

Pyrene Chromophores for the Photoreversal of Psoralen Interstrand Crosslinks

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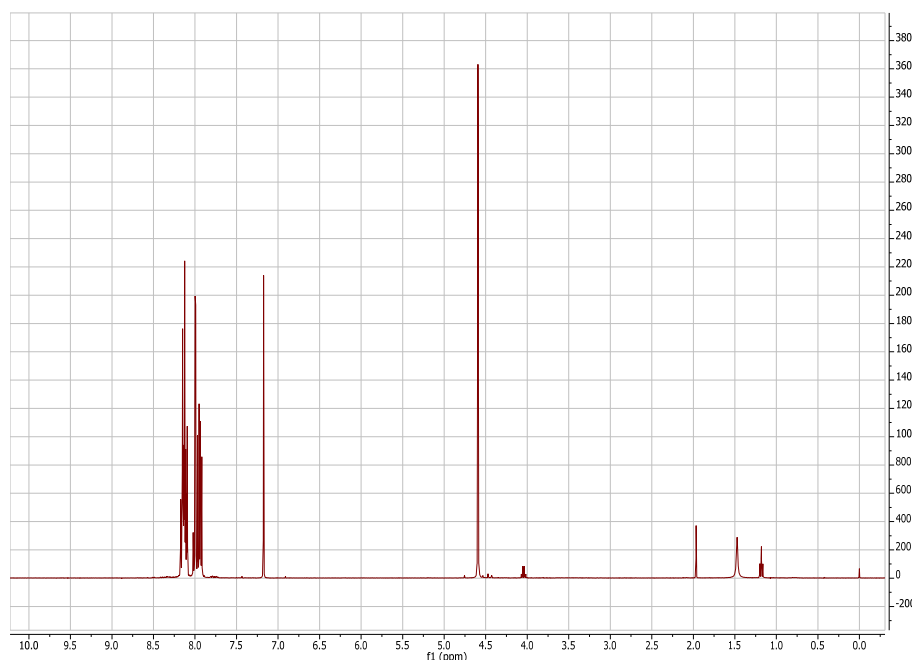
Supporting Information

Materials and methods

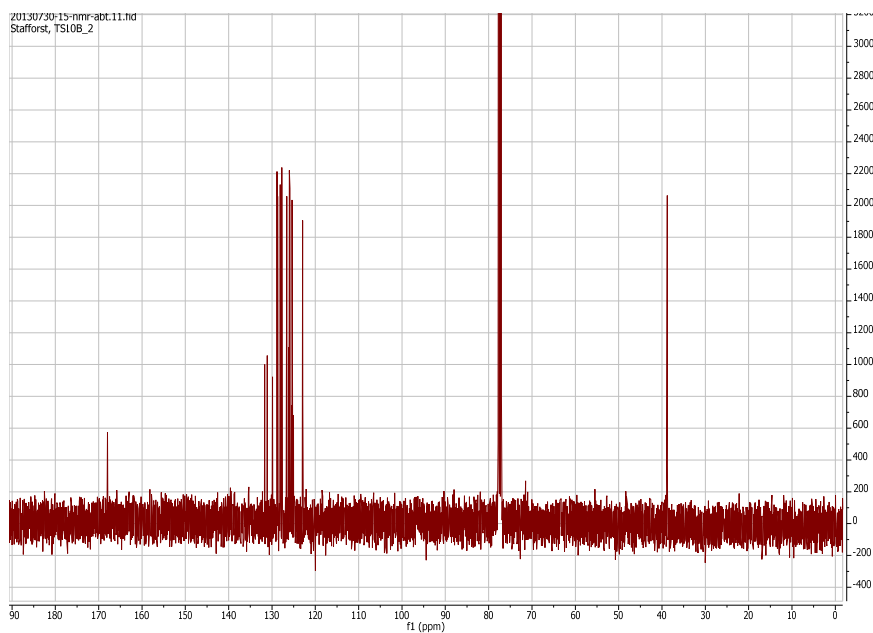
1-Pyrene acetic acid and pyrene-1-boronic acid were purchased from Sigma-Aldrich. 1-Ethynylpyrene and 5-iodouracil were obtained from Alfa Aesar. Resins, Fmoc-Dpr(Mtt)-OH, and Fmoc-Lys(Boc)-OH were purchased from Merck. Fmoc-PNA(Bhoc)-OH building blocks came from Link Technologies (UK). Solvents and reagents for solid phase peptide synthesis were purchased from ABCR (Germany). All chemicals were used without further purification.

