

Electronic Supporting Information

for

Thymine Photodimer Formation in DNA Hairpins. Unusual Conformations Favor (6-4) vs. (2+2)

Adducts^{†‡}

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Contents

page	content
2	Experimental Section
3	References
4	Tables S1-S3, MALDI, T _m , and NMR data for H₄2
5	Figure S1. Thermal dissociation profiles
6-7	Figures S2-4. NMR spectra for H₄2 and Figure S5 for H₆3
8	Figures S6-7 2D NMR spectra for (2+2) and (6-4) adducts of H₆3
9-14	Figures S8-S16 HPLC and UV data for irradiated solutions of hairpins
15	Figure S17. Calculated UV spectra

Experimental Section

Oligonucleotide conjugates containing TT steps (Chart 1) were prepared using standard phosphoramidite chemistry starting from 5'-phosphate CPG as solid support using a Millipore Expedite DNA synthesizer and following the procedure of Letsinger and Wu.¹ Following synthesis, the conjugates were isolated as trityl-on derivatives by reverse phase (RP) HPLC, detritylated in 80 % acetic acid for 30 min, and repurified by RP-HPLC as needed. RP-HPLC analysis was carried out on a Dionex chromatograph with a Hewlett-Packard Hypersil ODS-5 column (4.6 x 250 mm) and a 1% gradient of acetonitrile in 0.03 M triethylammonium acetate buffer (pH 7.0) with a flow rate of 1.0 mL/min. Molecular weights were determined following desalting by means of MALDI-TOF mass spectroscopy (Table S1, ElectSupporting Information).

Solutions containing ca. 1-1.2 μ M hairpin in 10 mM phosphate buffer (pH 7.2) with 1.0 M NaCl were irradiated at 280 nm using a Xenon arc lamp and monochromator (ca. 2 mW) at 10 °C in 1 cm path-length quartz cuvettes. Aliquots irradiated for different time intervals were analyzed by high performance liquid chromatography (HPLC; Waters 600) on a C18 reversed phase column (MICROSORB-MV 100-5 C18, 250 x 4.6 MM VALCO) equipped with a diode array detector (Waters PDA 996) using a column temperature of 60 °C with a UV detection wavelength of 260 nm, corresponding to the absorption maxima of the base pairs. A linear gradient of 20 mM ammonium acetate containing 0-30% CH₃CN with a flow rate of 1 mL/min for 40 min was used. Under these conditions, starting material and the product eluted with different characteristic retention time. The assignments of product peaks to cyclobutane adduct has been previously described.² Product yields were determined from the initial slopes of plots of peak area vs. irradiation time. Light intensities were determined using ferrioxalate actinometry.³

NMR Spectra: Samples of hairpin sequences used for NMR experiments were lyophilized four times from aqueous NH₄OH solution (10%) to remove residual triethylamine. The samples were dissolved in 200 μ L of H₂O/D₂O (9:1), containing 100 mM NaCl and 10 mM phosphate buffer (pH 7.2) and then transferred to NMR microtubes (Shigemi Co., Tokyo, Japan). The final concentration of the DNA-hairpin was 0.3-0.5 mM.

NMR spectra were recorded using a Varian Inova 600 spectrometer equipped with a Cold Probe. A series of 1D spectra was recorded at temperatures of 5, 10, 20, 30 and 40 °C. All spectra were acquired in 90% H₂O 10% D₂O using the WATERGATE pulse sequence for water suppression.⁴

Calculated UV Spectra. Minimum energy (AM1 Method) and electronic (ZINDO Hamiltonian) structure calculations of adenine, thymine, (2+2) and (6-4) photoadducts were carried using CAChe 6.1.10.⁵

References

1. R. L. Letsinger and T. Wu, Use of a stilbenedicarboxamide bridge in stabilizing, monitoring, and photochemically altering folded conformations of oligonucleotides, *J. Am. Chem. Soc.*, 1995, **117**, 7323-7328.
2. M. Hariharan and F. D. Lewis, Context-dependent photodimerization in isolated thymine-thymine steps in DNA, *J. Am. Chem. Soc.*, 2008, **130**, 11870-11871.
3. S. L. Murov, *Handbook of Photochemistry*, Marcel Dekker, New York, 1973.
4. M. Piotto, V. Saudek and V. Sklenar, Gradient-tailored excitation for single-quantum NMR-spectroscopy of aqueous solutions, *J. Biomol. NMR*, 1992, **2**, 661-665.
5. CAChe, CAChe Release 6.1.10, Fujitsu Limited, Miahama-Ku, Chiba City, Chiba, Japan, 2000.

Table S1. m/z values determined by MALDI-TOF mass spectrometry and melting temperatures (T_M) determined from the derivatives of thermal dissociation profiles.

