Supporting Information

Molecular Plumbing to Bend Self-Assembling Peptide Nanotubes
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### Table of contents:

1. Supporting schemes and figures (Scheme S1 and Fig. S1-S24) ..... S1-12

2. Materials................................................................................................................. S13
   2.1 Solid phase peptide synthesis. Preparation of **CP2** ................ S13
   2.2 Preparation of **CP3**
   2.3 Preparation of **CP3Z, **E,E-D3**
   2.4 Preparation of model anthracene derivative **Ant1**............. S35
   2.5 Photodimers thermal dissociation experiments ...................... S39

3. Methods................................................................................................................. S40
   3.1 Fluorescence Spectroscopy ............................................................... S40
   3.2 Circular Dichroism (CD) Spectroscopy ........................................ S40
   3.3 Nile red assay ......................................................................................... S40
   3.4 ThT fluorescence assay ........................................................................ S40
   3.5 Scanning Transmission Electron Microscopy (STEM) ............ S40
   3.6 Epifluorescence microscopy ............................................................... S41
   3.7 Atomic Force Microscopy (AFM) ....................................................... S41
   3.8 Kinetic studies of **CP3** dimerization ........................................... S41
   3.9 Procedure of irradiation under photo-oxidative conditions .... S41
   3.10 Procedure of irradiation-dark cycles ............................................. S41
   3.11 Head-to-Tail characterization of photodimers............................ S42

4. References............................................................................................................... S52
1. Supporting schemes and figures (Scheme S1 and Fig. S1-S19)

Scheme S1. Synthetic strategy for the preparation of CP2 and CP3.

Figure S1. a) RP-uHPLC chromatogram of purified CP3: [Agilent SB-C18 column, H₂O (0.1% TFA)/ACN (0.1% TFA). 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)] R₂ = 9.6 min; b) ESI-MS of the peak at 9.6 min.
Figure S2. a) CP3 fluorescence spectra at different pH obtained upon addition of NaOH (0.1 M) to aqueous solutions of CP3 at 350 µM (λ_{exc} = 385 nm, T = 20°C); b) Trend of fluorescence emission at 475 nm versus pH.

Figure S3. NR (1 µM) assay of CP3 in Hepes (10 mM, pH 7). Maximum intensity (data in purple) and corresponding wavelength at maximum intensity (data in green) were both plotted as a function of the logarithm of the peptide concentration. λ_{exc} = 550 nm and T = 20°C.
Figure S4. ThT assay of CP3 in Hepes (10 mM, pH 7). a) Overlaid ThT (20 µM) fluorescence spectra of solutions of CP3 at different concentrations; b) Intensity ratio I_{494}/I_o versus the concentration of CP3. I_o is the fluorescence intensity at 494 nm of ThT alone. Spectra were recorded at 20°C. The break point indicates a cac of 45 µM.

Figure S5. FT-IR spectrum of CP3 on a freeze-dried powder after basification with NaOH (0.1 M). T=298 K. The strong peak at 1633 cm^{-1} and a shoulder at 1664 cm^{-1} support the formation of a parallel β-sheet structure.
Figure S6. Trend of the ellipticity changes measured at 275 nm as a function of the pH (4-10) of the solution of CP3 (350 µM) in water. The data were fitted with adapted Henderson-Hasselbalch equation $(\text{pH} - pK_a) = \log(\frac{\theta_{\text{max}} - \theta}{\theta - \theta_{\text{min}}})$, providing a mean values of pKa = 7.3 and Hill coefficient of $n = 1.6 \pm 0.3$.

Figure S7. STEM micrographs of deposited solution of CP3 (400 µM) at native pH (3.5).

Figure S8. AFM topography micrographs of CP3 deposited silicon wafer from aqueous solutions (350 µM, pH 7). The height profiles (right) were achieved along the color lines shown in the image.
Figure S9. Time-course UV–VIS spectra of solutions of CP3 (250 µM) at (a) acidic (pH 1.85), (b) native (pH 4.95) and (c) neutral conditions (Hepes, 10 mM, pH 7) upon irradiation at 350 nm.

Figure S10. Representation of the photodimerization degree followed at 386 nm versus time for solutions of CP3 (250 µM) at acidic (pH 1.85, red), native (pH 4.95, orange) and neutral pH (Hepes, 10 mM, green) upon irradiation at 350 nm. The photodimerization degree was evaluated through the equation PD = ((A₀ − A)/A₀)*100, where A₀ is the initial intensity at 386 nm. The solid curve derived from the biexponential fit PD(t) = X₁(1 − e^{−k₁t}) + X₂(1 − e^{−k₂t}), where PD(t) corresponds to the time-dependent photodimerization degree, k₁ and k₂ are rate constants, X₁ and X₂ are the relative weighting fractions. Fitting the curves with this equation allowed to estimate a k₁ of 0.14 min⁻¹ at pH 7, of 0.06 min⁻¹ at pH 4.95, and 0.03 min⁻¹ at pH 1.85.
Figure S11. Time-course UV-VIS spectra of solutions of model Ant1 (250 μM, 1:3 MeOH/H₂O) at (a) native (pH 3.93) and (b) neutral conditions (Hepes, 10 mM, pH 7) upon irradiation at 350 nm.

Figure S12. Representation of the photodimerization degree followed at 386 nm versus time for solutions of Ant1 (250 μM, 1:3 MeOH/H₂O) at native (pH 3.93, orange) and neutral pH (Hepes, 10 mM, green) upon irradiation at 350 nm.
Figure S13. HPLC analysis (HPLC conditions: H₂O (0.1% TFA)/ACN (0.1% TFA), 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)) of the mixture of CP₃ (250 µM) irradiated at pH 7 at different time intervals. After 1 minute of irradiation (purple line), the formation of a new product (CP₃₋) with retention time 8.59 min is distinctly observable. After 90 min of irradiation, only the three dimeric structures can be observed.

Figure S14. HPLC traces and corresponding MS analysis of the different purified products obtained after irradiation, showing the formation of CP₃₋ (Rₚ = 8.59 min) and three dimeric species with retention time of 5.37 min (Z,Z-D₃₃), 8.19 min (E,E-D₃₃), and 8.26 min (E,Z-D₃₃), respectively.
Figure S15. UV-VIS spectra of a) CP3E (purple), CP3z (cyan), and b) the three photo-dimers [Z,Z-D3ht (blue), E,Z-D3ht (green), E,E-D3ht (orange)] obtained after irradiation in which the lack of the 368 nm band confirms the occurrence of the [4+4] photochemical cycloaddition among the anthracene moieties. In all cases, the sample concentration is 200 µM.

Figure S16. a) HPLC analysis [HPLC conditions: H2O (0.1% TFA)/ACN (0.1% TFA). 100:0 → 100:0 (2 min) and 100:0 → 50:50 (19 min)] of the mixture of CP3 (250 µM) irradiated at pH 7 (Hepes, 10 mM) under oxygen atmosphere at different time intervals. b) ESI-MS of CP3z and CP3E (●, 15.7 min and 15.9 min). c) ESI-MS of Z,Z-D3ht, E,E-D3ht and E,Z-D3ht (▲, 14.5 min, 15.4 min and 15.6 min). d) ESI-MS of endoperoxides (●, 12.8 min, 12.4 min and 11.7 min). e) ESI-MS of product at 8.6 min (a). f) ESI-MS of the N-O photolytic product (*, 8.2 min). g) ESI-MS of the oxyme hydrolysis product (♦, CP2, 8.0 min).
Figure S17. NMR spectra in water of: a) CP3E, b) CP3Z, c) E,Z-D3ht, d) E,E-D3ht, e) Z,Z-D3ht.

Figure S18. a) Evolution of the absorption at 386 nm of CP3 (250 μM) at neutral conditions (10 mM Hepes, pH 7), after cycles of irradiation (5 or 10 min) and darkness (40 min), b) Evolution of the absorption at 386 nm of Ant1 (250 μM) in a 3:1 mixture of MeOH and aqueous solution (10 mM Hepes, pH 7), after cycles of irradiation (10 or 20 min) and darkness (40 min).
Figure S19. Time-course UV-VIS spectra of thermal dissociation of dimers upon heating at 80°C of the corresponding aqueous solutions of a) $E,E$-D$_{3ht}$, b) $Z,Z$-D$_{3ht}$, and c) $E,Z$-D$_{3ht}$ at a concentration of 250 µM; d) Percentage of formed anthracene moieties (%) as a function of the heating time at 80°C. The percentage of free anthracene (%) was calculated through the equation $100\cdot \frac{(A_0 - A)}{A_0} \cdot 100$, where $A_0$ is the maximum absorption at 386 nm before irradiation. Concentration of free anthracene moieties calculated using an extinction coefficient at 386 nm of $e = 6300 \text{ M}^{-1}\text{cm}^{-1}$: $E,E$-D$_{3ht}$: 12% (blue), $Z,Z$-D$_{3ht}$: 25% (orange), and $E,Z$-D$_{3ht}$: 47% (green).