Syntheses

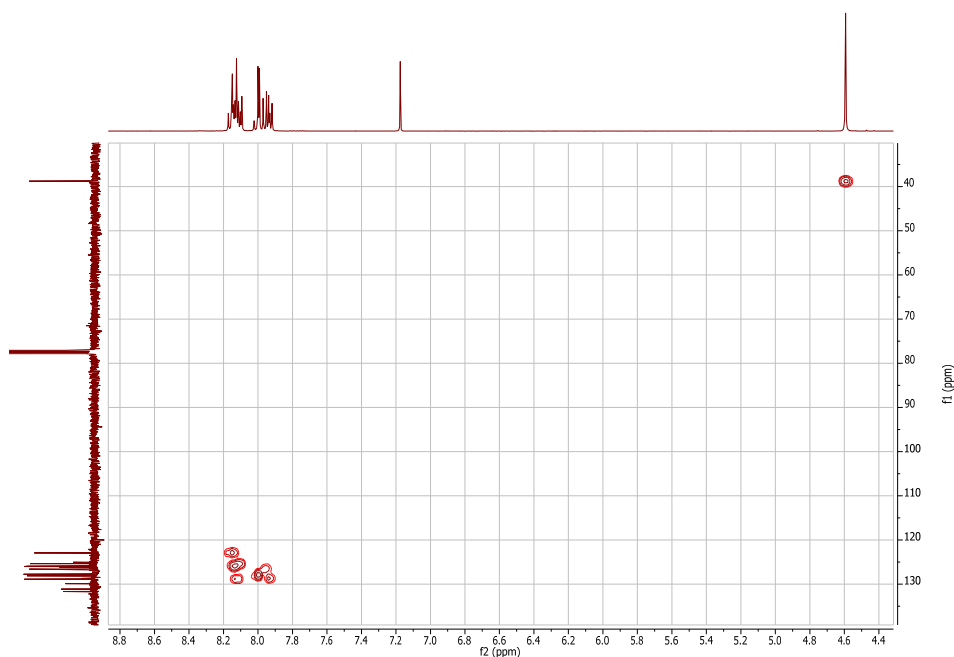
OPfp-activated 1-pyrene acetic acid (3). TLC (ethylacetate): R_f (product) = 0.91; R_f (free acid of product) = 0.05; $^1\text{H-NMR}$ (400.2 MHz, CDCl_3): δ = 4.59 (s, 2H; CH_2), 7.90-8.20 (m, 9H; pyrene CH); $^{13}\text{C-NMR}$ (100.6 MHz, CDCl_3 , ^1H uncoupled, not ^{19}F uncoupled): δ = 38.4 (CH_2), 122.5 (CH), 124.6 (CF), 124.9 (CH), 125.1 (CF), 125.4 (CH), 125.6 (CH), 125.7 (C), 126.2 (CH), 127.3 (CH), 127.7 (CH), 128.3 (CH), 128.5 (CH), 129.5 (CF), 130.7 (CF), 131.2 (CF), 131.3 (CF), 167.5 (CO) ppm, CH could be discriminated from CF via $^1\text{H}/^{13}\text{C}$ HSQC; **HR-ESI-TOF-MS**: 449.0571 expected for $\text{C}_{24}\text{H}_{11}\text{F}_5\text{O}_2\text{Na}_1$, 449.0573 found.



$^1\text{H-NMR}$ of compound **3**.

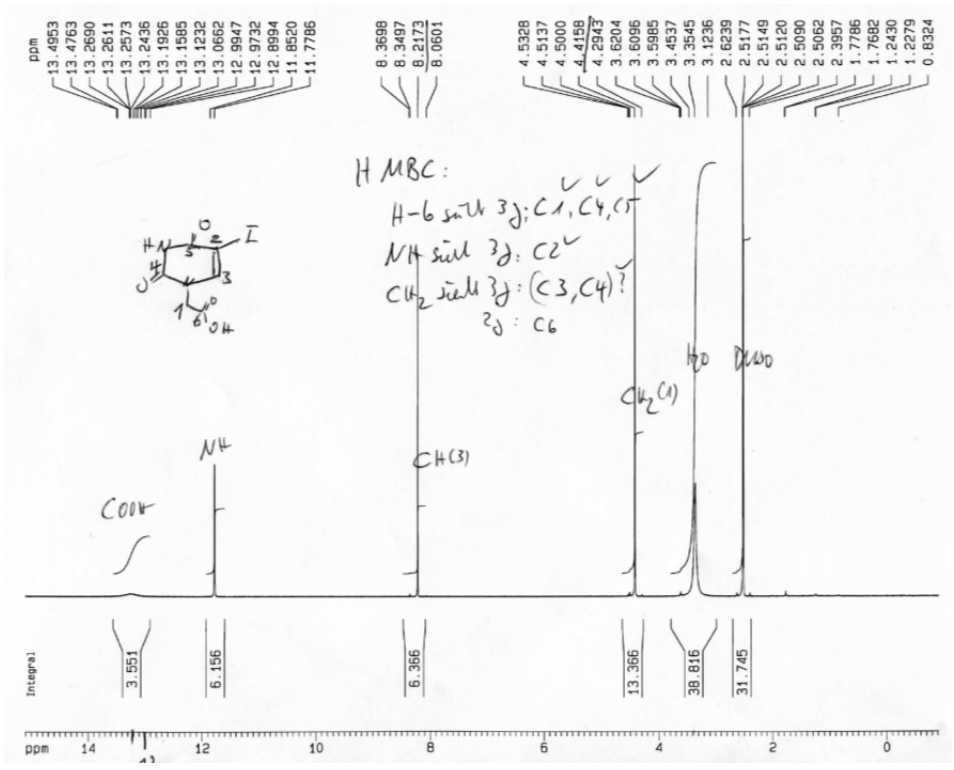


$^{13}\text{C-NMR}$ of compound 3.

