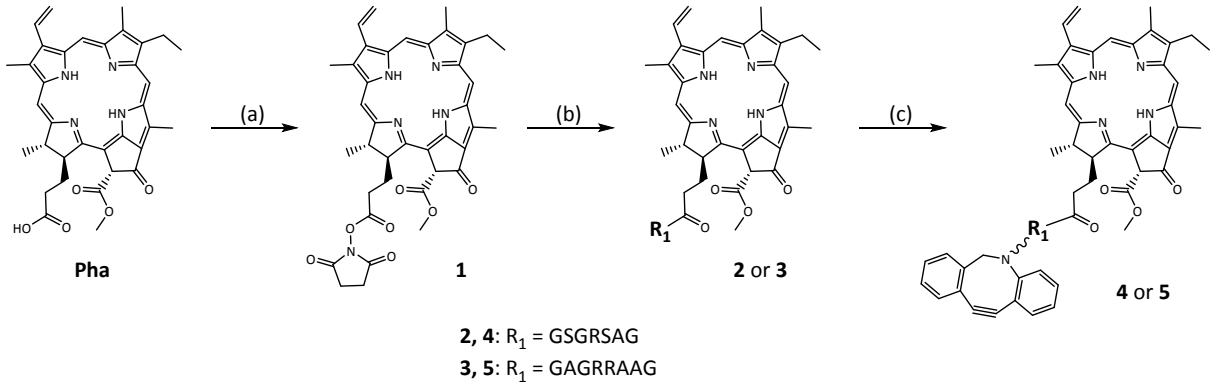
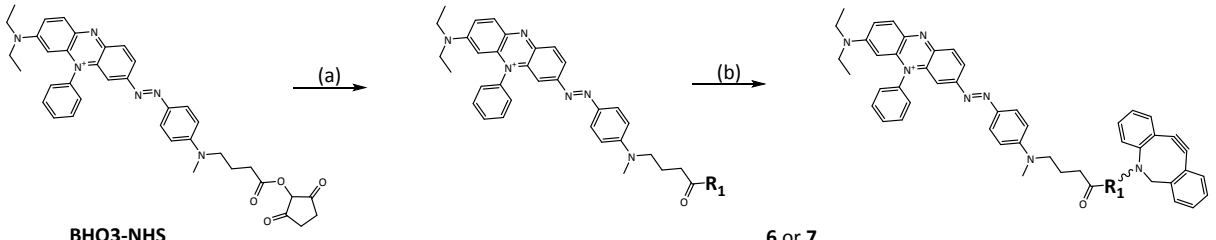



**2, 4**: $R_1$ = GSGRSAG
**3, 5**: $R_1$ = GAGRRAAG

Scheme S1 - Generic synthesis pathway for the Pha-peptide-DBCO conjugates. The text on the bottom depicts the different conjugates obtained depending on the nature of the amino acids of the linker. *Reagents and conditions*: (a) NHS, EDC, DMAP, argon (ar.), dark, room temperature (r. t.), overnight (o/n); (b) DIPEA, GSGRSAG (TFA salt) or GAGRRAAG (TFA salt), ar., dark, r. t., o/n; (c) DBCO-$NH_2$, HATU, DIPEA, ar., dark, r. t., o/n.




**6, 8:** $R_1$ **=** GSGRSAG
**7, 9:** $R_1$ = GAGRRAAG

Scheme S2 - Generic synthesis pathway for the BHQ3-peptide-DBCO conjugates. The text on the bottom depicts the different conjugates obtained depending on the nature of the amino acids of the linker. *Reagents and conditions*: (a) DIPEA, GSGRSAG (TFA salt) or GAGRRAAG (TFA salt), ar., dark, r. t., o/n; (b) DBCO-$NH_2$, HATU, DIPEA, ar., dark, r. t., o/n.

Table S1 – Fluorescence emission values ($\lambda_{ex}$ = 410 ± 9 nm and $\lambda_{em}$ = 670 ± 9 nm) and detection of $^1O_2$ ($\lambda_{ex}$ = 405 nm and $\lambda_{em}$ = 1270 nm) of the synthesized conjugates; N.D. means that no counts were detected under these conditions. [a] Conjugates at 3 μM of Pha equivalents; [b] conjugates at 30 μM of Pha equivalents.

| *Conjugate* | *F (a.u.)* [a] | *$^1O_2$ detected (counts per second)* [b] |
|---|---|---|
| *uPA-cPPP$_{1/5}$* | 15328 ± 240 | 185.5 ± 0.6 |
| *uPA-cPPP$_{4/5}$* | 201 ± 18 | 22.0 ± 0.7 |
| *uPA-cPPQ$_{2+2/5}$* | 26 ± 4 | N.D. |
| *CathB-cPPQ$_{2+2/5}$* | 18 ± 6 | N.D. |