Figure S20. HPLC traces obtained after the thermal dissociation experiments for of $E,E$-D$_{3ht}$ (green), $Z,Z$-D$_{3ht}$ (orange) and $E,Z$-D$_{3ht}$ (red) derivatives. The HPLC traces of CP$_3Z$ (purple) and CP$_3E$ (blue) were included for comparison.
**Figure S21.** Epifluorescence images of an aqueous solution of CP3 (350 µM) at pH 8.

**Figure S22.** Epifluorescence images of a single fibre of CP3 (350 µM, pH 8) exposed to different irradiation time in which the emission switching off suggest the structural changes on anthracene moiety. Scale bar: 10 µm.
**Figure S23.** a) Structure of CP bearing a pyrene moiety (CP3P); b) Overlaid NR (1 μM) fluorescence spectra of solutions of CP3P at different concentrations (Hepes, 10 mM, pH 7); c) Maximum intensity (data in purple) and corresponding wavelength at maximum intensity (data in magenta) were both plotted as a function of the logarithm of the peptide concentration allowing to estimate a cac around 10 µM. \( \lambda_{\text{exc}} = 550 \text{ nm} \) and \( T = 20^\circ\text{C} \). d) Overlaid CD spectra (\( T = 20^\circ\text{C} \)) of aqueous solution of CP3P (10 mM Hepes, pH 7) at different concentrations (5.7-500 µM) in which a strong bisignate Cotton effect for the \( \pi-\pi^* \) transition of pyrene was observed. e) Minimum intensity (data in purple) plotted as a function of the peptide concentration allowing to estimate the concentration at which the bisignate Cotton effect of Pyr band was observed (\(~100 \mu\text{M})

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**Figure S24.** STEM micrographs of grids resulting of the deposition of a solution of CP3 (250 µM, pH 7) that was irradiated for 90 min.
2. Materials

All chemicals were purchased from Sigma-Aldrich, Iris Biotech, TCI, Alfa Aesar, and Fischer Scientific and used without any further purification unless otherwise stated. All solvents used were HPLC or synthesis grade, except for dry CH$_2$Cl$_2$ that was distilled over CaH$_2$.

$^1$H NMR spectra were recorded on Varian Inova 500 in H$_2$O using D$_2$O as deuterated solvents. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS (δ = 0) or the solvent signals HOD (δ = 4.79 ppm). $^1$H NMR splitting patterns are reported as a singlet (s), doublet (d), triplet (t), multiplet (m), or broad (b). The coupling constants (J) are given in Hz. Not easily interpretable signals are indicated as multiple (m) or broad (br).

$^1$H NMR spectra signals were identified from the corresponding double-quantum-filled 2D COSY, TOCSY, and/or NOESY and ROESY at specified concentration and temperature in each case. Mixing times (~150 ms or 400 ms) were not optimized.

HPLC semi-preparative purification was performed on Hitachi D-7000 with a Phenomenex Luna 5 μm-C18 column using H$_2$O/MeCN (0.1% TFA) gradients. Ultrahigh-pressure liquid chromatography coupled with mass spectrometry (uHPLC-MS) analysis was accomplished on Agilent Technologies 1260 Infinity II connected with a 6120 Quadrupole LC-MS using an Agilent SB-C18 column.

Accurate mass determination (HR-MS) using ESI-MS were acquired in a Bruker MicroTof II mass spectrometer. Data are expressed in units of mass per unit of load (m/z).

FT-IR spectra were acquired in a PerkinElmer Spectrum Two ATR, placing the sample as a thin-film on the diamond plate (neat).

2.1 Solid phase peptide synthesis. Preparation of CP2

The synthesis of CP2 was performed on a Rink Amide resin (100–200 mesh; 0.7 mmol·g$^{-1}$) using manual solid phase synthesis according to the Fmoc-based strategy.[1] CP2 was synthesized at a 250 μmol scale. Briefly, Fmoc-Rink Amide resin was swelled in DCM (3 mL) for 30 min in a peptide synthesis vessel and washed with DMF (6 x 2 mL) and DCM (6 x 2 mL). Fmoc group was removed by treating the resin with piperidine in DMF (1:4, 3 mL) for 20 min. After DMF washings (3 x 2 mL),
couplings were carried out using a solution of Fmoc-protected amino acid (3 equiv), N-HBTU (3 equiv),
DIEA (6 equiv) in DMF (0.5 M in AA, 2 mL) under orbital shaking for 40 min at room
temperature. Once the reaction was completed, the resin was washed with DMF (3 x 2 mL) and DCM
(3 x 2 mL). The efficiency of each coupling and deprotection was checked by the TNBS test.

Cycles of deprotection and coupling are repeated using identical conditions until the desired linear peptide
was achieved. The last residue was maintained N-protected (Fmoc) to prevent side-reactions when
deprotection of the C-terminal allyl group. Deprotection of the allyl protecting group was achieved by
adding to the resin a degassed mixture of Pd(OAc)$_2$ (0.25 equiv), PPh$_3$ (1.25 equiv), N-
methylmorpholine (7 equiv), and phenylsilane (7 equiv) in dry DCM (8 mM in Pd, 2 mL) that previously
was stirred at rt until it took a bright-yellow color. The reaction was shaken for 3-4 hours at room
temperature. Afterwards, the resin was filtered and washed with DCM (3 x 2 mL), DMF (3 x 2 mL),
sodium diethylthiocarbamate in DMF (0.5%, 2 x 2 mL, 15 min), DMF (2 x 2 mL), LiCl in DMF (0.8 M, 3 x 2 mL). Subsequently, a solution of
TFE (2 x 2 mL) and finally DMF (3 x 2 mL) was added to the resin, followed by a dropwise addition of DIEA (5 equiv), and the mixture was shaken
for 30 min. Finally, the resin was thoroughly washed with DMF (3 x 2 mL).

Deprotection of CP side chains and cleavage was carried out by shaking the resin with a freshly
prepared solution of TFA, H$_2$O and TIS (95:2.5:2.5, 10 mL/g of resin) for 2 h. The mixture was collected
by filtration, concentrated with argon steam, and added dropwise over ice-cooled flask containing Et$_2$O
(20 mL of Et$_2$O for 1 mL of TFA). The precipitate was centrifuged (4000 rpm x 10 min) and the
supernant was discarded. After washing with diethyl ether, the resulting residue was dried under
vacuum.

For selective deprotection of the 4-methyltrityl group (Mtt), a freshly prepared mixture of DCM, HFIP,
TFE and TIS (6.5:2:1.05; 3 x 3 mL) was added to the resin and the mixture was stirred for 2 h, after
which was washed with DCM (3 x 3 mL) and DMF (3 x 3 mL). Subsequently, a solution of
(tert-butyloxycarbonylaminoxy)acetic acid (2.5 equiv) and 36.2 (CH, sulfonamide), 55.9 (CH, sulfo-
nitride), 55.8 (CH, sulfonamide), 53.7 (CH, sulfonamide), 53.6 (CH, sulfonamide), 53.4 (CH, sulfon-
nitride), 34.9 (CH, sulfonamide), 128.6 (C), 117.5 (CH), 117.4 (CH), 115.2 (C), 72.7 (CH$_2$), 61.5 (CH$_2$),
61.3 (CH$_2$), 59.9 (CH), 58.8 (CH), 53.7 (CH), 53.6 (CH), 53.4 (CH), 52.8 (CH), 52.4 (CH), 50.1 (CH), 38.9
(CH$_3$), 36.2 (CH$_3$), 35.9 (CH$_3$), 35.4 (CH$_3$), 34.9 (CH$_3$), 31.2 (CH$_3$), 30.7 (CH$_3$), 27.8 (CH$_2$),
26.8 (CH$_2$), 26.7 (CH$_2$), 26.5 (CH$_2$), 26.2 (CH$_2$), 22.4 (CH$_2$), 17.0 (CH$_3$), ESI-MS m/z (%) for
C$_{45}$H$_{71}$N$_{18}$O$_{16}$, [M+H]$^+$, 560.3 (100) [M+2H]$^{2+}$, 374.0 (8) [M+3H]$^{3+}$. HRMS (ESI) m/z: [M+H]$^+$ calcd for C$_{45}$H$_{71}$N$_{18}$O$_{16}$,
1119.5213; found, 1119.5290 **FTIR (neat):** ν = 3279.4 (amide A), 1654, 1647 (amide I), 1532.8 (amide II) cm⁻¹.