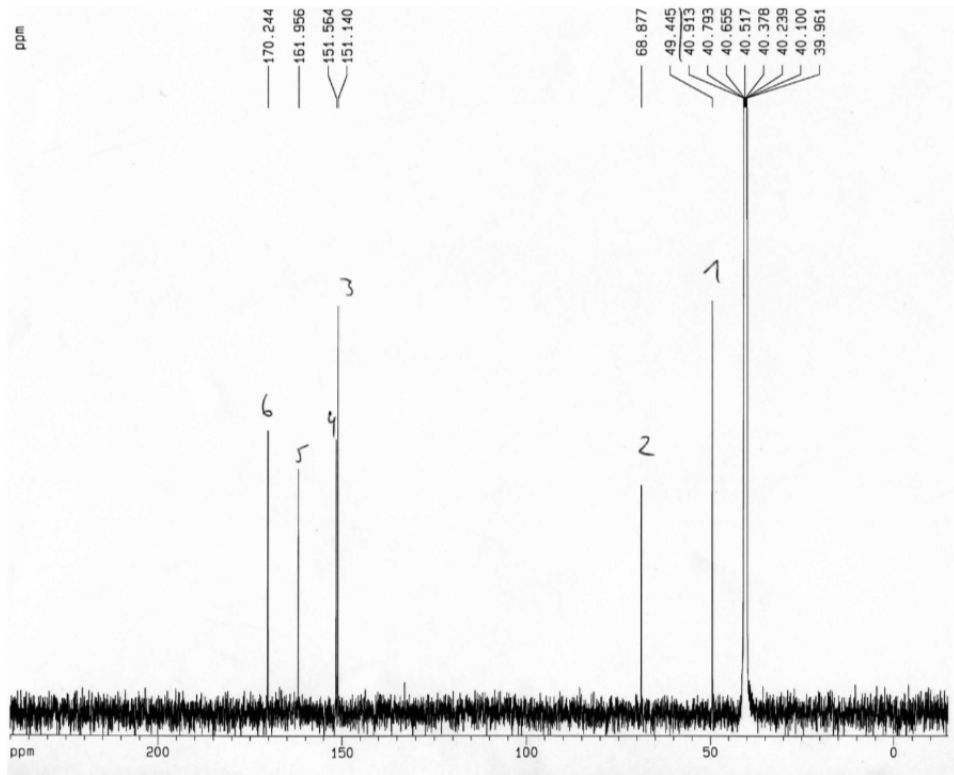


$^1\text{H}/^{13}\text{C-HSQC}$ NMR spectra of compound 3.

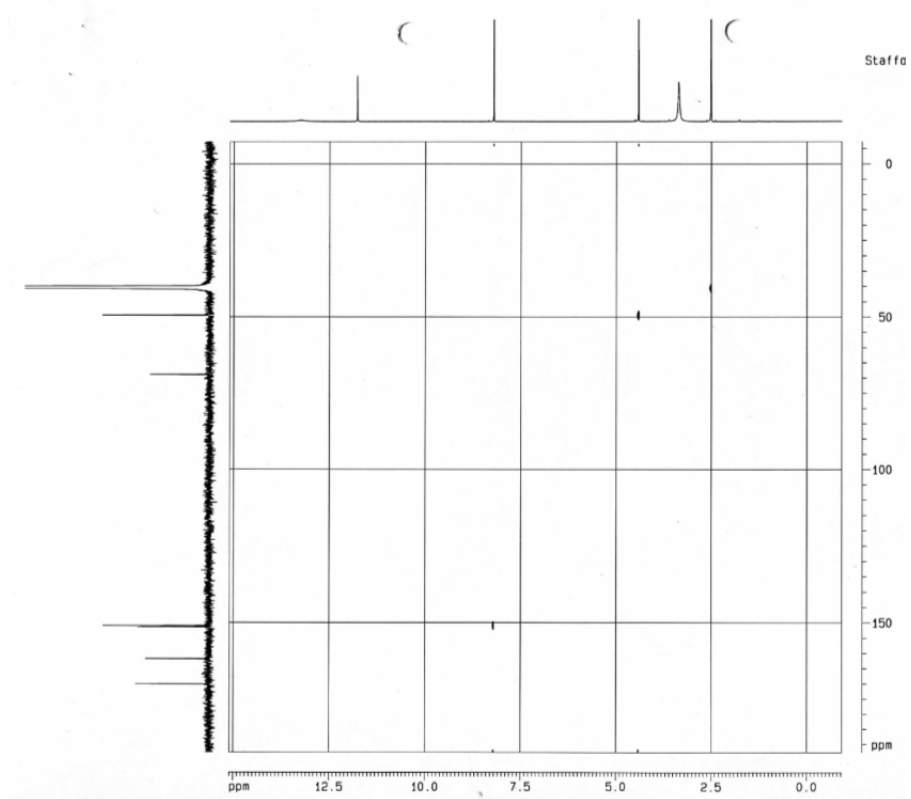
5-Iodo-uracil-N1-acetic acid (4): TLC (ethyl acetate / MeOH 9:1 + 1% AcOH): $R_F = 0.35$; $^1\text{H-NMR}$ (600.13 MHz, D6-DMSO): $\delta = 4.42$ (s, 2H; CH_2); 8.22 (s, 1H; H-6); 11.78 (s, 1H; NH); 13.30 (s br, 1H; OH) ppm; $^{13}\text{C-NMR}$ (150.9 MHz, D6-DMSO): $\delta = 49.4$ (CH_2); 68.9 (C-5); 151.1 (C-6); 151.6 (C-2); 162.0 (C-4); 170.2 (COOH) ppm; C-H connectivity was assigned via $^1\text{H}/^{13}\text{C-HSQC}$; the N1-regioisomer was clearly assigned via $^1\text{H}/^{13}\text{C-HMBC}$; **HR-ESI-TOF-MS:** 296.9367 expected for $\text{C}_6\text{H}_6\text{I}_1\text{N}_2\text{O}_4$, 296.9363 found.



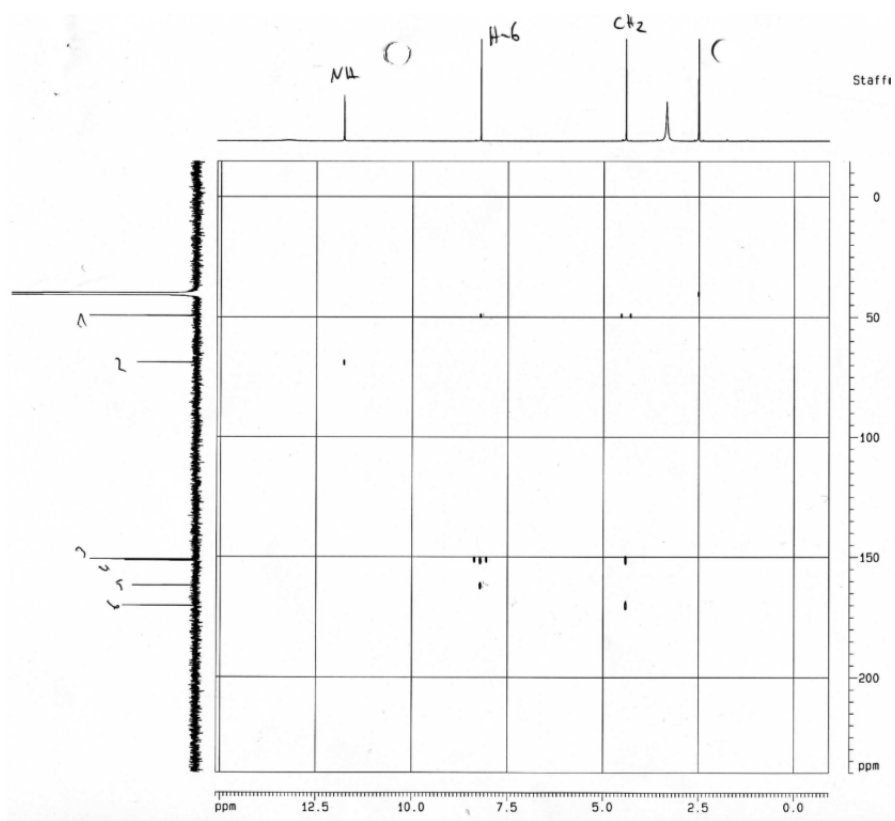
¹H-NMR spectra of compound 4.