Sequence	m/z Calculated	m/z Found	T_M , °C
H ₄ 1	2674.4	2673.8	25.4
H ₄ 2	2674.4	2672.4	26.1
H ₄ 3	2674.4	2673.5	27.5
2H ₄	2674.4	2674.1	26.7
H ₆ 1	3904.76	3903.6	49.5
H ₆ 2	3904.76	3905.9	50.5
H ₆ 3	3904.76	3908.1	48.8
2H ₆	3904.76	3905.1	49.1
L ₆ 1	5503.7	5502.41	38.4
L ₆ 2	5503.7	5504.84	44.6
L ₆ 3	5503.7	5501.89	35.5
SS1	1791.26	1791.71	

Table S2. Proton assignments for chemical shifts of H₄2 at 17 °C

Base	H1'	H2'	H2''	H3'	H4'	H6	H8	H2	M7
A1	6.267	2.898	2.779	4.914	4.323		8.246	7.985	
T2	6.116	2.531	2.175	4.909	4.282	7.471			1.432
T3	5.885	2.265	1.841	4.801	4.177	7.369			1.752
A4	6.315	2.864	2.755	5.082	4.453		8.409	7.914	
T6	5.646	2.447	2.249	4.862	4.137	7.56			1.912
A7	6.02	2.925	2.825	5.078	4.424		8.318		
A8	6.286	2.882	2.703	5.032	4.501		8.236		
T9	6.124	2.206	2.19	4.553	4.077	7.261			1.46

Table S3. Assignments of C12 linker proton chemical shifts (see Table 1 for numbering). Two values are given if two peaks were assigned for the methylene group, the second field is left blank if only one resonance was assigned.

C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
3.864	1.438	1.020	0.808	0.440			0.638 ^a	0.729	0.915	1.334	
			0.730	0.318			0.468 ^a	0.638 ^a	0.808 ^a		

^a Assignments of these protons are less certain.

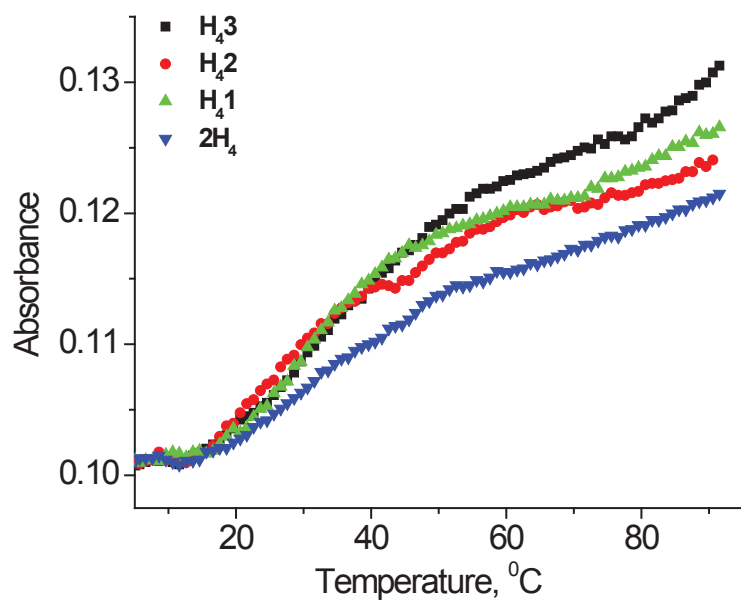


Fig. S1 Thermal dissociation profiles for the a) alkane-linked hairpins having 4 AT base pairs in 10 mM phosphate buffer (pH 7.2) containing 100 mM NaCl.

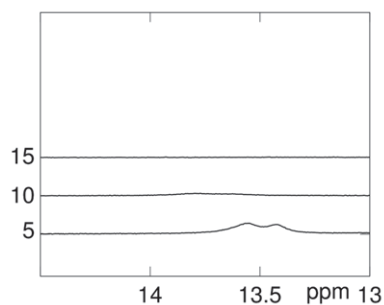


Fig. S2 Temperature dependent 1D NMR spectra for the thymine H3 imino protons of conjugates **H₄₂** in 90 % H₂O, 10 % D₂O (10 mM phosphate buffer, 100 mM NaCl).

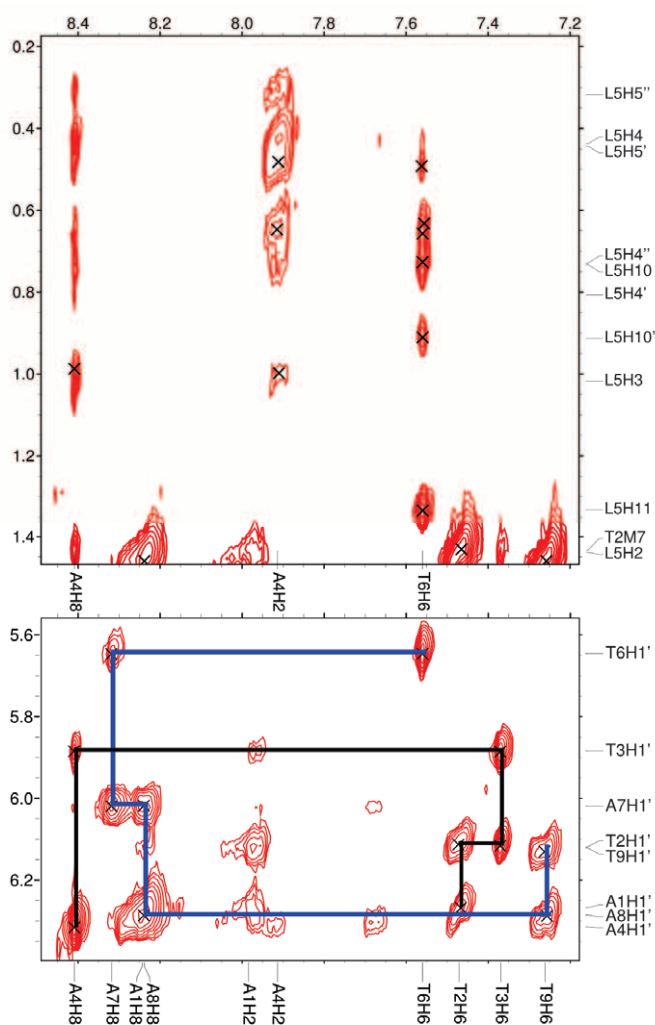


Fig. S3 Sequential assignments for **H₄₂** obtained using base protons (7.2-9.8ppm), H1' (5.5-6.5 ppm) and linker protons (0.2 -1.4 ppm). The 5' strand is drawn in black, 3' strand drawn in blue. The NOESY spectra for **H₄₂** (10 mM phosphate buffer, 100 mM NaCl) is recorded at 20 °C with a mixing time of 250 ms.