![Graph a) Abs at 222 nm (mÅ)](image)

![Graph b) Relative abundance (%)](image)

*a) RP-uHPLC: Agilent SB-C18 column, H₂O (0.1% TFA)/ACN (0.1% TFA). 100:0 → 100:0 (2 min) and 100:0 → 70:30 (19 min)] Rt = 8.4 min; b) ESI-MS of the main peak.

![NMR spectrum](image)

**¹H NMR of CP2 at 3 mM (500 MHz, D₂O/H₂O, 298K)**
DEPT-135 and $^{13}$C NMR of CP2 at 5.5 mM (500 MHz, D$_2$O/H$_2$O, 298K).

TOCSY of CP2 at 3 mM (500 MHz, D$_2$O/H$_2$O, 298K)
FT-IR spectrum of CP2 (solid, 298 K).

2.2 Preparation of CP3E

CP2 (15 mg, 11.1 μmol) was dissolved in a solution of 9-anthracencarboxaldehyde (2.8 mg, 13.4 μmol) in DMSO (100 μL) and the mixture was warmed at 60°C for approximately 2h, until no starting substrate was detected by HPLC-MS analysis. The mixture was poured into an eppendorf containing cold diethyl ether (2 mL) and then powerfully shaken to obtain a yellow precipitate. After centrifugation, the organic phase was discarded, and the residue was sonicated for 15 minutes with fresh diethyl ether (2 mL), spined down and decanted. This step was repeated three times. The product was dried under reduced pressure to provide CP3E as a yellow solid (16 mg, 93%).

$^1$H NMR (D$_2$O, 500 MHz, δ, ppm): 9.31 (s, 1H, CH$_{ox}$), 8.59 (d, J = 8.3 Hz, 1H, NH$_{His}$), 8.55 (s, 1H, $^1$H$_{Ant}$), 8.45 (s, 1H, CH$_{in}$), 8.40 (s, 1H, CH$_{in}$), 8.34 (d, J = 8.0 Hz, 1H, NH$_{His}$), 8.30–8.24 (m, 3H, $^6$NH$_{Lys}$ and 2H$_{Ant}$), 8.22 (d, J = 7.9 Hz, 1H, $^6$NH$_{Gln}$), 8.20 (d, J = 6.8 Hz, 2H, NH$_{Ser}$, $^6$NH$_{Gln}$), 8.16 (d, J = 6.2 Hz, 1H, NH$_{Ala}$), 8.09–8.00 (m, 3H, NH$_{Ser}$, 2 H$_{Ant}$), 7.95–7.84 (m, 2H, $^6$NH$_{Lys}$, NH$_{Pala}$), 7.74 (t, J = 6.2 Hz, 1H, NH$_{Pala}$), 7.58–7.47 (m, 4H, H$_{Ant}$), 7.38 (brs, 2H, NH$_{2-Gln}$), 7.08 (s, 1H, CH$_{im}$), 7.04 (s, 1H, CH$_{im}$), 6.74 (d, J = 17.3 Hz, 2H, NH$_{2-Gln}$), 3.86 (m, 1H, $^6$CH$_{Lys}$), 3.72–3.56 (m, 6H, CH$_{2-Ser}$, CH$_{2-OH}$), 3.34–3.10 (m, 7H, CH$_{2-Lys}$, $^5$CH$_{2-His}$, $^5$CH$_{2-Pala}$), 3.06 (dd, J = 15.8 and 5.4 Hz, 1H, $^5$CH$_{2-His}$), 2.86–2.85 (m, 2H, $^5$CH$_{2-His}$), 2.38–2.18 (m, H, $^6$CH$_{2-Pala}$), 2.16 (t, J = 7.0 Hz, 2H, $^6$CH$_{2-Pala}$), 2.16–2.00 (m, 4H, $^7$CH$_{2-Glu}$), 1.92 (m, 1H, $^7$CH$_{2-Glu}$), 1.80–1.65 (m, 3H, $^7$CH$_{2-Glu}$), 1.60–1.34 (m, 4H, CH$_{2-Lys}$), 1.17 (d, J = 7.6 Hz, 3H, CH$_{3-Ala}$), 1.18 (m, 2H, CH$_{2-Lys}$). $^{13}$C NMR (D$_2$O, 75 MHz, δ, ppm): 177.7 (CO), 177.6 (CO), 175.8 (CO), 174.8 (CO), 174.0 (CO), 173.5 (CO), 172.8 (CO), 172.3 (CO), 172.0 (CO), 171.6 (CO), 171.2 (CO), 171.4 (CO), 171.3 (CO), 151.5 (CH), 133.7 (CH), 133.6 (CH), 130.9 (C), 130.0 (CH), 129.6 (C), 129.0 (CH), 128.5 (C), 128.4 (C), 127.4 (CH), 125.9 (CH), 124.5 (CH), 117.5 (C), 117.4 (CH), 117.3 (CH), 72.5 (CH$_2$), 61.3 (CH$_2$), 61.2 (CH$_2$), 55.9 (CH), 55.7 (CH), 53.8 (CH), 53.5 (CH), 53.4 (CH), 52.7 (CH), 52.3 (CH), 50.0 (CH), 38.9 (CH$_2$), 36.1 (CH$_2$), 35.7 (CH$_2$), 35.2 (CH$_2$), 34.7 (CH$_2$), 31.2 (CH$_2$), 30.9 (CH$_2$).
30.3 (CH₂), 28.2 (CH₂), 26.9 (CH₂), 26.6 (CH₂), 26.2 (CH₂), 26.1 (CH₂), 22.4 (CH₂), 16.9 (CH₃). 

**ESI-MS** m/z (%): 1307.6 (15) [M+H]⁺, 654.4 (100) [M+2H]²⁺, 436.9 (2) [M+3H]³⁺. 

**HRMS** (ESI) m/z: [M+H]⁺ calcd for C₆₀H₇₉N₁₈O₁₆, 1307.5921; found, 1307.5916. 

**FTIR (neat):** ν = 3292.5 (amide A), 1664.6, 1633.3 (amide I), 1539.4 (amide II) cm⁻¹.

**a) RP-uHPLC:** [Agilent SB-C18 column, H₂O (0.1% TFA)/ACN (0.1% TFA). 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)] Rₜ = 9.6 min; b) ESI-MS of the main peak.

**¹H NMR of CP₃:** at 1.3 mM (500 MHz, D₂O/H₂O, 298K).
DEPT-135 and $^{13}$C NMR of CP3$_E$ at 5.5 mM (500 MHz, D$_2$O/H$_2$O, 298K).

TOCSY of CP3$_E$ at 1.3 mM (500 MHz, D$_2$O/H$_2$O, 298K).
CP3E FT-IR spectrum (solid, 298 K).

2.3 Preparation of CP3E, E,E-D3ht, Z,Z-D3ht, and E,Z-D3ht derivatives.

Aqueous solution of CP3E (400 μM) was neutralized with NaOH (0.1 M) to pH 7-8 and the resulting mixture was degassed with argon for 10 min. Then, the solution was sealed in a quartz cuvette under argon atmosphere to prevent the formation of endoperoxide species during the irradiation experiments. After equilibration for 20 min, the solutions were irradiated at 350 nm with a Xenon Light Source 300W Max-303 through a XUS0350 Shortpass Filter for 40 min. The distance between the sample solutions and the light source was set to be around 5 cm. The resulting mixture was collected and freeze-dried. The solid obtained (20 mg) was solubilized in MilliQ-H2O with 0.1% TFA (0.05 mL/mg of crude) and purified by semipreparative RP-HPLC using a Phenomenex Luna C18 (2) 100Å column [gradient: H2O (0.1% TFA)/ACN (0.1% TFA), 90:10 → 76:24 (10 min); 76:24 → 65:35 (35 min), Rf(Z,Z-D3ht) = 13.71 min, Rf(E,E-D3ht) = 17.86 min, Rf(E,E-D3ht)+ = 18.43 min, Rf(CP3E) = 25.69 min, Rf(CP3E) = 27.50 min]. After freeze-drying of the proper fractions, Z,Z-D3ht, E,E-D3ht, E,Z-D3ht, CP3Z, and CP3E were obtained with a yield of 5.5% (white solid, 2.2 mg), 9.6% (white solid, 3.8 mg), 17.1% (white solid, 6.8 mg), 13% (light yellow solid, 2.8 mg) 14.1% (yellow solid, 3 mg), respectively. The pure products were stored at -20 °C until further use.