¹³C-NMR spectra of compound 4.

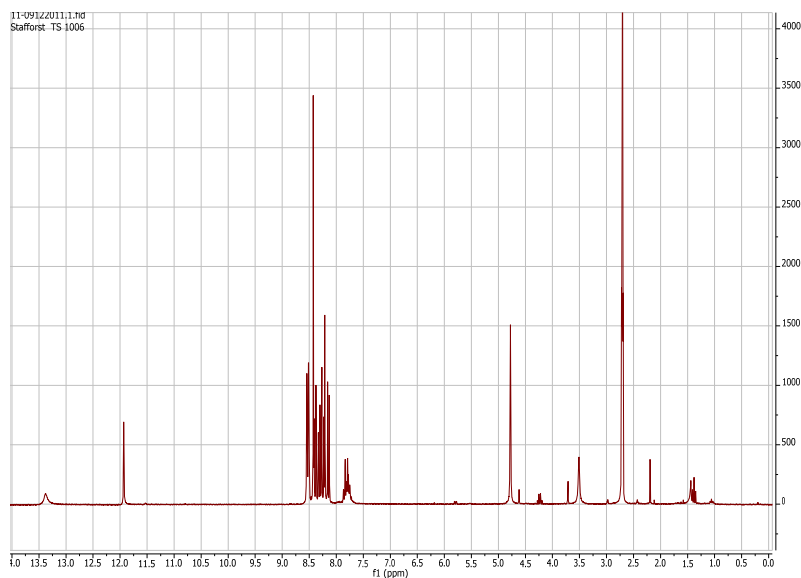


$^1\text{H}/^{13}\text{C}$ -HSQC spectra of compound 4.

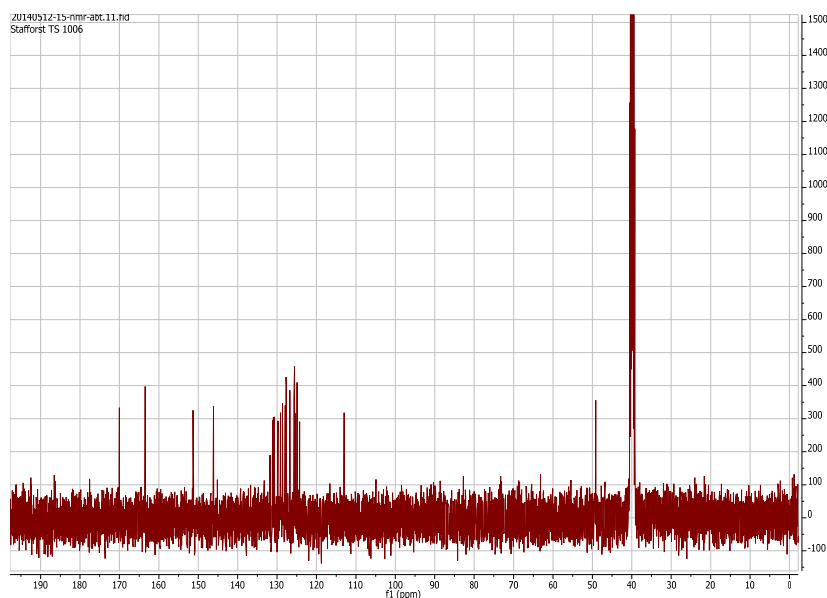


$^1\text{H}/^{13}\text{C}$ -HMBC spectra of compound 4.

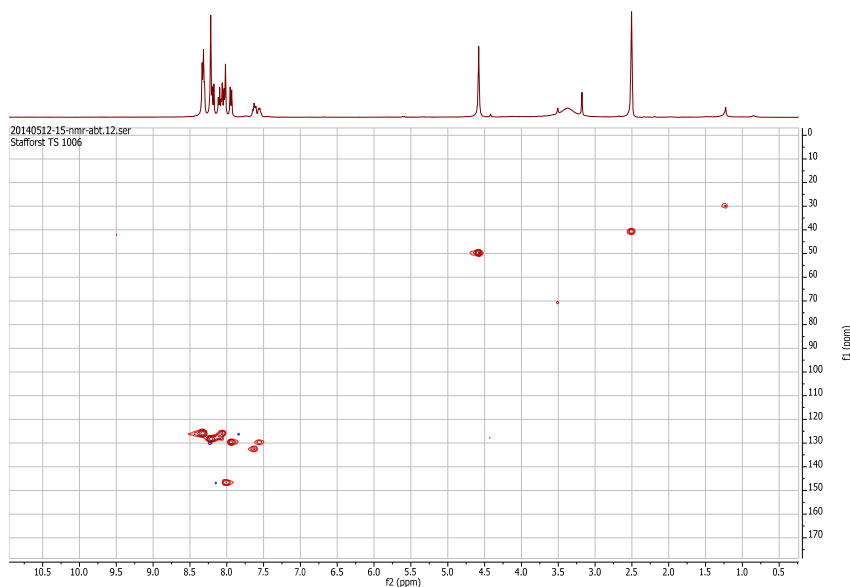
OPfp-activated uracil acetic acid directly linked to pyrene (6): TLC (ethylacetate): R_f (product) = 0.91; R_f (free acid of product) = 0.05; $^1\text{H-NMR}$ (250.1 MHz, D6-DMSO): free acid: δ = 4.57 (s, 2H; CH₂); 7.92-8.35 (m, 10H; H-6, pyrene CH); 13.21 (s br, 1H; COOH); $^{13}\text{C-NMR}$ (101 MHz, D6-DMSO) δ = 170.0 (COOH), 163.5 (C-4), 151.3 (C-2), 146.1 (CH, C-6), 131.1 (C), 131.0 (C), 130.7 (C), 129.8 (C), 129.2 (C), 128.6 (CH), 128.0 (CH), 127.7 (CH), 127.7 (CH), 126.8 (CH), 125.8 (CH), 125.6 (CH), 125.4 (C), 125.0 (CH), 124.3 (CH), 124.1 (C), 113.0 (C-5), 49.2 (CH₂); **HR-ESI-TOF-MS:** free acid: 369.0881 expected for C₂₂H₁₃N₂O₄, found 369.0876.



