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Accelerating Chemical Reactions by Molecular Sledding

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Materials

Peptides (>95% purity) were purchased from CASLO ApS (Denmark). Cy3 labelled pVIc was acquired from Bio-Synthesis Inc (United States). All peptides were used without further purification. 100 bp DNA was purchased from Integrated DNA Technologies. Verteporfin was purchased from MedChem Express Company (United States). All other chemicals were obtained from Sigma-Aldrich and used without further purification. During all experiments, ultrapure water (18.2 MΩ) purified by a MilliQ-Millipore system (Millipore, Germany) was used.

General methods

Reaction kinetics were analyzed by reverse-phase High-Performance Liquid Chromatography (HPLC) performed on a Shimadzu VP instrument using a C18 X Bridge BEF column. Verteporfin-peptide conjugates were purified on the same instrument. Mass spectrometric analysis of the synthesized conjugates was performed using a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems, Foster City, CA, USA) with 3-hydroxypicolinic acid (HPA) as matrix. LC-MS analyses were performed on a Waters Xevo G2 UPLC/TOF instrument. All UV-vis spectra were measured on a JASCO V-630 spectrophotometer. Analysis of DNA cleavage was carried out by agarose gel electrophoresis (1.5 weight%) with subsequent ethidium bromide staining, and photographs of the gels were taken with a GenoSmart Image Reader (VMR, The Netherlands).

Synthesis

Standard procedure for substitution reactions:

(S) or (R)-bromide-pVIc, pVIc-cy3 and 100 bp double-stranded (ds) DNA (if needed) were mixed in 50 mM Tris-HCl buffer (pH 8.5) containing 2 mM sodium chloride and 5 mM ethylenediaminetetraacetic acid (EDTA). The final concentration of pVIc-Cy3, bromide-pVIc and DNA are 20 μM, 16 μM and 4.4 μM, respectively. The mixtures were degased by purging with argon and reacted at room temperature. After reaction times ranging from 30 min to 24 hours, the reaction mixtures were treated with DNase I to digest DNA catalyst, and then they were analyzed by HPLC.

Syntheses of verteporfin-peptide conjugates:

Verteporfin (6 mg, 8.35 μmol) was dissolved in 1 ml anhydrous dichloromethane, to which 1 mg (8.67 μmol) N-hydroxysuccimide and 1.8 mg (8.72 μmol) N,N'-dicyclohexylcarbodiimide were added. The reaction was stirred at room temperature and was completed after one hour as moniterd by TLC (5% methanol in dichloromethane). The dichloromethane was evaporated under reduced pressure and the crude mixture was re-dissolved in tetrahydrofuran and methanol (v/v = 4:1, 1.6 ml). 2 equiv. of N-(2-aminoethyl)maleimide trifluoroacetate salt (4.5 mg) was added to the mixture, which was kept overnight at room temperature. The verteporfin-maleimide (verteporfin-M) compound was purified by silica gel column chromatography, using 2.5% methanol in chloroform as eluent. To obtain verteporfin-peptide conjugates, veteporfin-M (2 mg, 2.46 μmol) and pVIc or scrambled peptide (S) (2 mg, 1.48 μmol) were mixed in tetrahydrofuran and methanol (v/v = 1:3, 600 μL). Then 6 μL trimethylamine was introduced, and the reaction mixture was shaken overnight. The crude products were purified by gradient reverse phase HPLC (buffer A: 0.1% trifluoroacetic acid (TFA) in 5% acetonitrile and 95% MilliQ water, buffer B: 0.1% TFA in 95% acetonitrile and 5% MilliQ water). The final conjugates were lyophilized and kept in freezer for further use (yield:~60%).

Preparation of supercoiled pUC 19 DNA:

Escherichia coli strain DH5α (Life Technologies) was transformed with the circular 2686 base pair vector pUC19 (New England Biolabs) as described by Sambrook et al.[1] The vector was

isolated from a 2 L bacterial culture in LB Lennox Broth (Sigma-Aldrich) using the GenElute HP Plasmid DNA Maxiprep Kit (Sigma-Aldrich). pUC 19 DNA was characterized by 1.5% agrose gel electrophoresis (Figure S6).