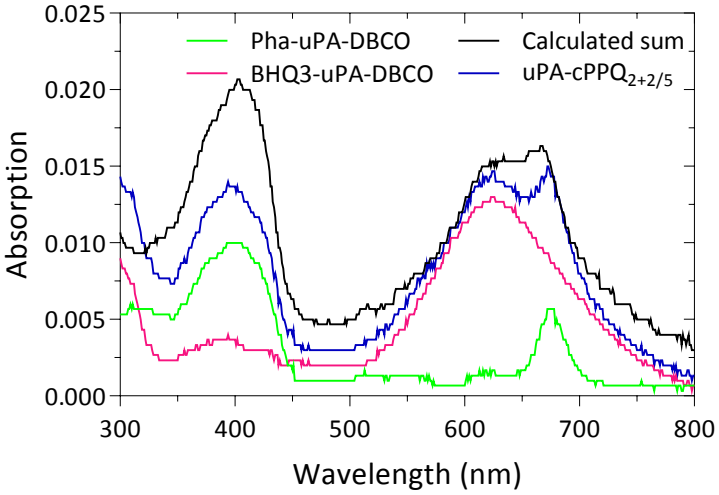



Figure S1 - Absorption spectra of Pha-uPA-DBCO (green line), BHQ3-uPA-DBCO (purple line) and uPA-cPPQ$_{2+2/5}$ (blue line) at 3 μM of Pha (or BHQ3) equivalents. The black line depicts the sum of the absorbance of both Pha-uPA-DBCO and BHQ3-uPA-DBCO for comparison.

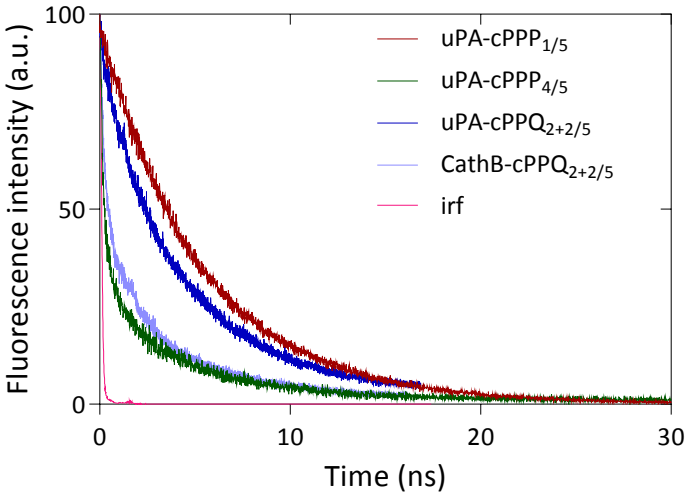



Figure S2 – Normalized fluorescence decays of the considered compounds in water at 3 μM of Pha equivalents; $\lambda_{ex}$ = 395 nm and $\lambda_{em}$ = 670 nm.

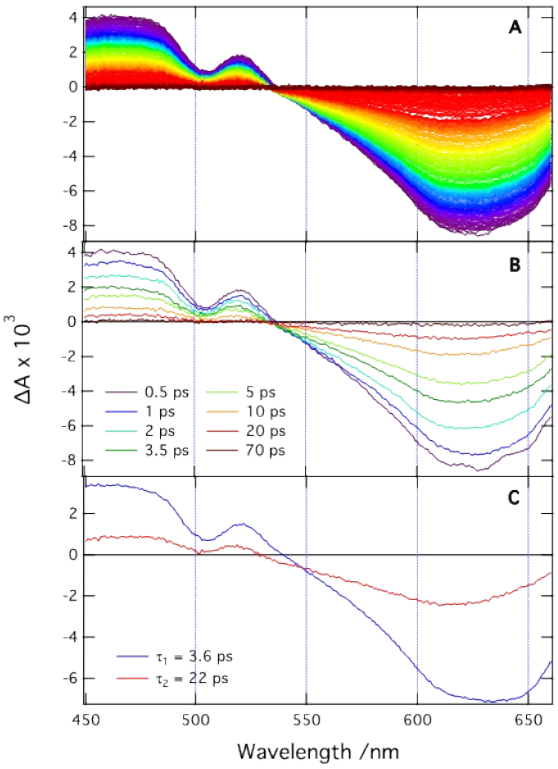



Figure S3 – (A) TA spectra recorded with CathB-cPPQ$_{2+2/5}$ in water at various time delays and (B) selected time delays after 385 nm excitation, (C) decay-associated difference absorption spectra obtained from a global analysis using the sum of two exponential functions.