$^1\text{H-NMR}$ spectra of the free acid intermediate of compound **6**. A minor PPh₃ impurity is seen around 7.75 ppm.

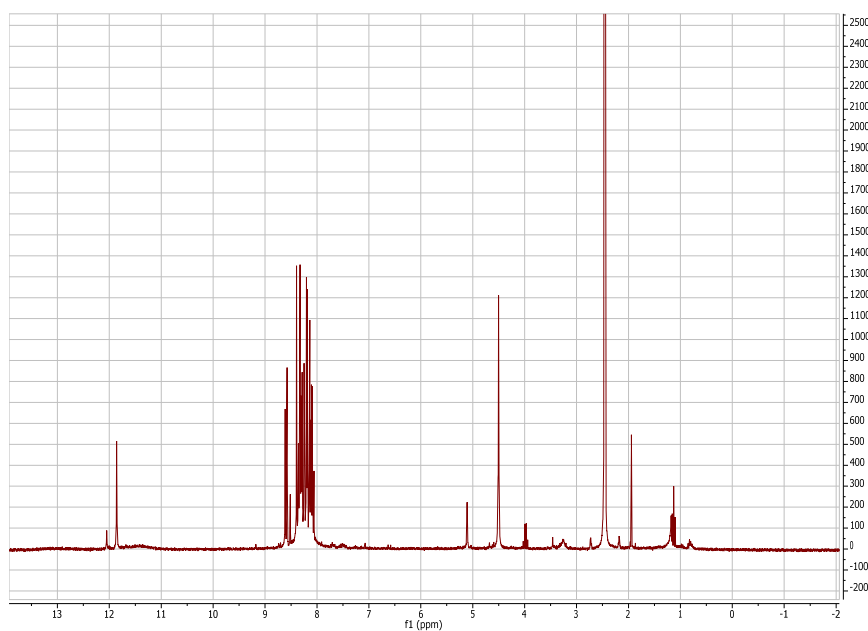


$^{13}\text{C-NMR}$ spectra of the free acid intermediate of compound **6**. A minor PPh₃ impurity is seen around 129.2 and 131.8 ppm.

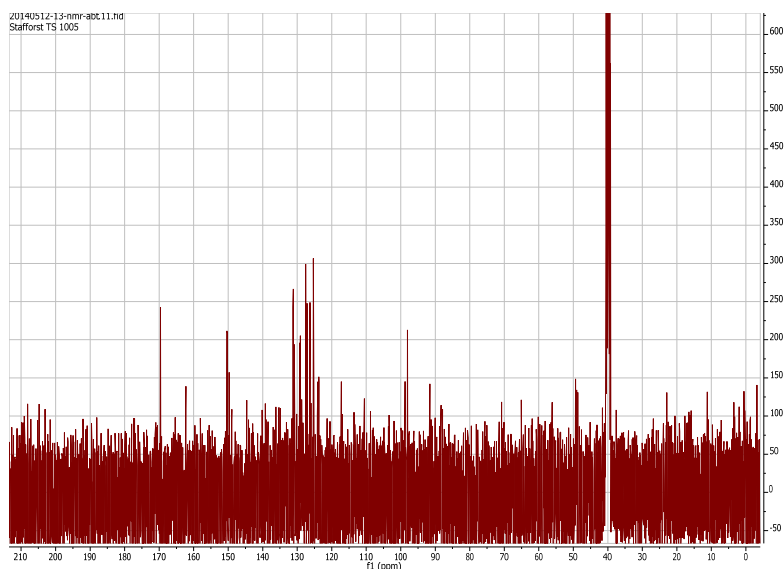


$^1\text{H}/^{13}\text{C}$ -HSQC spectra of compound **6**.

OPfp-activated uracil acetic acid ethynyl-linked to pyrene (8): TLC (ethylacetate 100%): R_F (product) = 0.95; R_F (free acid of product) = 0.05; $^1\text{H-NMR}$ (250.1 MHz): δ = 4.50 (s, 2H; CH_2); 8.06-8.63 (m, 10H; H-6, pyrene CH); 11.86 (NH) ppm; $^{13}\text{C-NMR}$ (101 MHz, $\text{D}_6\text{-DMSO}$) δ = 169.6 (COO), 162.3 (C4), 150.4 (C2), 149.7 (C6), 131.3 (C), 131.1 (C), 130.9 (C), 129.4 (C), 129.3 (C), 129.1 (CH), 129.1 (CH), 128.7 (CH), 127.6 (CH), 127.1 (CH), 126.3 (CH), 126.3 (CH), 126.0 (C), 125.3 (C), 125.2 (CH), 124.0 (CH), 123.7 (C), 98.8 (C5), 98.1 (ethynyl C), 91.6 (ethynyl C), 49.4 (CH_2); **HR-ESI-TOF-MS**: 583.0688 expected for $\text{C}_{30}\text{H}_{13}\text{F}_5\text{N}_2\text{O}_4\text{Na}_1$: 583.0687 found.