Photocleavage of supercoiled plasmid DNA:

DNA photocleavage by all verteporfin derivatives was conducted in Tris buffer containing 21 μM pUC 19 plasmid DNA. The samples were irradiated by a white light through a band-pass filter (680±10 nm, 0.5 mW/cm^2). After different irradiation times, the efficiency of photocleavage was analyzed by gel electrophoresis on 1.5% agarose gel.

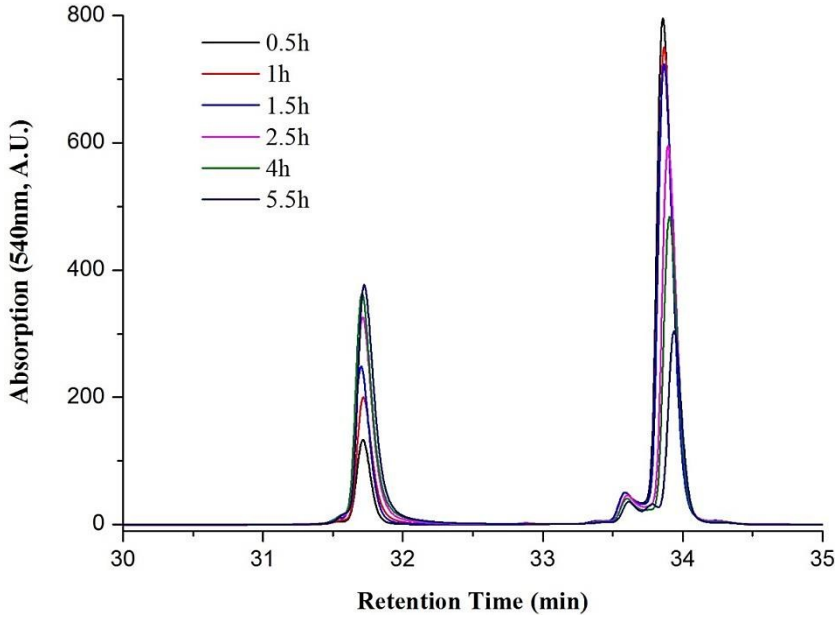


Fig. S1. *HPLC traces indicating product (retention time 31.7 min) formation at different reaction times between the substrates (R)-Br-pVIc and Cy3-pVIc (retention time 33.9 min).*