CP3E: 1H NMR (D2O, 500 MHz, δ, ppm): 8.60 (d, J = 8.0 Hz, 1H, NHHis), 8.48 (s, 1H, CHim), 8.45 (d, J = 8.0 Hz, 1H, NHHis), 8.43 (s, 1H, 1HAm), 8.42 (s, 1H, CHim), 8.38 (s, 1H, Hox), 8.26 (d, J = 7.5 Hz, 1H, αNHGin), 8.21 (d, J = 6.8 Hz, 4H, αNHGin, 2NHSer, NHAla), 8.10 (d, J = 7.2 Hz, 1H, αNHLys), 7.97 (d, J = 9.3 Hz, 2H, 2HAla), 7.92 (t, J = 6.3 Hz, 1H, NHβAla), 7.82–7.74 (m, 3H, NHβAla, 1HAnl), 7.54–7.44 (m, 4H, HAnl), 7.39 (brs, 2H, NH2Gin), 7.09 (s, 2H, CHim), 6.90 (t, J = 6.3 Hz, 1H, αNH Gly), 6.74 (d, J = 13.4 Hz, 2H, NH2Gin), 4.28–4.01 (m, 3H, αCHGin, αCHLys, αCHHis), 3.72 (Abd, J = 11.6 and 5.2 Hz, 1H, 1Hβ2Ser), 3.68 – 3.58 (m, 3H, βCH2Ser), 3.38 – 2.84 (m, 10H, CH2Lys, CH2His, βCH2βAla), 2.32 (m, 2H, CH2βAla), 2.28 (m, 2H, CH2βAla), 1.64 (m, 1H, CH2Lys), 1.48 (m, 1H, CH2Lys), 1.22 (d, J = 7.4 Hz, 3H, CH3Ala), 1.18 (m, 2H, βCH2Lys), 1.00 (m, 2H, 1Hβ2Lys). 13C NMR (D2O, 75 MHz, δ, ppm): 177.7 (CO), 177.5 (CO), 175.0 (CO), 174.1 (CO), 174.0 (CO), 173.6 (CO), 172.8 (CO), 172.1 (CO), 171.7 (CO), 171.5 (CO), 171.3 (CO), 163.1 (CO), 162.8 (CO), 150.4 (CH), 133.8 (CH), 130.7 (C), 129.1 (CH), 129.0 (CH), 128.7 (C), 128.6 (C), 127.7 (C), 127.3 (CH), 126.0 (CH), 124.7 (CH), 124.6 (CH), 117.5 (C), 117.4 (CH), 117.3 (CH), 72.4 (CH2), 61.3 (CH2), 61.2 (CH2), 55.9 (CH), 55.7 (CH), 53.8 (CH), 53.6 (CH), 53.5 (CH), 52.7 (CH), 52.3 (CH), 50.1 (CH), 38.6 (CH2), 36.1 (CH2), 35.8 (CH2), 35.3 (CH2), 34.7 (CH2), 31.2 (CH2), 30.9 (CH2), 30.5 (CH2), 27.6 (CH2), 26.8 (CH2), 26.6 (CH2), 26.3 (CH2), 26.1 (CH2), 22.2 (CH2), 16.9 (CH3). ESI-MS m/z (%): 1307.5 (13) [M+H]+, 654.5 (100)
[M+2H]²⁺. HRMS (ESI) m/z: [M+H]⁺ calcd for C₆₀H₇₈N₁₈O₁₆, 1307.5921; found, 1307.5916. FTIR (neat): ν = 3279.9 (amide A), 1638.5 (amide I), 1540.7 (amide II) cm⁻¹.

a) RP-uHPLC: [Agilent SB-C18 column, H₂O (0.1% TFA)/ACN (0.1% TFA). 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)] Rₜ = 8.59 min; b) ESI-MS of the main peak.

¹H NMR of CP₃₂ at 2.5 mM (500 MHz, D₂O/H₂O, 298K).
DEPT-135 and $^{13}$C-NMR of CP3$_2$ at 2.6 mM (500 MHz, D$_2$O/H$_2$O, 298K).

TOCSY of CP3$_2$ at 2.5 mM (500 MHz, D$_2$O/H$_2$O, 298K).

CP3$_2$ FT-IR spectrum (solid, 298 K).
Z-Z-D3het. $^1$H NMR (D$_2$O, 500 MHz, δ, ppm): 8.68 (d, J = 7.9 Hz, 2H, NH$_{His}$), 8.58–8.45 (m, 6H, NH$_{His}$, CH$_{im}$), 8.34–8.25 (Overlaped d, 10H, 2NH$_{Ser}$, 4NH$_{Gin}$, 2NH$_{Ala}$, 2$^2$NH$_{Lys}$), 8.18–8.13 (m, 4H, NH$_{Ser}$, NH$_{Lys}$), 8.08 (s, 2H, H$_{ox}$), 7.98 (t, J = 6.3 Hz, 2H, NH$_{βAla}$), 7.87 (t, J = 6.2 Hz, 2H, NH$_{βAla}$), 7.42 (br, 4H, NH$_{2-Gin}$), 7.23 (s, 2H, CH$_{im}$), 7.16 (s, 2H, CH$_{im}$), 7.04 (d, J = 7.5 Hz, 4H, H$_{Ar}$), 6.92–6.69 (m, 14H, H$_{Ar}$ and NH$_{2-Gin}$), 6.76 (br, 2H, NH$_{2-Gin}$), 4.28–4.04 (m, 10H, $^6$CH$_{Gin}$, $^3$CH$_{Lys}$, $^6$CH$_{His}$, CH$_{2}$ONH), 3.75 Abd, J = 11.6 and 5.3 Hz, 2H, $^5$CH$_{2-Ser}$), 3.72 (d, J = 5.1 Hz, 4H, $^5$CH$_{2-Ser}$), 3.66 (Abd, J = 11.6 and 4.7 Hz, 2H, $^5$CH$_{2-Ser}$), 3.43–3.20 (m, 12H, $^6$CH$_{βAla}$, $^2$CH$_{2-His}$), 3.05 (td, J = 16.0 and 9.4 Hz, 4H, $^6$CH$_{2-His}$), 2.74–2.56 (m, 4H, $^1$CH$_{2-Lys}$), 2.39 (m, 4H, $^6$CH$_{2-βAla}$), 2.31 (t, J = 7.0 Hz, 4H, $^6$CH$_{2-βAla}$), 2.24–2.08 (m, 8H, CH$_{2-Gin}$), 2.00 (m, 2H, CH$_{2-Gin}$), 1.54 (m, 2H, CH$_{2-Lys}$), 1.40 (m, 2H, CH$_{2-Lys}$), 1.24 (d, J = 7.4 Hz, 6H, CH$_{3-Ala}$), 0.90 (m, 4H, CH$_{2-Lys}$), 0.77 (m, 2H, CH$_{2-Lys}$), 0.68 (m, 2H, CH$_{2-Lys}$). $^{13}$C NMR (D$_2$O, 75 MHz, δ, ppm): 177.7 (CO), 177.6 (CO), 175.0 (CO), 174.1 (CO), 174.0 (CO), 173.7 (CO), 172.9 (CO), 172.1 (CO), 171.7 (CO), 171.5 (CO), 171.4 (CO), 163.1 (CO), 162.8 (CO), 157.7 (CH), 140.8 (C), 140.7 (C), 133.9 (CH), 133.8 (CH), 128.7 (C), 128.6 (C), 128.4 (CH), 126.5 (CH), 126.3 (CH), 126.1 (CH), 119.8 (C), 117.5 (CH), 117.4 (CH), 115.2 (C), 71.6 (CH$_2$), 61.4 (CH$_2$), 61.2 (CH$_2$), 61.1 (CH), 60.9 (C), 55.8 (CH), 53.7 (CH), 53.6 (CH), 53.5 (CH), 52.8 (CH), 52.4 (CH), 52.3 (CH), 50.1 (CH), 38.2 (CH$_2$), 36.2 (CH$_2$), 35.9 (CH$_2$), 35.4 (CH$_2$), 34.8 (CH$_2$), 31.2 (CH$_2$), 31.0 (CH$_2$), 30.4 (CH$_2$), 27.4 (CH$_2$), 26.9 (CH$_2$), 26.7 (CH$_2$), 26.4 (CH$_2$), 26.2 (CH$_2$), 22.0 (CH$_2$), 16.9 (CH$_3$). ESI-MS m/z (%): 1307.9 (14) [M+2H]$^{2+}$, 872.5 (100) [M+3H]$^{3+}$, 654.7 (8) [M+4H]$^{4+}$. HRMS (ESI) m/z: [M+2H]$^{2+}$ calcd for C$_{120}$H$_{158}$N$_{35}$O$_{32}$, 1307.5921; found, 1307.5916. FTIR (neat): ν = 3274.6 (amide A), 1654 (amide I), 1540.7 (amide II) cm$^{-1}$.

a) RP-uHPLC: [Agilent SB-C18 column, H$_2$O (0.1% TFA)/ACN (0.1% TFA). 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)] $R_t$ = 5.36 min; b) ESI-MS of the main peak.
$^1$H NMR of $Z,Z$-D$_3$ht at 3.2 mM (500 MHz, D$_2$O/H$_2$O, 298K).