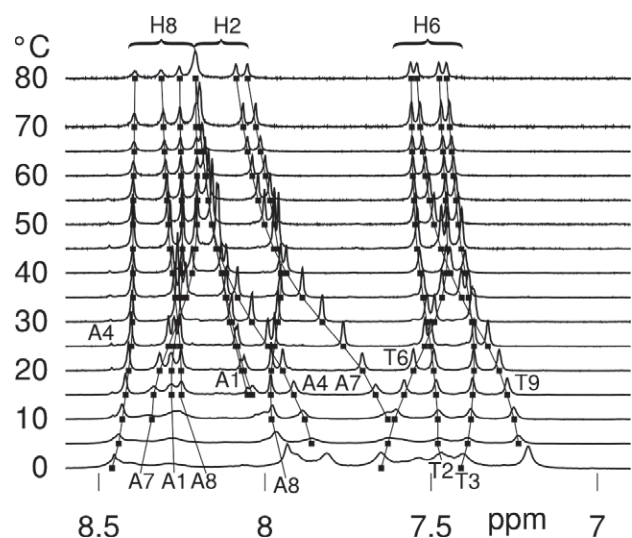


Fig. S4 Temperature dependent 1D NMR spectra for aromatic region of **H₄₂** (10 mM phosphate buffer, 100 mM NaCl). Assignments of nucleotide type and number are shown next to the peaks and are reported in Table S2.

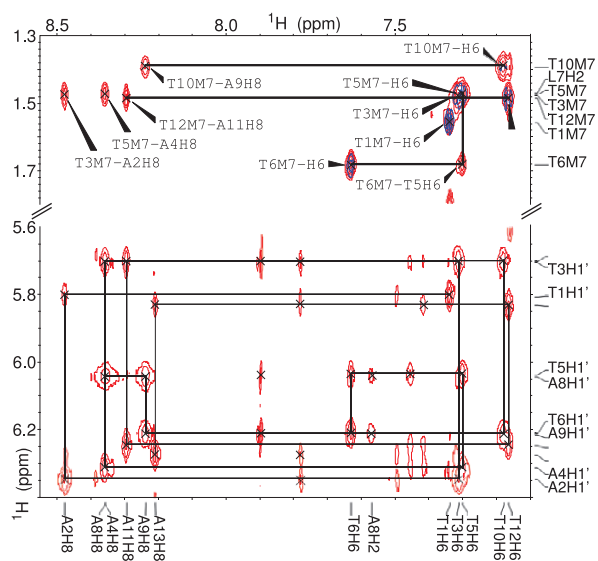


Fig. S5 Overlay of the NOESY spectrum (red) and TOCSY spectrum (blue) of **H₆₃** recorded at 20 °C. Shown is the region containing crosspeaks for the sequential assignment (H1' and H6/H8) and the region of crosspeaks with H6/H8 with thymidine methyl groups (M7).The sequential H1'-H6/H8 and M7-H6/H8 connectivities of close proton contact peaks are shown as lines.

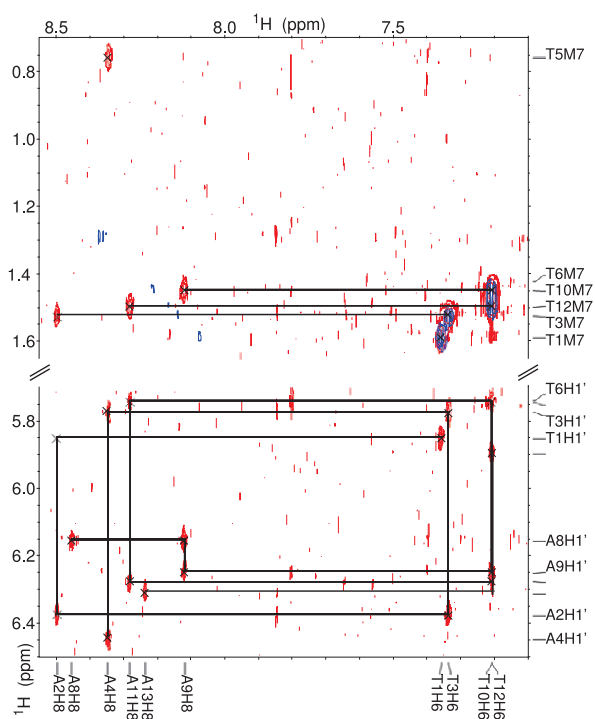


Fig. S6 Overlay of NOESY spectrum (red) and TOCSY spectra (blue) of the (2+2) photoadduct of **H₆3** recorded at 20°C. Shown is the region containing crosspeaks for the sequential assignment (H1' and H6/H8) and the region of crosspeaks with H6/H8 with thymidine methyl groups (M7). The sequential H1'-H6/H8 and M7-H6/H8 connectivities of close proton contact peaks are shown as lines.