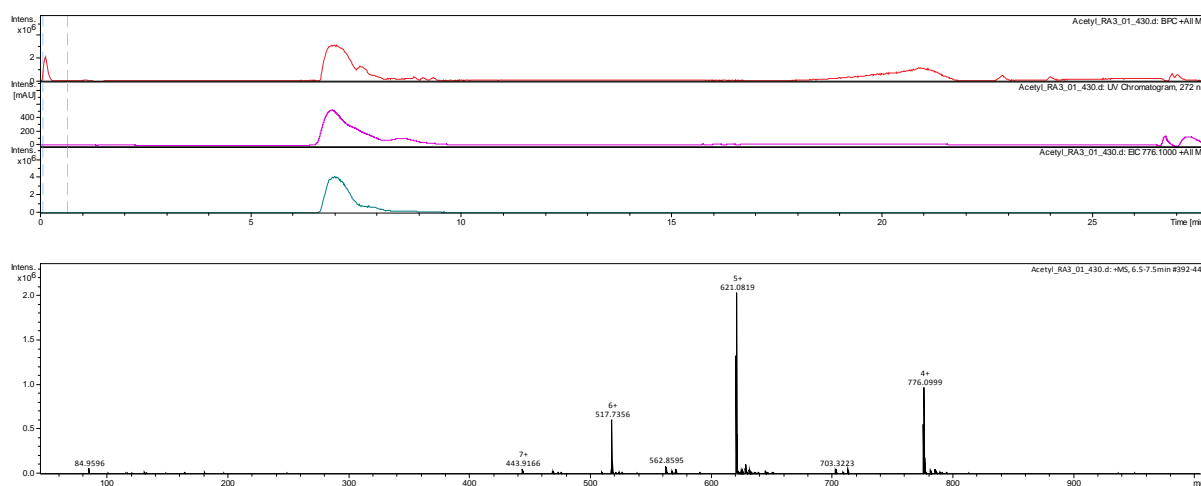
$^1\text{H-NMR}$ spectra of compound **8**.

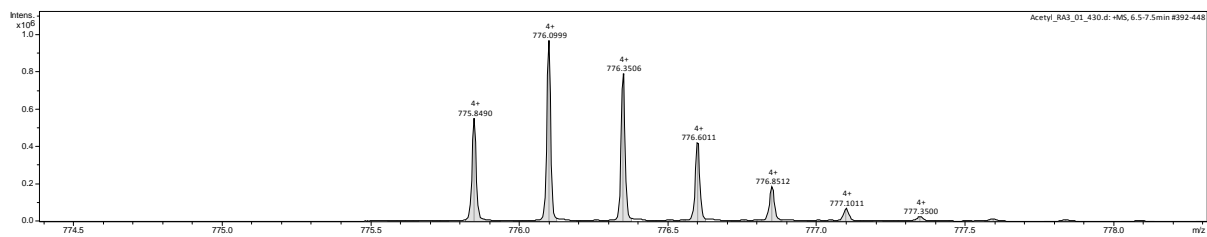


^{13}C -NMR spectra of compound **8**.

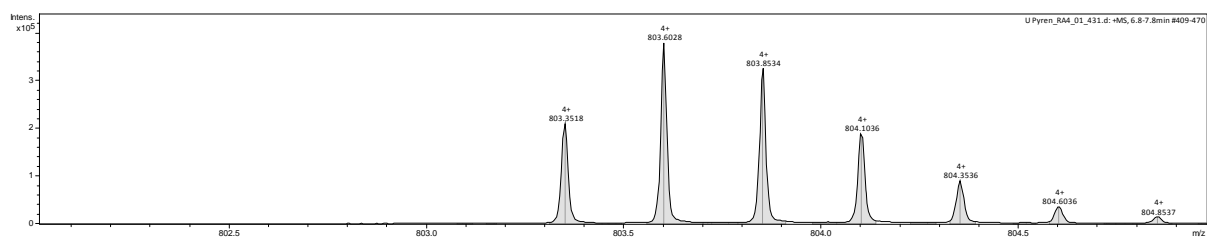
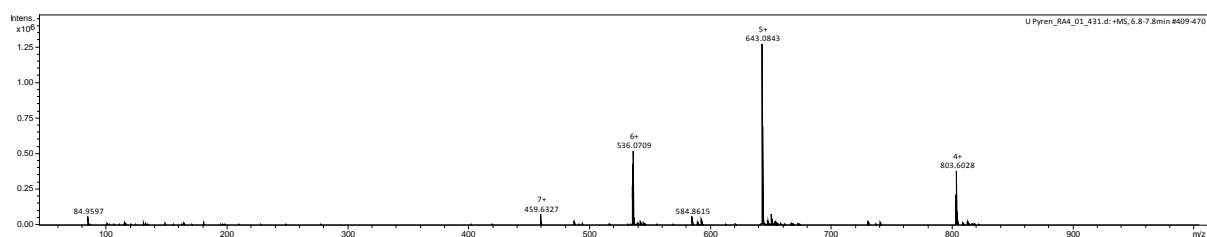
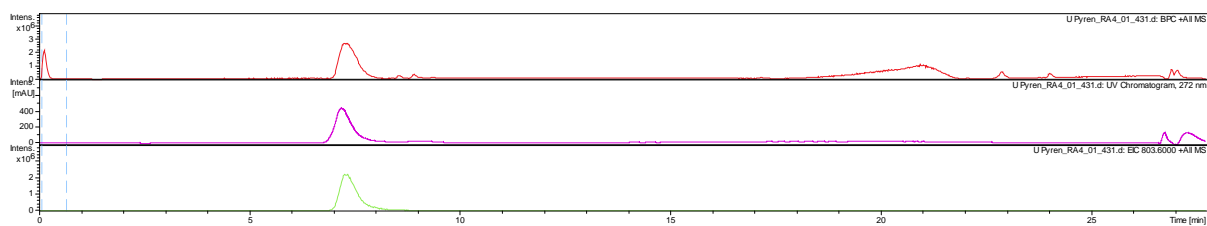
Pyrene-PNAs **9-11**