DEPT-135 and $^{13}$C NMR of $Z,Z$-D$_3$ht at 3.2 mM (500 MHz, D$_2$O/H$_2$O, 298K).
TOCSY of $\text{Z,Z-D}3_{H}$ at 3.2 mM (500 MHz, D$_2$O/H$_2$O, 298K).

ROESY of $\text{Z,Z-D}3_{H}$ at 3.2 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HSQC of $Z,Z$-D$_{3}t$ at 3.2 mM (500 MHz, D$_2$O/H$_2$O, 298K).

HMBC of $Z,Z$-D$_{3}t$ at 3.2 mM (500 MHz, D$_2$O/H$_2$O, 298K).
**Z,Z-D3_{nt}** FT-IR spectrum (solid, 298 K).

**E,E-D3_{nt}:** $^1$H NMR (D$_2$O, 500 MHz, δ, ppm): 8.64 (d, $J = 7.9$ Hz, 2H, NH$_{His}$), 8.45 (overlapped s, 4H, CH$_{im}$), 8.42 (d, $J = 7.8$ Hz, 2H, NH$_{His}$) 8.40 (7, $J = 6.5$ Hz, 2H, 4NH$_{Lys}$), 8.30–8.18 (m, 12H, NH$_{Ser}$, 4NH$_{Gln}$, NH$_{Abu}$, 4NH$_{lys}$), 8.09 (s, 2H, H$_{ox}$), 7.90 (t, $J = 6.3$ Hz, 2H, NH$_{Bala}$), 7.76 (t, $J = 6.2$ Hz, 2H, NH$_{Bala}$), 7.38 (brs, 4H, NH$_{2-Gin}$), 7.11 (s, 2H, CH$_{im}$), 7.06 (s, 2H, CH$_{im}$), 6.84–6.70 (m, 20H, H$_{Ar}$, NH$_{2-Gln}$), 5.44 (s, 2H, H$_{10-Amt}$), 4.38–4.02 (m, 6H, 6CH$_{Gln}$, 6CH$_{Lys}$, 6CH$_{His}$), 3.76–3.66 (m, 6H, CH$_{2-Ser}$), 3.63 (dd, $J = 11.7$ and 4.7 Hz, 2H, CH$_{2-Ser}$), 3.38–3.10 (m, 16H, CH$_{2-His}$, 6CH$_{Bala}$, 6CH$_{2-Lys}$), 2.97 (dd, 2H, $J = 15.8$ and 9.4 Hz, CH$_{2-His}$), 2.91 (dd, 2H, $J = 15.7$ and 9.4 Hz, CH$_{2-His}$), 2.35–2.26 (m, 4H, 6CH$_{2-Bala}$), 2.22 (t, $J = 7.1$ Hz, 4H, 6CH$_{2-Bala}$), 2.18–1.88 (m, 12H, CH$_{2-Gin}$), 1.82–1.54 (m, 16H, CH$_{2-Lys}$, CH$_{2-Gin}$), 1.38 (m, 4H, 4CH$_{2-Lys}$), 1.28 (d, $J = 7.4$ Hz, 6H, CH$_{3-Aba}$). $^{13}$C NMR (D$_2$O, 75 MHz, δ, ppm): 177.7 (CO), 177.5 (CO), 174.9 (CO), 174.0 (CO), 173.7 (CO), 173.6 (CO), 172.8 (CO), 172.5 (CO), 172.0 (CO), 171.7 (CO), 171.4 (CO), 163.0 (CO), 162.7 (CO), 156.2 (CH), 143.2 (C), 142.5 (C), 133.9 (CH), 133.7 (CH), 128.6 (C), 128.5 (C), 128.4 (CH), 126.6 (CH), 126.0 (CH), 125.7 (CH), 125.6 (CH), 117.5 (C), 117.4 (CH), 115.2 (C), 72.8 (CH$_2$), 61.4 (CH$_2$), 61.2 (CH$_2$), 57.7 (C), 55.8 (CH), 55.7 (CH), 53.8 (CH), 53.6 (CH), 53.5 (CH), 52.8 (CH), 52.4 (CH), 52.0 (CH), 50.1 (CH), 39.0 (CH$_2$), 36.1 (CH$_2$), 35.8 (CH$_2$), 35.3 (CH$_2$), 34.7 (CH$_2$), 31.2 (CH$_3$), 31.0 (CH$_3$), 30.6 (CH$_3$), 28.7 (CH$_2$), 26.8 (CH$_2$), 26.3 (CH$_2$), 26.0 (CH$_2$), 22.6 (CH$_2$), 17.0 (CH$_3$). ESI-MS m/z (%): 1307.5 (31) [M+2H]$^{2+}$, 872.5 (100) [M+3H]$^{3+}$, 654.7 (9) [M+4H]$^{4+}$. HRMS (ESI) m/z: [M+2H]$^{2+}$ calcd for C$_{120}$H$_{158}$N$_{30}$O$_{32}$, 1307.5921; found, 1307.5916. FTIR (neat): ν = 3274.6 (amide A), 1659 (amide I), 1535.6 (amide II) cm$^{-1}$.

**a) RP-uHPLC:** [Agilent SB-C18 column, H$_2$O (0.1% TFA)/ACN (0.1% TFA). 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)] $R_t = 8.19$ min; b) ESI-MS of the main peak.
$^1$H NMR of E,E-D$_3$ht at 3.37 mM (500 MHz, D$_2$O/H$_2$O, 298K).

DEPT-135 and $^{13}$C-NMR of E,E-D$_3$ht at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).
TOCSY of $E,E$-D$_3$h at 3.37 mM (500 MHz, D$_2$O/H$_2$O, 298K).

ROESY of $E,E$-D$_3$h at 3.37 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HSQC of \textbf{E,E-D3}_{nt} at 3.7 mM (500 MHz, D\textsubscript{2}O/H\textsubscript{2}O, 298K).

HMBC of \textbf{E,E-D3}_{nt} at 3.7 mM (500 MHz, D\textsubscript{2}O/H\textsubscript{2}O, 298K).
$E,E$-D$_3$ht FT-IR spectrum (solid, 298 K).

$Z,E$-D$_3$ht: $^1$H NMR (D$_2$O, 500 MHz, δ, ppm): 8.66 (d, $J = 7.5$ Hz, 1H, NH$_{His}$), 8.65 (d, $J = 7.5$ Hz, 1H, NH$_{His}$), 8.54–8.48 (m, 6H, NH$_{His}$, CH$_{Im}$), 8.43–8.12 (m, 14H, NH$_{Ser}$, $^6$NH$_{Gln}$, NH$_{Ala}$, $^6$NH$_{Lys}$, $^6$NH$_{Lys}$), 8.13 (s, 1H, H$_{ax}$), 8.09 (s, 1H, H$_{ax}$), 7.96 (m, 2H, NH$_{Ala}$), 7.84 (m, 2H, NH$_{Ala}$), 7.40 (m, 4H, NH$_{2-Gln}$), 7.20 (s, 1H CH$_{Im}$), 7.18 (s, 1H CH$_{Im}$), 7.15 (s, 2H, CH$_{Im}$), 7.04 (m, 2H, H$_{ax}$), 6.90–6.70 (m, 18H, H$_{Ar}$, NH$_{2-Gln}$), 5.44 (s, 1H, $^1$H$_{N}$), 4.40–4.04 (m, 10H, $^6$CH$_{Gln}$, $^6$CH$_{Lys}$, $^6$CH$_{His}$, CH$_{2}$ONH), 3.84–3.64 (m, 10H, CH$_{2-Ser}$), 3.40–3.16 (m, 14H, CH$_{2-His}$, $^6$CH$_{2-\beta Ala}$, $^6$CH$_{2-Lys}$), 3.04 (m, 4H, CH$_{2-His}$), 2.70 (m, 2H, $^6$CH$_{2-Lys}$), 2.45–2.32 (m, 4H, $^6$CH$_{2-\beta Ala}$), 2.30 (m, 4H, $^6$CH$_{2-\beta Ala}$), 2.25–2.05 (m, 8H, CH$_{2-Gln}$), 1.98 (m, 2H, CH$_{2-Gln}$), 1.84–1.64 (m, 8H, CH$_{2-Lys}$, CH$_{2-Gln}$), 1.59 (m, 2H, CH$_{2-Lys}$), 1.46–1.36 (m, 4H, CH$_{2-Lys}$), 1.32 (d, $J = 7.4$ Hz, 3H, CH$_{3-Ala}$), 1.26 (d, $J = 7.4$ Hz, 3H, CH$_{3-Ala}$), 1.02–0.70 (m, 4H, CH$_{2-Lys}$).