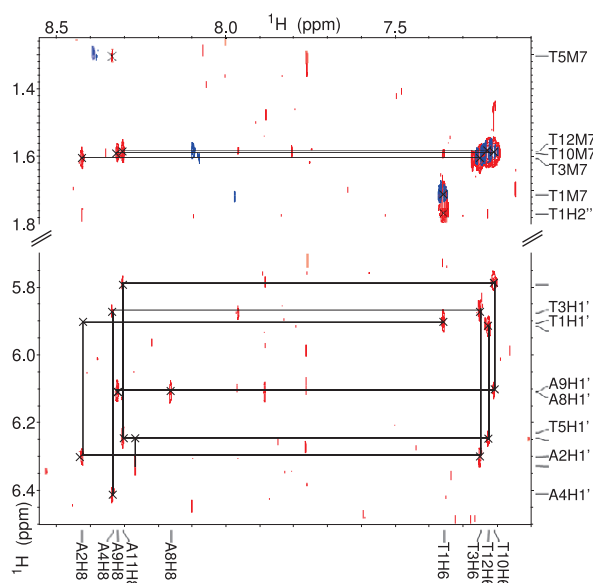


Fig. S7 Overlay of NOESY spectrum (red) and TOCSY spectrum (blue) of the (6-4) photoadduct of **H₆3** recorded at 20°C. Shown is the region containing crosspeaks for the sequential assignment (H1' and H6/H8) and the region of crosspeaks with H6/H8 with thymidine methyl groups (M7). The sequential H1'-H6/H8 and M7-H6/H8 connectivities of close proton contact peaks are shown as lines.

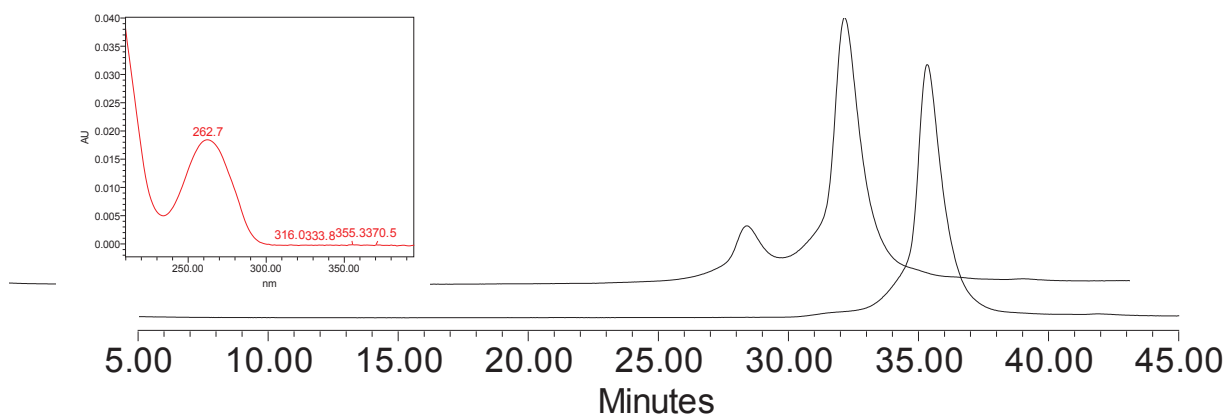


Fig. S8 HPLC trace for hairpin **H₆₁** at 280 nm at irradiation times of 0 and 45 min. First peak after 45 min irradiation is (2+2) adduct, second peak is starting material. UV of the (2+2) adduct is shown in red.

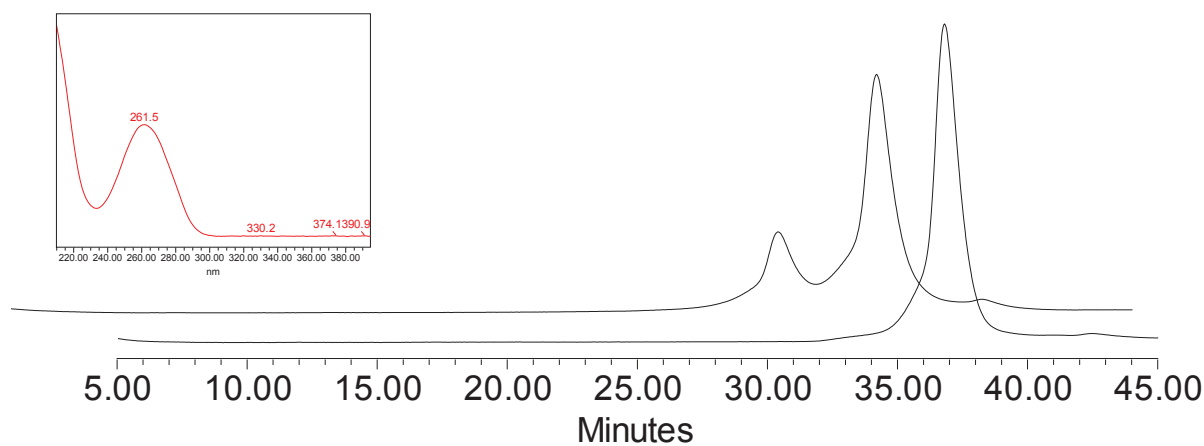


Fig. S9 HPLC trace for hairpin **H₆₂** at 280 nm at irradiation times of 0 and 45 min. First peak after 45 min irradiation is (2+2) adduct, second peak is starting material. UV of the (2+2) adduct is shown in red.