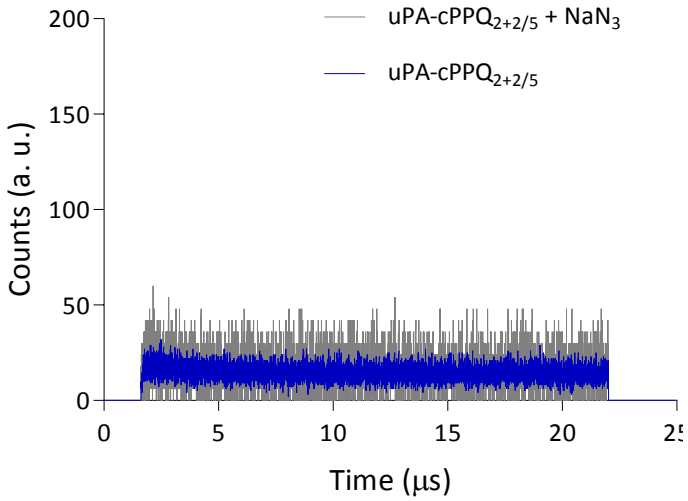



Figure S4 – Singlet oxygen luminescence decay of uPA-cPPQ$_{2+2/5}$ at 30 µM of Pha equivalents (blue curve), and after addition of a 2 M solution of $NaN_3$ (grey curve). Excitation occurred at 405 nm (40 kHz, 5 mW) over 10 minutes.

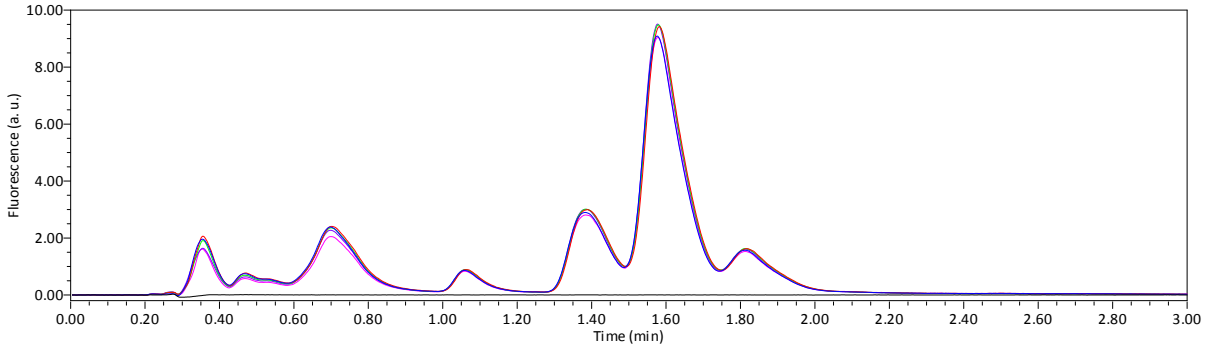



Figure S5 – Digestion by trypsin (42 µL of trypsin-EDTA solution 10x) of uPA-cPPQ$_{2+2/5}$ at 30 µM of Pha equivalents followed by UPLC (isocratic gradient over 3 minutes, 47.5% solvent B with $H_2O$ + 0.1% FA as solvent A and ACN + 0.1% FA as solvent B, fluorescence detection with $\lambda_{ex}$ = 410 nm and $\lambda_{em}$ = 670 nm, gain of 10) at 0 (black curve), 5 (blue curve), 15 (red curve), 30 (green curve), 60 (purple curve) and 120 minutes (pink curve).

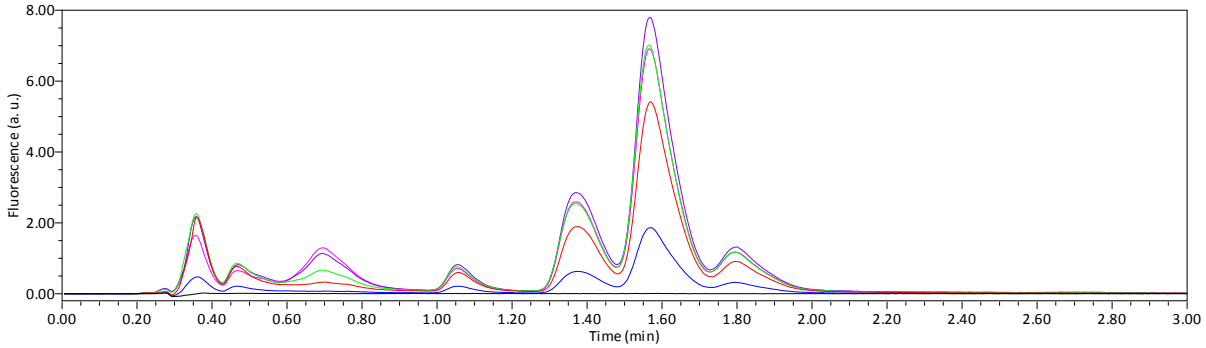



Figure S6 – Digestion by uPA (1000 U) of uPA-cPPQ$_{2+2/5}$ at 30 µM of Pha equivalents followed by UPLC (isocratic gradient over 3 minutes, 47.5% solvent B with $H_2O$ + 0.1% FA as solvent A and ACN + 0.1% FA as solvent B, fluorescence detection with $\lambda_{ex}$ = 410 nm and $\lambda_{em}$ = 670 nm, gain of 10) at 0 (black curve), 5 (blue curve), 15 (red curve), 30 (green curve), 60 (purple curve) and 120 minutes (pink curve).