$^{13}$C NMR (D$_2$O, 75 MHz, δ, ppm): 117.7 (CO), 117.6 (CO), 177.5 (CO), 177.5 (CO), 175.0 (CO), 174.8 (CO), 174.1 (CO), 174.0 (CO), 173.7 (CO), 173.6 (CO), 172.9 (CO), 172.8 (CO), 172.5 (CO), 172.4 (CO), 172.1 (CO), 172.0 (CO), 171.7 (CO), 171.6 (CO), 171.5 (CO), 171.4 (CO), 171.3 (CO), 171.2 (CO), 163.1 (CO), 163.0 (CO), 162.8 (CO), 162.7 (CO), 156.5 (CH), 156.4 (CH), 143.2 (C), 142.7 (C), 141.9 (C), 141.2 (C), 134.9 (CH), 134.8 (CH), 134.8 (CH), 134.7 (CH), 128.7 (C), 128.6 (C), 128.6 (C), 128.5 (C), 128.5 (CH), 128.3 (CH), 126.7 (CH), 126.6 (CH), 126.5 (CH), 126.4 (CH), 126.3 (CH), 126.1 (CH), 125.9 (CH), 125.7 (CH), 117.5 (CO), 117.5 (CH), 117.4 (C), 117.4 (CH), 115.2 (C), 115.1 (C), 115.1 (C), 72.9 (CH$_2$), 72.8 (CH$_2$), 61.5 (CH$_2$), 61.4 (CH$_2$), 61.3 (CH$_2$), 61.2 (CH$_2$), 60.5 (C), 58.0 (C), 56.0 (CH), 55.9 (CH), 55.8 (CH), 55.7 (CH), 53.8 (CH), 53.7 (CH), 53.6 (CH), 53.6 (CH), 53.5 (CH), 53.4 (CH), 53.2 (CH), 52.7 (CH), 52.4 (CH), 52.3 (CH), 51.7 (CH), 51.6 (CH), 50.1 (CH), 50.0 (CH), 39.1 (CH$_2$), 38.9 (CH$_2$), 38.6 (CH$_2$), 36.1 (CH$_2$), 35.9 (CH$_2$), 35.8 (CH$_2$), 35.4 (CH$_2$), 35.3 (CH$_2$), 34.8 (CH$_2$), 34.7 (CH$_2$), 34.7 (CH$_2$), 31.2 (CH$_2$), 31.1 (CH$_2$), 31.0 (CH$_2$), 30.9 (CH$_2$), 30.7 (CH$_2$), 30.5 (CH$_2$), 28.6 (CH$_2$), 28.5 (CH$_2$), 26.8 (CH$_2$), 26.7 (CH$_2$), 26.7 (CH$_2$), 26.6 (CH$_2$), 26.4 (CH$_2$), 26.3 (CH$_2$), 26.1 (CH$_2$), 26.0 (CH$_2$), 22.6 (CH$_2$), 22.5 (CH$_2$), 17.0 (CH$_2$), 16.9 (CH$_3$).

ESI-MS m/z (%): 1307.5 (12) [M+2H]$^{2+}$, 872.5 (100) [M+H]$^{3+}$, 654.7 (8) [M+4H]$^{4+}$.

HRMS (ESI) m/z: [M+2H]$^{2+}$ calcld for C$_{120}$H$_{158}$N$_{56}$O$_{32}$, 1307.5921; found, 1307.5916. FTIR (neat): v = 3269.4 (amide A), 1643.4 (amide I), 1535.6 (amide II) cm$^{-1}$. 

S-31
a) **RP-uHPLC:** [Agilent SB-C18 column, H₂O (0.1% TFA)/ACN (0.1% TFA). 80:20 → 80:20 (2 min) and 80:20 → 50:50 (19 min)] \(R_t = 8.26\) min; b) ESI-MS of the main peak.

\(^1\)H NMR of \(E,Z-D3\) at 3.4 mM (500 MHz, \(D_2O/H_2O\), 298K).
DEPT-135 and $^{13}$C-NMR of $E,Z$-D$_3$ht at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

TOCSY of $E,Z$-D$_3$ht at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).
ROESY of $E,Z$-D3$_{het}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

HSQC of $E,Z$-D3$_{het}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HMBC of $E,Z$-$D3_{nt}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

$E,Z$-$D3_{nt}$ FT-IR spectrum (solid, 298 K).

2.4 Preparation of model anthracene derivative **Ant1**

![Chemical diagram](image)
Preparation of Boc-O2Oc-OMe. A solution of Boc-O2Oc-OH (185 mg, 0.703 mmol) in dry MeOH (7 mL) was treated with EDC.HCl (200 mg, 1.05 mmol), HOBT (142 mg, 1.05 mmol) and DMAP (128 mg, 1.05 mmol). The reaction mixture was stirred for 24 hours at room temperature under argon atmosphere. Then, the solvent was removed under reduced pressure. The resulting residue was redissolved in CH2Cl2 and washed with aqueous solutions of HCl (5%, 3x5 mL) and NaHCO3 (sat. sol., 3x5 mL). The organic phase was dried over anhydrous MgSO4, filtered and concentrated under vacuum. Flash chromatography (0-2% MeOH) purification provided Boc-O2Oc-OMe as a light-yellow oil [152 mg, 78%, Rf = 0.75 (10% MeOH/ CH2Cl2)]. ¹H NMR (CDCl3, 300 MHz, δ, ppm): 5.02 (br, 1H, NH), 4.14 (s, 2H, CH2CO), 3.73 (s, 3H, OMe), 3.68 (m, 2H, CH2), 3.63 (m, 2H, CH2), 3.52 (m, 2H, CH2), 3.29 (m, 2H, CH2), 1.41 (s, 9H, Boc). ¹³C NMR (CDCl3, 75 MHz, δ, ppm): 170.9 (CO), 156.1 (CO), 79.3 (C), 71.0 (CH2), 70.4 (CH2), 70.3 (CH2), 68.7 (CH2), 51.9 (CH3), 40.4 (CH2), 28.5 (CH3). MS (ESI) [m/z (%)]: 300.1 ([M+Na]+, 23), 278.2 ([M+H]+, 1), 178.1 ([M-Boc+H]⁺, 100). HRMS (ESI) Calculated for C12H23NNaO6: 300.1418, found 300.1411.
Preparation of Boc-NO-Ac-O2Oc-OMe. A solution of Boc-O2Oc-OMe (142 mg, 0.512 mmol) in CH₂Cl₂ (2 mL) was treated with TFA (2 mL) and stirred for 15 minutes. The solvent was removed under reduced pressure and the resulting oil was dissolved in CH₂Cl₂ and further evaporated; the process was repeated three times. The residue was dried at high vacuum for 2 hours. Then, it was dissolved in dry CH₂Cl₂ (5 mL) and treated with DIEA (351 μL, 2.05 mmol), N-HBTU (243 mg, 0.640 mmol) and (Boc-aminooxy)acetic acid (108 mg, 0.563 mmol). The reaction mixture was stirred for 1.5 hours at room temperature under argon atmosphere and then washed with aqueous solutions of HCl (5%, 3x5 mL) and NaHCO₃ (sat., 3x5 mL). The organic phase was dried over anhydrous MgSO₄, filtered and concentrated under vacuum. The resulting residue was purified by flash chromatography (0-3% MeOH/CH₂Cl₂) and the product was obtained as a light-yellow oil [128 mg, 72%, Rᵣ = 0.68 (10% MeOH/CH₂Cl₂)].

\(^1\)H NMR (CDCl₃, 300 MHz, δ, ppm): 7.99 (br, 1H, NH), 7.86 (br, 1H, NH), 4.32 (s, 2H, COCH₂O), 4.15 (s, 2H, COCH₂ON), 3.73 (s, 3H, OMe), 3.70 (m, 2H, CH₂), 3.67 (m, 2H, CH₂), 3.57 (m, 2H, CH₂), 3.49 (m, 2H, CH₂), 3.49 (m, 2H, CH₂), 1.45 (s, 9H, Boc).

\(^1^3\)C NMR (CDCl₃, 75 MHz, δ, ppm): 171.1 (CO), 169.1 (CO), 157.6 (CO), 82.6 (C), 76.0 (CH₂), 71.0 (CH₂), 70.4 (CH₂), 69.6 (CH₂), 68.6 (CH₂), 52.0 (CH₃), 38.9 (CH₂), 28.2 (CH₃).

MS (ESI) [m/z (%)]: 373.2 ([M+Na]\(^+\), 51), 351.2 ([M+H]\(^+\), 17), 295.1 ([M-tBu+H]\(^+\), 62), 251.1 ([M-Boc+H]\(^+\), 100).