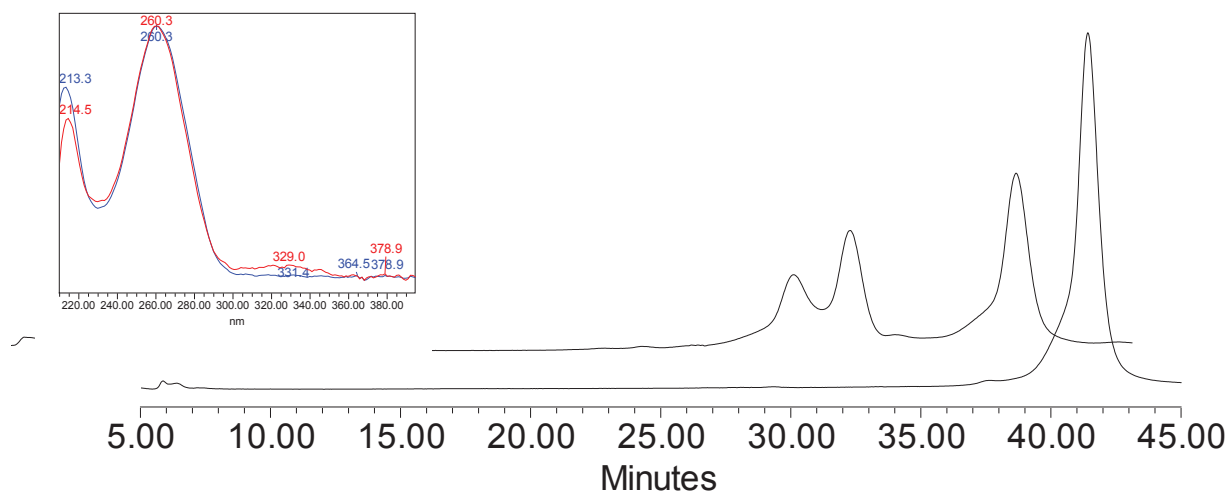


Fig. S10 HPLC traces for hairpin **H₆₃** at 280 nm at irradiation times of 0 and 45 min. First peak after 45 min is (6-4) adduct, second peak is (2+2) adduct, third peak is starting material. UV of the (6-4) and (2+2) adducts are shown in red and blue, respectively.

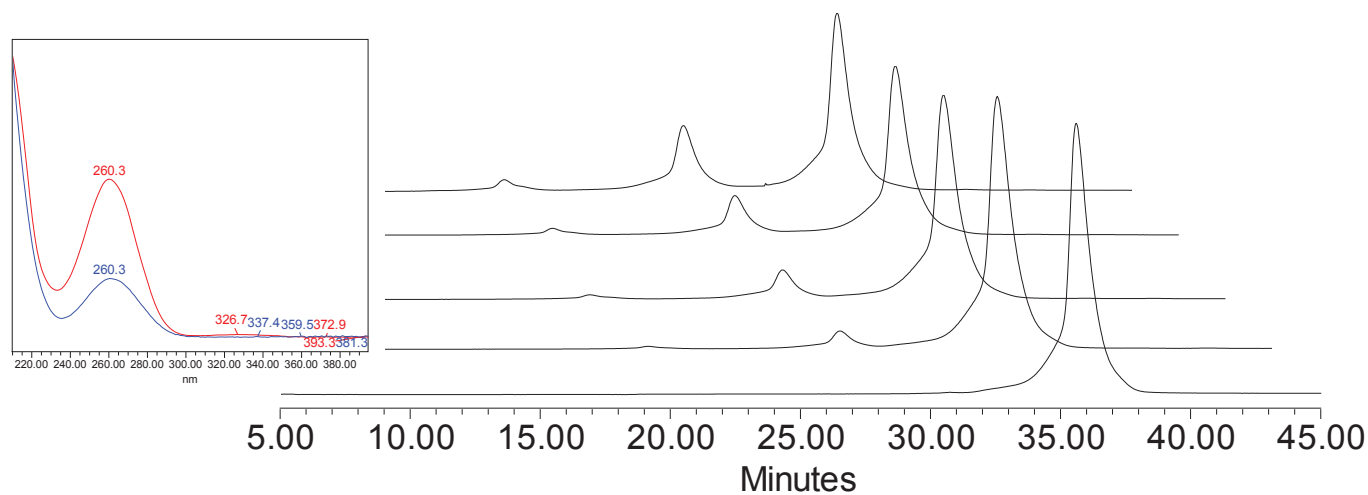


Fig. S11 HPLC trace for hairpin **H₄₁** at 280 nm with increasing irradiation time (0, 10, 20, 30 and 40 min). First peak is the (6-4) adduct and second peak is the starting material. UV of the (6-4) adduct is shown in red.