PNA-sequences: PNA **9**: H-Lys-ctgctcgcc-Dpr(β -pyrene **3**)-Lys-Lys-NH: **UV**: ϵ ($\text{mM}^{-1}\text{cm}^{-1}$) = 90 (260 nm), 21 (334 nm), 29 (350 nm), 2.0 (365nm); **MS**: 775.8475 expected for $[\text{M}+4\text{H}]^{4+}$: $\text{C}_{133}\text{H}_{178}\text{N}_{56}\text{O}_{34}$, found: 775.8490; PNA **10**: H-Lys-ctgctcgcc-Dpr(β -pyrene **6**)-Lys-Lys-NH: **UV**: ϵ ($\text{mM}^{-1}\text{cm}^{-1}$) = 100 (260 nm), 26 (350 nm), 12 (365 nm); **MS**: 803.3504 expected for $[\text{M}+4\text{H}]^{4+}$: $\text{C}_{137}\text{H}_{180}\text{N}_{58}\text{O}_{36}$, found: 803.3518; PNA **11**: H-Lys-ctgctcgcc-Dpr(β -pyrene **8**)-Lys-Lys-NH: **UV**: ϵ ($\text{mM}^{-1}\text{cm}^{-1}$) = 100 (260 nm), 20 (365 nm), 36 (400 nm), 25 (430 nm); **MS**: 809.3504 expected for $[\text{M}+4\text{H}]^{4+}$: $\text{C}_{137}\text{H}_{180}\text{N}_{58}\text{O}_{36}$, found: 809.3513. Extinction coefficients used for PNA: t ($8.4 \text{ mM}^{-1}\text{cm}^{-1}$), a ($15.2 \text{ mM}^{-1}\text{cm}^{-1}$), c ($7.05 \text{ mM}^{-1}\text{cm}^{-1}$), g ($12.0 \text{ mM}^{-1}\text{cm}^{-1}$).

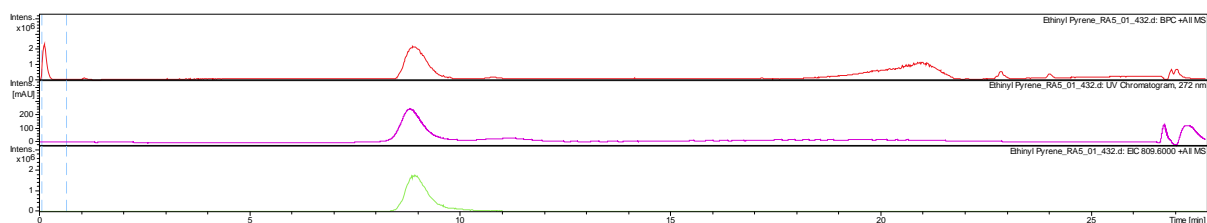


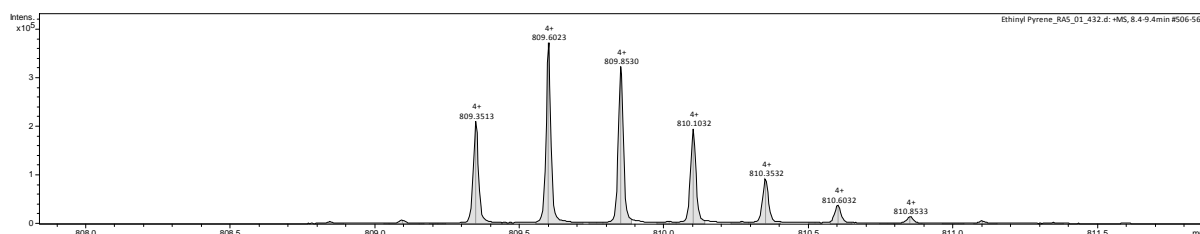
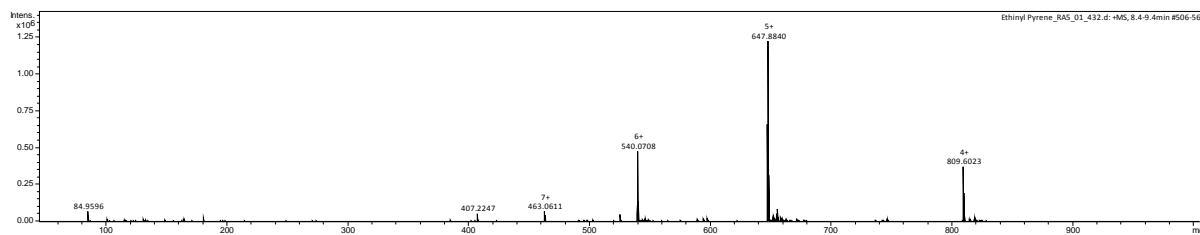


HPLC-HR-MS analysis of PNA 10 H-Lys-ctgctgcc-Dpr(β -pyrene 3)-Lys-Lys-NH. 1.) UV-trace 272 nm, 2.) total ion current, 3.) extracted ion current 776.1 m/z, 4.) integrated MS over 6.5-7.5 min, 5) close-up at $[M+4H]^{4+}$.



HPLC-HR-MS analysis of PNA 10 H-Lys-ctgctgcc-Dpr(β -pyrene 6)-Lys-Lys-NH. 1.) UV-trace 272 nm, 2.) total ion current, 3.) extracted ion current 803.6 m/z, 4.) integrated MS over 7.0-8.0 min, 5) close-up at $[M+4H]^{4+}$.





HPLC-HR-MS analysis of PNA **11** H-Lys-ctgctgcc-Dpr(β -pyrene **7**)-Lys-Lys-NH. 1.) UV-trace 272 nm, 2.) total ion current, 3.) extracted ion current 809.6 m/z, 4.) integrated MS over 8.5-9.5 min, 5) close-up at $[M+4H]^{4+}$.