HRMS (ESI) Calculated for C\(_{14}\)H\(_{27}\)N\(_2\)O\(_8\): 351.1762, found 351.1769.

\(^1\)H NMR (CDCl₃, 300 MHz) of Boc-NO-Ac-O2Oc-OMe
Preparation of Ant1. A solution of Boc-NO-Ac-O2Oc-OMe (99 mg, 0.283 mmol) in MeOH/H$_2$O (3:1, 6 mL) was treated with LiOH (34 mg, 1.42 mmol). After stirring for 3 hours, MeOH was evaporated under reduced pressure and the aqueous phase was washed with Et$_2$O (3x2 mL). Then, the aqueous phase was acidified with aqueous HCl (5%) to pH 2 and extracted with CH$_2$Cl$_2$ (3x5 mL). The organic phase was dried over anhydrous MgSO$_4$, filtered and concentrated under vacuum. The resulting light-yellow oil was dissolved in a mixture of TFA/CH$_2$Cl$_2$ and stirred for 30 minutes. The mixture was concentrated under reduced pressure, diluted in CH$_2$Cl$_2$ and further evaporated for three times. The residue obtained was dried under high vacuum for 2 hours. After this time, it was dissolved in CHCl$_3$ (3 mL), treated with 9-anthracenecarboxaldehyde (58 mg, 0.283 mmol) and sonicated for 15 minutes protected from light. Then CHCl$_3$ was concentrated under reduced pressure and the residue was solubilized in ACN/MilliQ-H$_2$O (1:4). This solution was purified by semipreparative RP-HPLC using a Phenomenex Luna C18 (2) 100Å column [gradient: H$_2$O (0.1% TFA)/ACN (0.1% TFA), 5 min; 80:20 → 5:95 (40 min), R$_t$ = 24.5 min]. The proper fractions were freeze-dried to provide Ant1 as a yellow solid with an overall yield of 33% (40 mg). The pure product was stored at -20 °C until further use. $^1$H NMR (MeOD-d$_4$, 300 MHz, $\delta$, ppm): 9.38 (s, 1H, CH$_{Ar}$), 8.56 (s, 1H, CH$_{Ox}$), 8.48 (d, $J$ = 8.5 Hz, 2H, 2 CH$_{Ar}$), 8.05 (d, $J$ = 8.2 Hz, 2H, 2 CH$_{Ar}$), 7.51 (m, 4H, 4 CH$_{Ar}$), 4.76 (s, 2H, COCH$_2$ON), 3.92 (s, 2H, COCH$_2$O), 3.58 (m, 2H, CH$_2$), 3.49 (m, 6H, 3 CH$_2$). $^{13}$C NMR (MeOD-d$_4$, 75 MHz, $\delta$, ppm): 174.2 (CO), 172.7 (CO), 151.0 (CH), 132.6 (C), 131.3 (C), 130.8 (CH), 129.9 (CH), 128.0 (CH), 126.4 (CH), 126.0 (CH), 124.1 (C), 73.8 (CH$_2$), 71.4 (CH$_2$), 71.1 (CH$_2$), 70.5 (CH$_2$), 69.0 (CH$_2$), 40.1 (CH$_2$). MS (ESI) [m/z (%)], 447.2 ([M+Na]$^+$, 51), 425.2 ([M+H]$^+$, 100). HRMS (ESI) Calculated for C$_{23}$H$_{25}$N$_2$O$_6$: 425.1707, found 425.1706.
2.5 Photodimers thermal dissociation experiments
The thermal reversibility of the photocycloaddition reaction was monitored by UV-Vis measurements using a Varian Cary 500 Scan UV-Vis spectrophotometer equipped with a temperature controller. In
these experiments, aqueous solutions of $E,E$-D$_3$ht, $Z,Z$-D$_3$ht, and $E,Z$-D$_3$ht derivatives at a concentration of 250 µM were sealed in a 0.2 cm quartz cuvette under argon atmosphere and heated at 80°C for 800 min. Every 5 min, UV-VIS spectra were collected in the 500-200 nm spectral range at T=80°C to follow the changes in the characteristic absorption bands of anthracene.

3. Methods

3.1 Circular Dichroism (CD) Spectroscopy
Circular Dichroism (CD) spectra were acquired in a Jasco J-1100 CD Spectrometer supplied with a Jasco MCB-100 Mini Circulation Bath for temperature control using a 0.1 cm quartz cuvette. Spectra were acquired in the 500-190 nm spectral range at 20°C, at scanning speed of 100 nm·min$^{-1}$ (1 s response time, 1 nm bandwidth, 0.2 nm data pitch). Spectra at fix concentration of CP3 (350 µM) were run at native pH (pH ~ 3.6) or varying the solution pH by adding aqueous NaOH solution (0.1M). Each spectrum corresponds to the average of 12 scans and is baseline corrected.

3.2 Fluorescence Spectroscopy
Fluorescence measurements were performed in a Varian Cary Eclipse spectrophotometer. Fluorescence spectra were acquired in the 400-700 nm spectral range at 20 °C with an averaging time of 0.5 s, an excitation wavelength of 385 nm, and using a Hellma® fluorescence quartz cell (10 × 2 mm). To evaluate the pH-induced supramolecular self-assembly, solutions of CP3 at a fixed concentration (350 µM) were titrated with aqueous NaOH (0.1M), and the resulting pH was measured with a pH meter. The solutions were allowed to equilibrate 30 minutes before measurements are performed.

3.3 Nile red assay
Nile red fluorescence emission spectra$^4$ were acquired with Varian Cary Eclipse fluorescence spectrophotometer provided with a temperature controller and employing a Hellma® fluorescence quartz cell (10 × 2 mm). The spectra were collected from 570 to 800 nm using an excitation wavelength of $\lambda_{ex} = 550$ nm. Briefly, solutions of CP3 in Heps (10 mM) at pH 7 and 8, in the concentration range 6 µM – 500 µM, were prepared and NR in MeOH (0.5 mM) was diluted 500-fold into solutions to obtain a final concentration of 1 µM. Therefore, all samples contain 0.2% MeOH content that is not expected to influence the self-assembly process. The solutions were allowed to equilibrate 1h before performing the measurements. The maximum emission intensity and the respective wavelength at maximum intensity were both reported as a function of the logarithm of the CP3 concentration.

3.4 ThT fluorescence assay
ThT fluorescence emission$^5$ spectra were carried out with a Cary Eclipse Fluorescence Spectrophotometer (Agilent) equipped with a temperature-controlled cell chamber and a Hellma® fluorescence quartz cell (10 × 2 mm). Spectra were recorded from 465 to 650 nm at 20 °C using an excitation wavelength $\lambda_{ex} = 450$ nm. Briefly, CP3 solutions in Heps buffer (10 mM, pH 7) in the concentration range 1 µM – 500 µM were prepared. Then, ThT aqueous solution (4 mM) was diluted 200-fold into solutions to obtain a final concentration of 20 µM. Solutions were kept under shaking for 30 minutes before measurements to attain a constant emission intensity value.

3.5 Scanning Transmission Electron Microscopy (STEM)
STEM micrographs were obtained using a ZEISS FESEM ULTRA Plus with EDX operating at an extra high-tension of 20 kV. Briefly, 10 µL of peptide solution in the proper buffer was drop-casted over a 400-mesh carbon-coated copper grid (Electron Microscopy Sciences). After 3 minutes, excess of the sample was removed by blotting with a filter paper and air-dried. Then, the samples were stained with 2% w/v phosphotungstic acid (PTA) at pH 7.4 and washed with H$_2$O (10 µL) once.
3.6 Epifluorescence microscopy
Epifluorescence measurements were performed on an Olympus BX51 microscope at magnifications of 20x and 40x. Epifluorescence images were acquired on CP3 aqueous solution basified with NaOH (0.1 M) to pH 8. Small imaging chambers were achieved by attaching an imaging spacer (Secure-Seal Imaging Spacers, 0.12 mm deep, Grace Bio-Labs) to a clean glass slide. Then, 10 µL of the sample was deposited on the glass, and the whole system was sealed with a second glass slide prior to microscopic visualization. All images were acquired at room temperature and analysed using ImageJ.[6]

3.7 Atomic Force Microscopy (AFM)
Atomic force microscopy measurements were performed at room temperature and ambient atmosphere using a Park Systems XE-100 in non-contact mode. ACTA tips were utilized (silicon tips, nominal values: spring constant = 40 N/m, frequency = 300 kHz, ROC less than 10 nm). Briefly, 10 µL of water solutions of CP3 (350 µM) at pH 7 were dropped on a silicon wafer substrate. After 5 min, the silicon wafer was thoroughly washed with MilliQ-H2O and dried under argon flow. Image analysis was carried out with Gwyddion.[7]

3.8 Kinetic studies of CP3 dimerization
The photodimerization reaction of the anthracene groups was investigated by UV-Vis measurements using a Jasco V-630 spectrophotometer. The spectra were collected from 200 to 500 nm at T= 20°C. Briefly, CP3 solutions at a concentration of 250 µM at acidic pH (1.85), native pH (4.95), and neutral pH (Hepes Buffer, 10 mM, pH 7) were degassed with argon for 5 min and sealed in a quartz cuvette (0.2 cm) under argon atmosphere to avoid the formation of endoperoxide species during the irradiation experiments. After equilibration, the solutions were irradiated at 350 nm with a Xenon Light Source 300W Max-303 through a XUS0350 Shortpass Filter, and the photodimerization reaction was monitored by UV-VIS measurements every 5-10 min. During the irradiation, the distance between the sample solutions and the light source was set to be around 5 cm. Product conversion was followed at different time intervals (1, 3, 5, 10, 20, 40 and 90 min) by taking aliquots that were analysed by HPLC (Fig. S9 and S10).