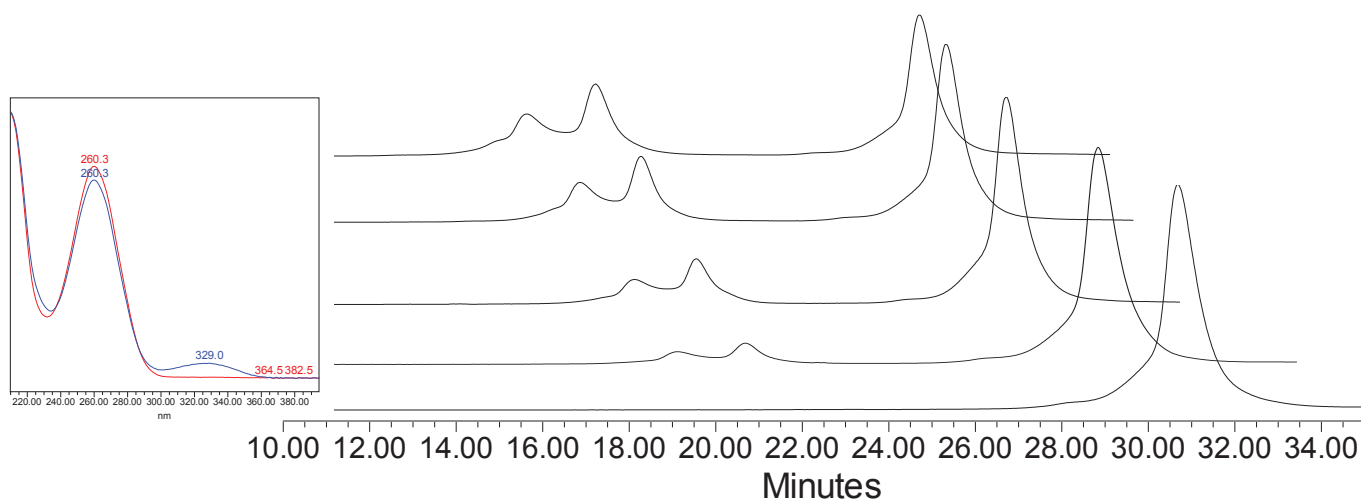


Fig. S12 HPLC trace for hairpin **H₄₂** at 280 nm with increasing irradiation time (0, 10, 20, 30 and 40 min). First peak is the (2+2) adduct and second peak is the starting material. UV of the (2+2) adduct is shown in blue and starting material in red.

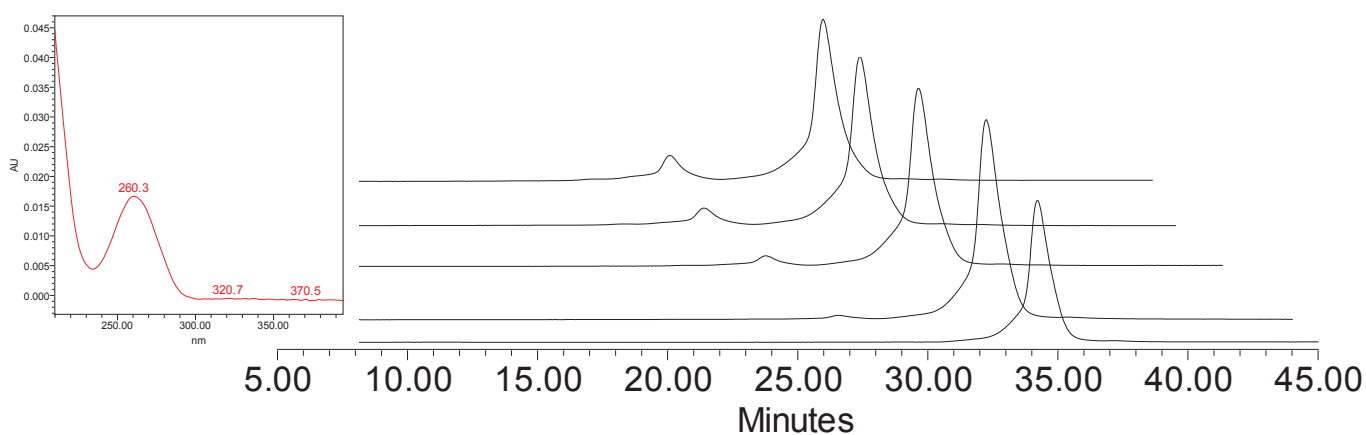


Fig. S13 HPLC trace for hairpin **H₄₃** at 280 nm with increasing irradiation time (0, 10, 20, 30 and 40 min). First peak is the (6-4) adduct, second peak the (2+2) adduct and third peak is the starting material. UV of the (2+2) adduct is shown in red and (6-4) adduct in blue.