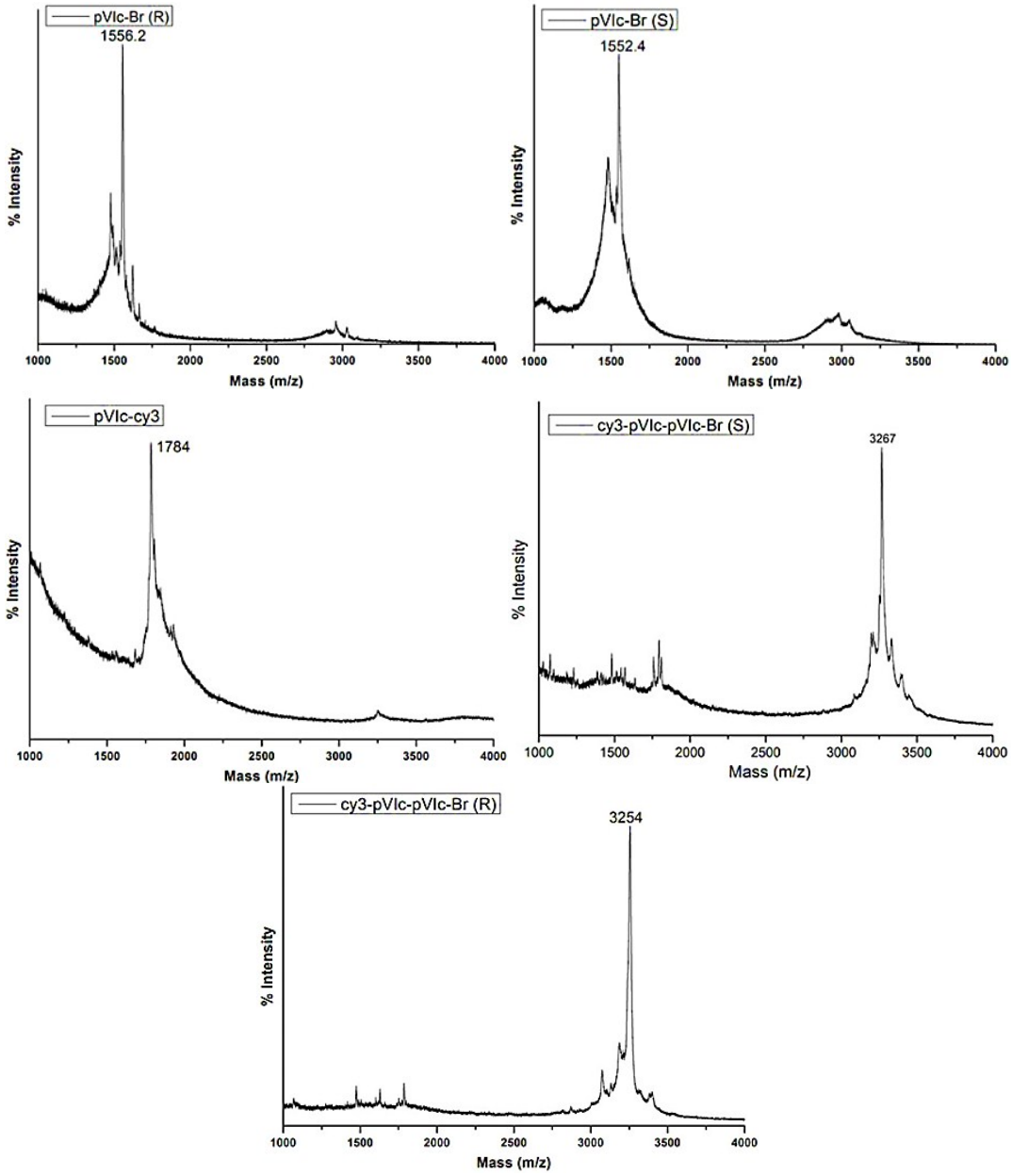


Fig. S2. *MALDI-TOF mass spectra of pure (R)-bromide pVIc (calculated M.W. 1554), (S)-bromide pVIc, pVIc-cy3 (calculated M.W. 1784) and their conresponding products(calculated M.W. 3257).*