3.9 Procedure of irradiation under photo-oxidative conditions. The photodimerization reaction of the anthracene groups was investigated by UV-Vis measurements using a Jasco V-630 spectrophotometer. Aqueous solutions of CP3ε (250 µM) or Ant1 (250 µM, 1:3 MeOH/aqueous media) in Hepes (10 mM, pH 7.0) were degassed with argon for 10 min. Then, the resulting solution was sealed in a quartz cuvette under oxygen atmosphere and after equilibration for 20 min, the solution was irradiated at 350 nm with a Xenon Light Source 300W Max-303 through a XUS0350 Shortpass Filter for sort period of time. The distance between the sample solutions and the light source was set to be around 5 cm. The product conversion was followed at different time intervals (1, 3, 5, 10, 20, 40 and 90 min) by taking aliquots that were analysed by HPLC (Fig. S16).

3.10 Procedure of irradiation-dark cycles. The photodimerization reaction of the anthracene groups was investigated by UV-Vis measurements using a Jasco V-630 spectrophotometer. Aqueous solutions of CP3ε (250 µM) or Ant1 (250 µM, 1:3 MeOH/aqueous media) in Hepes (10 mM, pH 7.0) were degassed with argon for 10 min. Then, the resulting solutions were sealed in a quartz cuvette under argon atmosphere. After equilibration for 20 min, the solutions were irradiated at 350 nm with a Xenon Light Source 300W Max-303 through a XUS0350 Shortpass Filter for sort periods of time. The distance between the sample solutions and the light source was set to be around 5 cm. The samples were initially irradiated for a sort time (5-10 min) and after three cycles the irradiation times were doubled (10-20 min). After each irradiation cycle the solutions were maintained at room temperature in the dark at the UV-vis spectrophotometer chamber for 40 min. The amount of converted material...
was followed by the disappearance of the absorption band at 386 nm (Fig. S18) by taking UV spectra every 2 min.

### 3.11 Head-to-Tail characterization of photodimers.

#### NMR analysis for Z,E-D$_3$ht

![Diagram of Z,E-D$_3$ht](image)

The $^1$H-NMR spectrum displays a singlet at $\delta = 5.44$ ppm that can be assigned to the bridge hydrogen $H^{10}$ according to the integral value (2H) and the chemical shift. DEPT-135 spectrum shows that the signal at $\delta = 51.78$ ppm should be attributed to carbon C$^{10}$ of the bridge. This assignment is additionally supported by HSQC spectrum of Z,E-D$_3$ht because a cross-signal is found between the $^1$H-NMR signal at 5.44 ppm and the $^{13}$C-NMR signal at 51.78 ppm. Comparing the DEPT-135 and $^{13}$C-NMR spectra, the $^{13}$C-NMR signal at 57.94 ppm can be assigned to the bridge carbon connected to oxyme C$^9$. However, in the HMBC spectrum, a cross-signal is present between $^1$H-NMR signal at 5.44 ppm and the $^{13}$C-NMR signal at 57.94 ppm, but no cross-signal is found between the $^1$H-NMR signal at 5.44 ppm and the $^{13}$C-NMR signal at 51.78 ppm. This result could indicate that only the h-t photodimer was obtained.

Moreover, in the HMBC, the bridge hydrogen at $\delta = 5.44$ ppm shows further cross signals:

1) 128.56 ppm: C atom that gives a signal in $^{13}$C and DEPT; and in the HSQC gives 2 cross-signals at 7.04 and 6.82 that from the $^1$H-NMR spectrum can be assigned to the aromatic anthracene moieties.

2) 141.25 ppm: C atom that gives a signal in $^{13}$C but not in DEPT, so is a quaternary carbon.

3) 156.42 ppm: C atom that gives a signal in $^{13}$C and DEPT; and in the HSQC gives 2 cross-signals at 8.13 and 8.09 ppm that from the $^1$H-NMR spectrum can be assigned to proton of the oxyme moieties. Moreover, these two proton signals at 8.13 and 8.09 ppm in the ROESY show 2 cross-signals with the anthracene moiety at around 6.82 ppm

Finally, from the HMBC a cross-signal between the bridge carbon connected to oxyme C$^9$ and the $^1$H-NMR signal at 8.13 ppm of the oxyme is also found.
$^1$H NMR of $E, Z$-D$_3$ht at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

DEPT-135 and $^{13}$C-NMR of $E, Z$-D$_3$ht at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).
Comparison of $^{13}$C-NMR and DEPT-135 for $\text{E, Z-D3}_h$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

HSQC of $\text{E, Z-D3}_h$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HSQC magnification of $E,Z$-$D_{3ht}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

HMBC of $E,Z$-$D_{3ht}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HMBC magnification of $E,Z$-$\text{D}3_{ht}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

ROESY of $E,Z$-$\text{D}3_{ht}$ at 3.4 mM (500 MHz, D$_2$O/H$_2$O, 298K).

NMR analysis for $E,E$-$\text{D}3_{ht}$
The $^1$H-NMR spectrum displays a singlet at $\delta = 5.45$ ppm that can be assigned to the bridge hydrogen $H^{10}$ (of any of the dimers) according to the integral value (2H) and the chemical shift. DEPT-135 spectrum shows that the signal at $\delta = 52.03$ ppm should be attributed to carbon $C^{10}$ of the bridges. This assignment is additionally supported by HSQC spectrum of $E,E$-D$_3$ht because a cross-signal is found between the $^1$H-NMR signal at 5.45 ppm and the $^{13}$C-NMR signal at 52.03 ppm. Comparing the DEPT-135 and $^{13}$C-NMR spectra, the $^{13}$C-NMR signal at 57.68 ppm can be assigned to the bridge carbon connected to oxyme $C^9$. However, in the HMBC spectrum, a cross-signal is present between $^1$H-NMR signal at 5.44 ppm and the $^{13}$C-NMR signal at 57.68 ppm, but no cross-signal is found between the $^1$H-NMR signal at 5.44 ppm and the $^{13}$C-NMR signal at 52.03 ppm. This result could indicate that only the $h-t$ photodimer was obtained.

Moreover, in the HMBC, the bridge hydrogen at $\delta = 5.44$ ppm shows further cross signals:
1) 128.48 ppm: C atom that gives a signal in $^{13}$C and DEPT; and in the HSQC gives a cross-signals at 6.81 that from the $^1$H-NMR spectrum can be assigned to the aromatic anthracene moieties.
2) 142.55 and 143.28 ppm: C atoms that give a signal in $^{13}$C but not in DEPT, so it is a quaternary carbon.
3) 156.23 ppm: C atom that gives a signal in $^{13}$C and DEPT; and in the HSQC gives a cross-signals at 8.12 ppm that from the $^1$H-NMR spectrum can be assigned to proton of the oxyme moieties. Moreover, this proton signal at 8.12 ppm in the ROESY show a cross-signals with the anthracene moiety at around 6.81 ppm.

Finally, from the HMBC, cross-signals between the bridgehead carbon connected to oxyme $C^9$ and the $^1$H-NMR signal at 6.81 (anthracene) and 8.12 ppm of the oxyme are also found.
$^1$H NMR of $E,E$-D$_3$ht at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).

DEPT-135 and $^{13}$C-NMR of $E,E$-D$_3$ht at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).
Comparison of $^{13}$C-NMR and DEPT-135 for $E,E$-$D_3ht$ at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).

HSQC of $E,E$-$D_3ht$ at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HSQC magnification of $E,E$-D$_3$nt at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).

HMBC of $E,E$-D$_3$nt at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).
HMBC magnification of $E,E$-$D3_{nt}$ at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).

ROESY of $E,E$-$D3_{nt}$ at 3.7 mM (500 MHz, D$_2$O/H$_2$O, 298K).
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