Irradiation experiments

Irradiation experiments were performed as described in the main text. We here report only additional experiments and the densitometric data used for Figure 2.

Results

1.) 350 ± 20 nm, xl24 **12** alone, no PNA

Controls:

Exp.	μ OD	mean gray
A	2.5	8.187
B	1.25	9.81
C	0.625	14.24
D	0.313	18,85
	0	24.98

xl24 irradiation

time	mean gray	calc. μ OD
0	24.98	0
2.5	24.20	0.033
5.0	23.57	0.060
10	22.77	0.098
20	21.17	0.177
40	18.07	0.349
80	15.72	0.512

2.) 350 ± 20 nm, **12** + PNA **9**

To improve the accuracy of the kinetic data / quantum yield, the average of two experiments was used

Controls Experiment 1:

Exp.	μ OD	mean gray
A	2.5	9.54
B	1.25	12.93
C	0.625	16.99
D	0.313	20.68
	0	25.24

xl24 irradiation Experiment 1

time	mean gray	calc. μ OD
0	25.24	0
2.5	19.36	0.45
5.0	15.24	0.858
10	13.26	1.16
20	12.04	1.49
40	11.57	1.49
80	12.58	1.36

Controls Experiment 2:

Exp.	μ OD	mean gray
A	2.5	7.27
B	1.25	12.95
C	0.625	16.92
D	0.313	20.7
	0	26.5

xl24 irradiation Experiment 2

time	mean gray	calc. μ OD
0	26.50	0
2.5	18.45	0.479
5.0	17.81	0.530
10	15.24	0.783
20	13.48	1.07
40	12.44	1.36
80	12.13	1.41

averaged data Exp.1 / Exp.2 for kinetic analysis

time	calc. μ OD
0	0
2.5	0.464
5.0	0.694
10	0.972
20	1.280
40	1.425
80	1.39

3.) 350±20 nm, 12 + PNA 10

Controls Experiment:

Exp.	μ OD	mean gray
A	2.5	6.94
B	1.25	11.22
C	0.625	15.18
D	0.313	17.53
	0	22.84

xl24 irradiation Experiment

time	mean gray	calc. μ OD
0	22.84	0
2.5	21.40	0.070
5.0	21.38	0.070
10	20.04	0.163
20	19.08	0.223

40	17.68	0.325
80	16.55	0.432

4.) 350±20 nm, **12** + PNA **11**

Controls Experiment:

Exp.	μOD	mean gray
A	2.5	7.78
B	1.25	12.13
C	0.625	17.33
D	0.313	19.14
	0	23.53

xl24 irradiation Experiment

time	mean gray	calc. μOD
0	23.53	0
2.5	22.87	0.024
5.0	21.69	0.135
10	20.42	0.253
20	18.83	0.380
40	17.08	0.600
80	16.41	0.680

5.) Additional Experiment

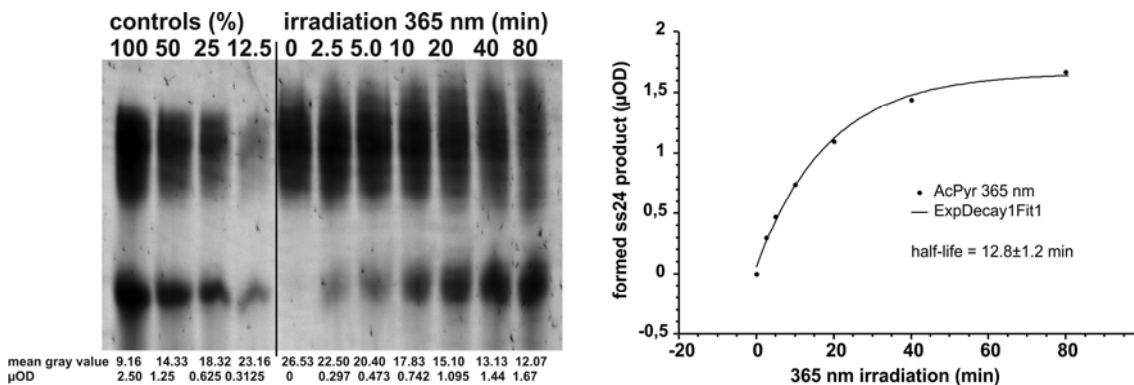
4.) **365±20 nm**, **12** + PNA **9**

Controls Experiment:

Exp.	μOD	mean gray
A	2.5	9.16
B	1.25	14.33
C	0.625	18.32
D	0.313	23.16
	0	26.53

xl24 irradiation Experiment

time	mean gray	calc. μOD
0	26.53	0
2.5	22.50	0.297
5.0	20.40	0.473
10	17.83	0.742
20	15.10	1.095
40	13.13	1.44
80	12.07	1.67



PAGE gel and kinetic analysis for xl24 **12** + PNA **9**, 365±20 nm irradiation

Determination of the quantum yield

Quantum yield was determined exactly as described before. Commercial DMNB-caged cAMP was diluted to 10 μM in irradiation buffer, as described above. Aliquots of 55 μL each were put in PCR tubes and irradiated inside the fluorescence spectrometer at 350 ± 20 nm as described above. Each full aliquot (55 μL) was subjected to analytical HPLC (20 μL loop, 2.5fold overload). Aliquots were generated that were 0 min, 38 sec, 75 sec, 150 sec, 5.0 min, 10 min, or 20 min irradiated. The half-life for the formation of cAMP was determined from the area of the 260 nm HPLC trace and was directly plotted against the irradiation time and fitted according to 1st-order kinetics. The obtained half-life was 3.5 ± 0.2 min and equals an uncaging efficiency $\epsilon\phi$ of $600 \text{ M}^{-1}\text{cm}^{-1}$.

Data used for the plot:

irradiation time	HPLC area
0	< 4.0
38 sec	16.448
75 sec	27.526
150 sec	46.849
5.0 min	85.642
10.0 min	115.204
20.0 min	128.826