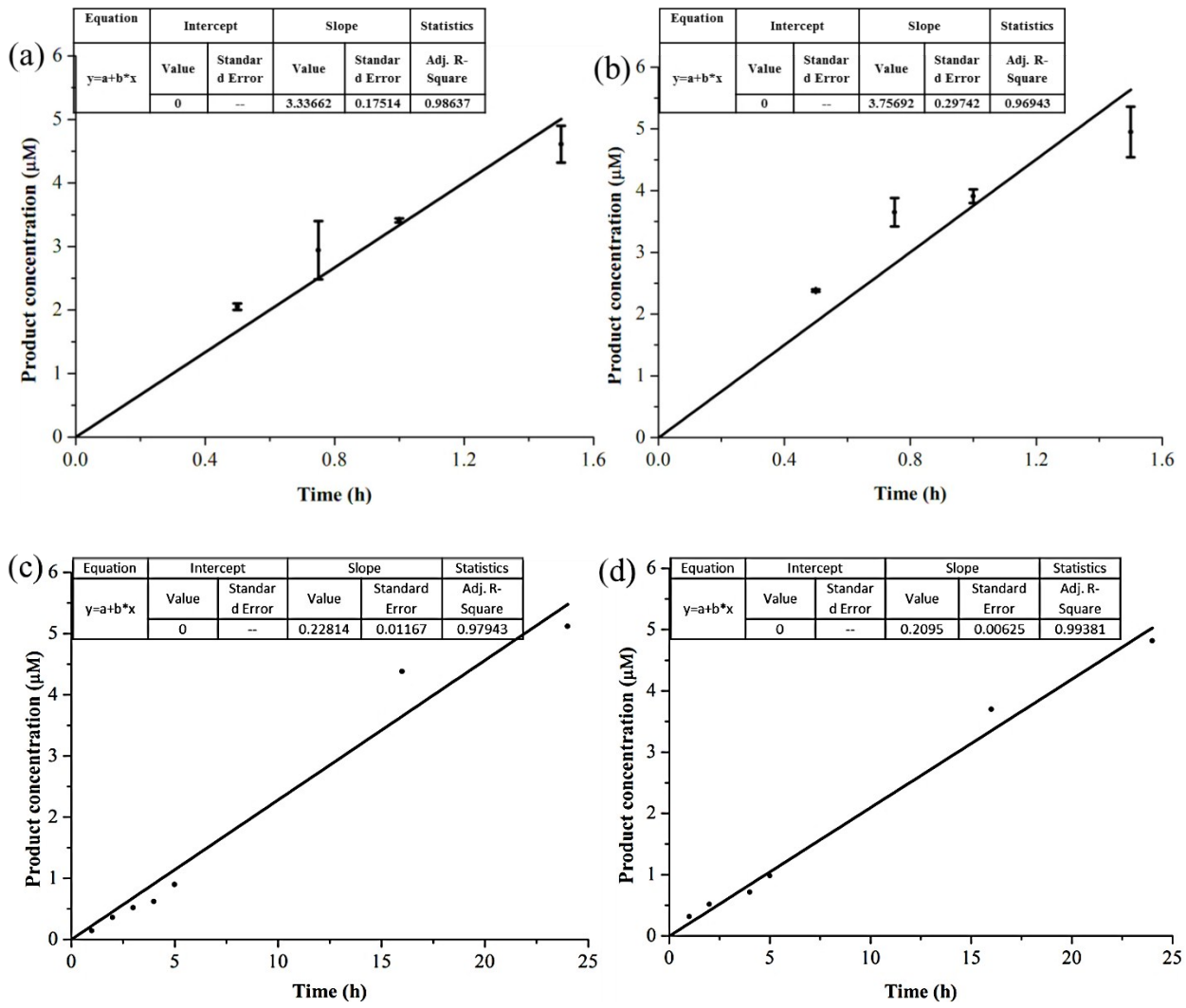


Fig. S3. *Determination of initial rates for a) (R)- and b) (S)-product formation in the presence of DNA, c) (R)- and d) (S)-product formation in the absence of DNA.*