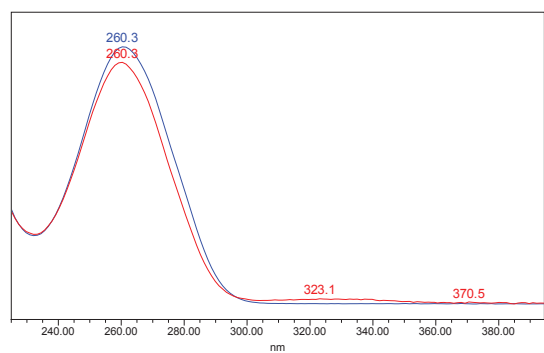
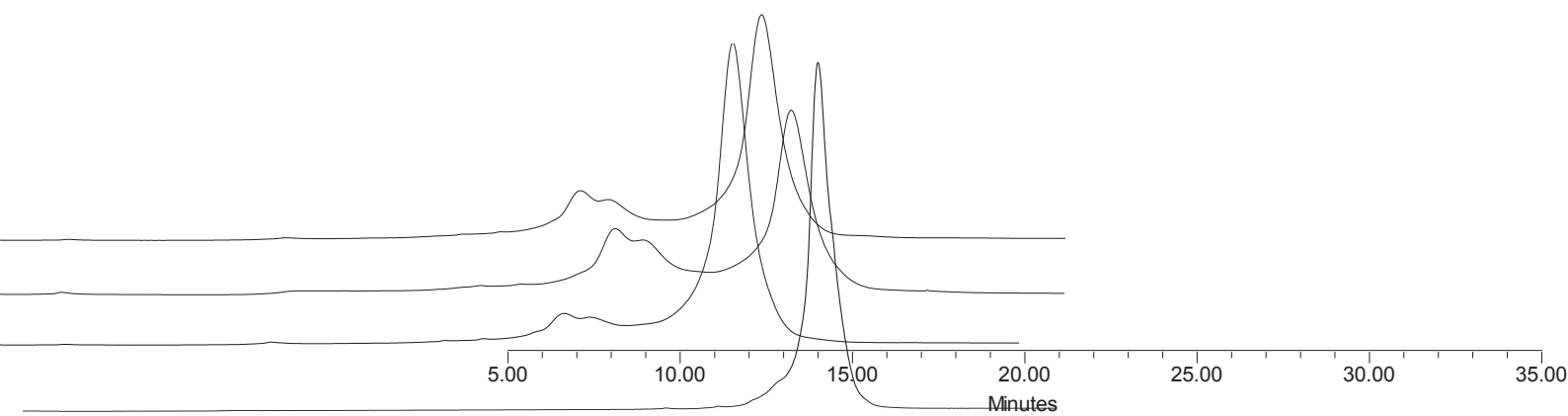


Fig. S14 HPLC trace for hairpin **L₆1** at 280 nm with increasing irradiation time (0, 10, 20 and 30 min). First peak is the (2+2) adduct, second peak the (6-4) adduct and third peak is the starting material. UV of the (2+2) adduct is shown in blue and (6-4) adduct in red.

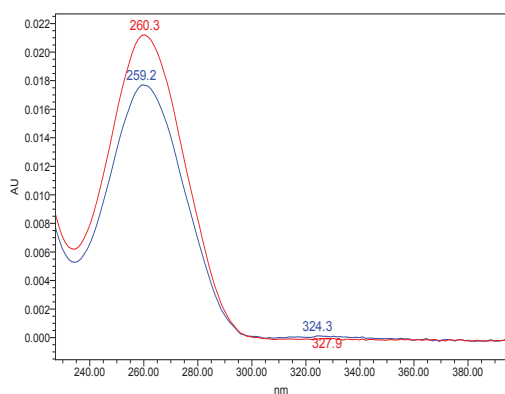
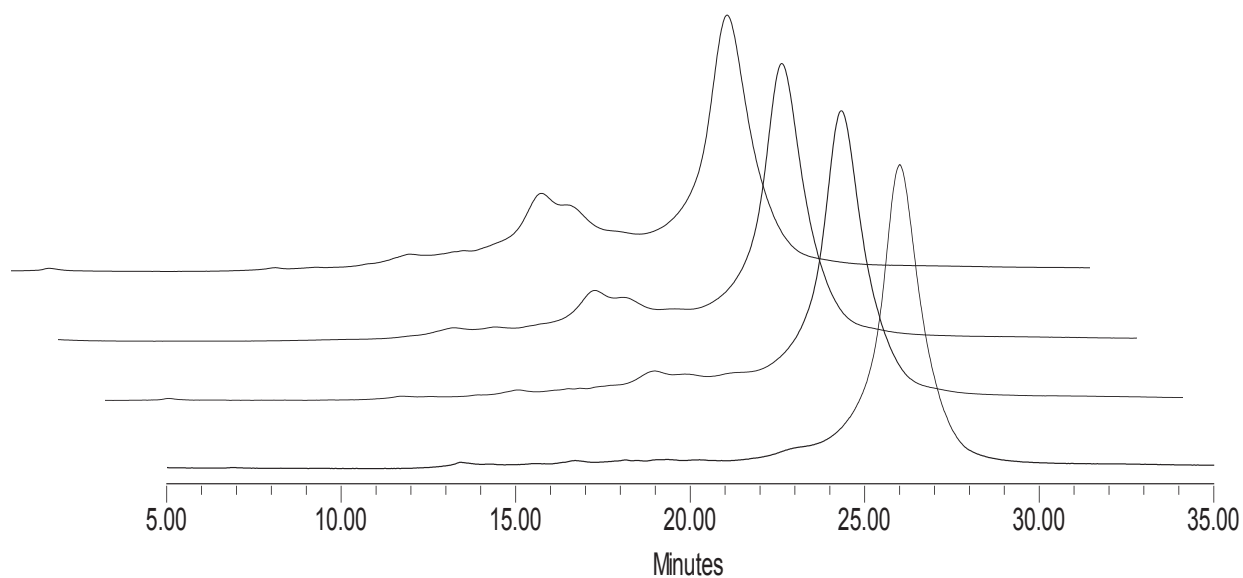


Fig. S15 HPLC trace hairpin **L₆2** at 280 nm with increasing irradiation time (0, 10, 20 and 30 min). First peak is the (2+2) adduct, second peak the (6-4) adduct and third peak is the starting material. UV of the (2+2) adduct is shown in blue and (6-4) adduct in red.

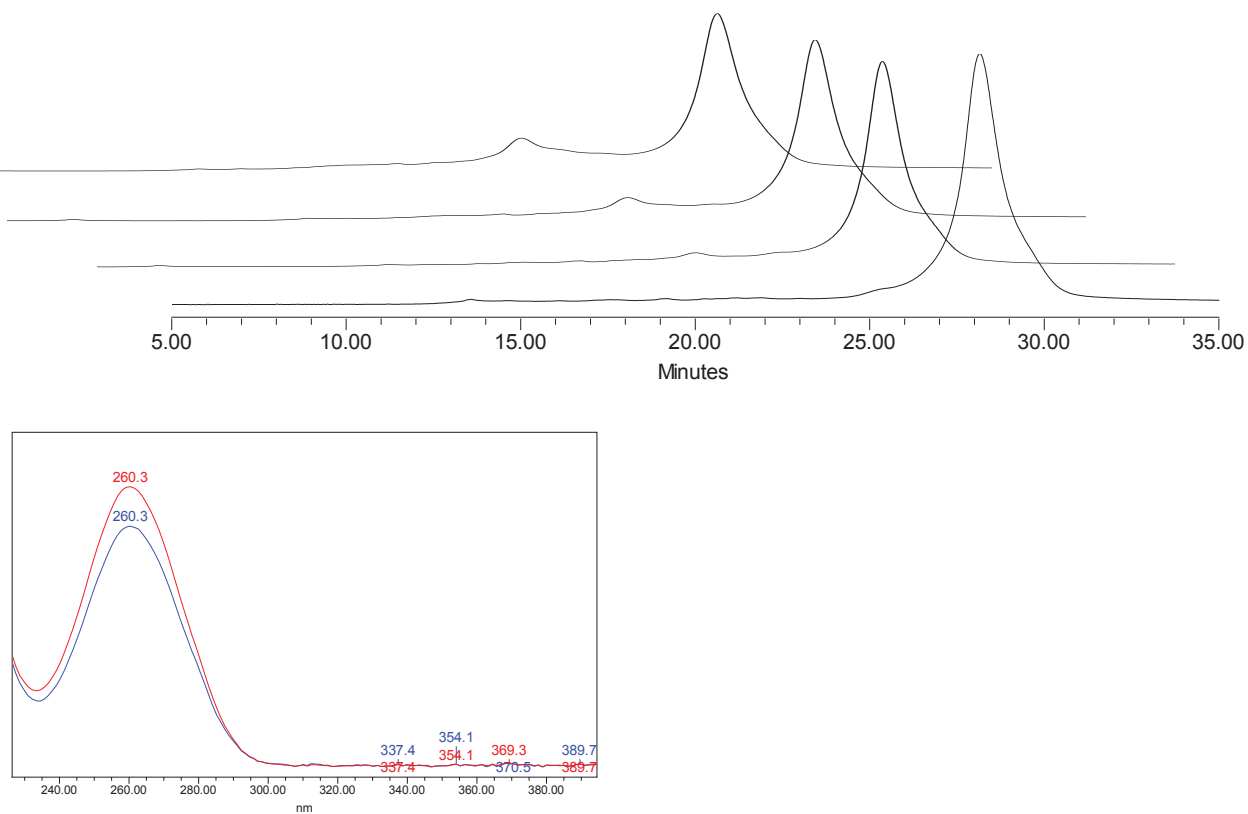


Fig. S16 HPLC trace for hairpin **L₆3** at 280 nm with increasing irradiation time (0, 10, 20 and 30 min). First peak is the (2+2) adduct, the second peak is the starting material. UV of the (2+2) adduct is shown in blue and (the starting material adduct in red).

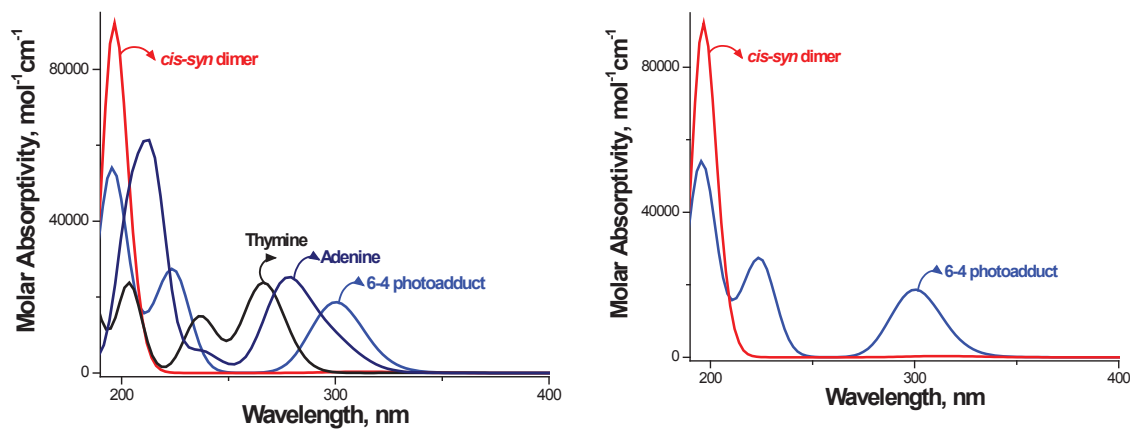


Fig. S17 Calculated electronic absorption spectra of thymine, adenine, 2+2 and 6-4 photoproducts.