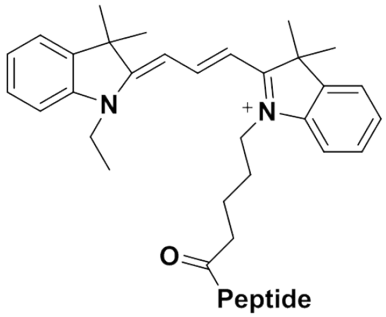


Fig. S4. *Structure of Cy3 dye*

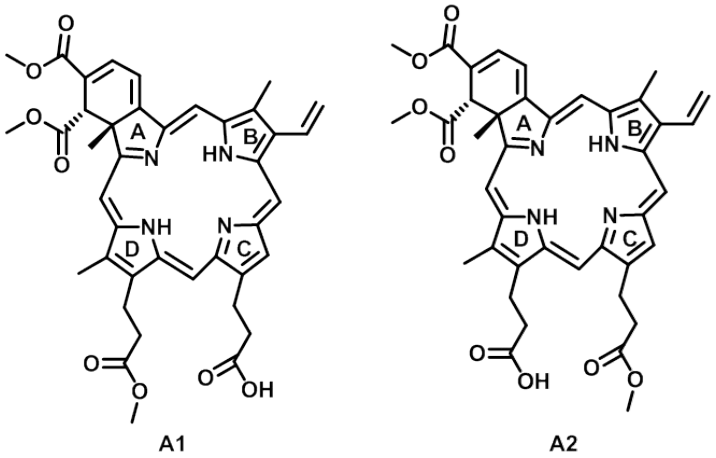
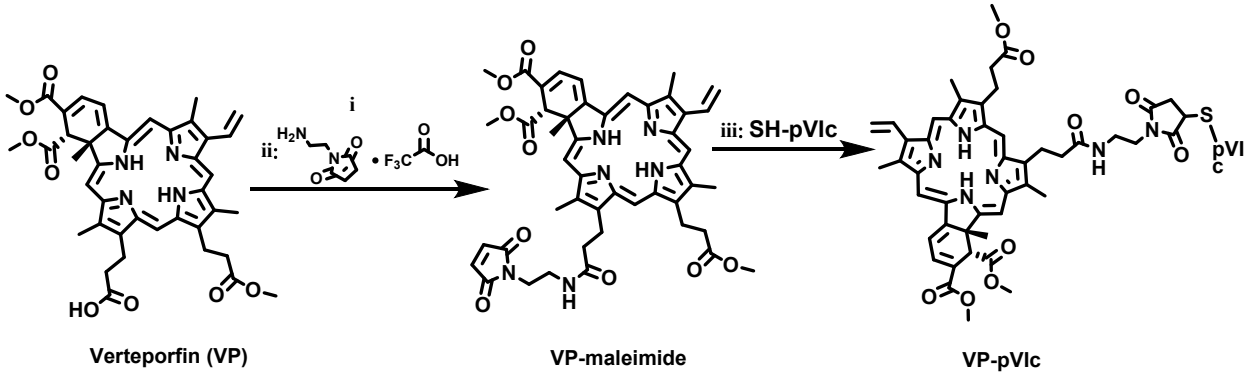


Fig. S5. *Structures of two regioisomers (A1 and A2) of verteporfin (differing by the position of the propionic ester group either at ring C or D).*



Scheme S1. Synthesis route of verteporfin-pVIc conjugate (i: DCC, NHS, CH_2Cl_2, 1h; ii: THF, MeOH, overnight; iii: Et_3N, THF, MeOH, overnight). Verteporfin functionalized with a scrambled peptide vp-S was synthesized analogously.

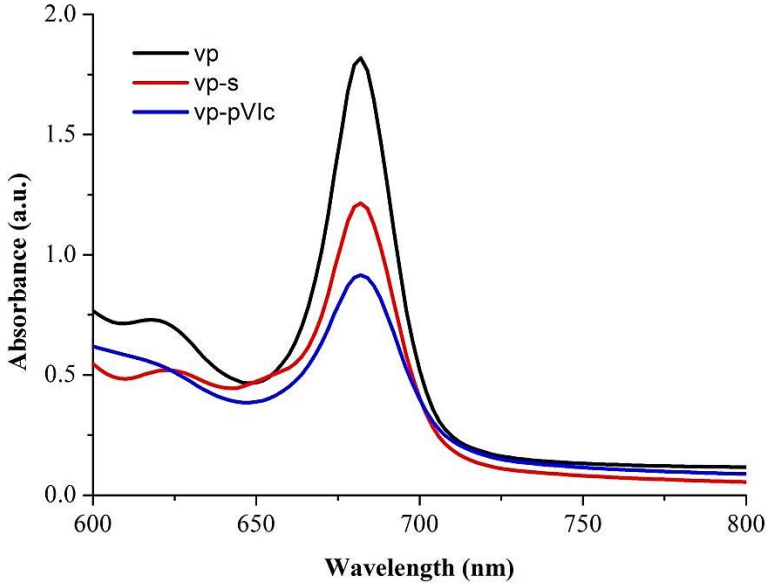


Fig. S6. *UV-vis spectra of verteporfin and verteporfin-peptide conjugates. All compounds show a typical absorption wavelength at 683 nm.*

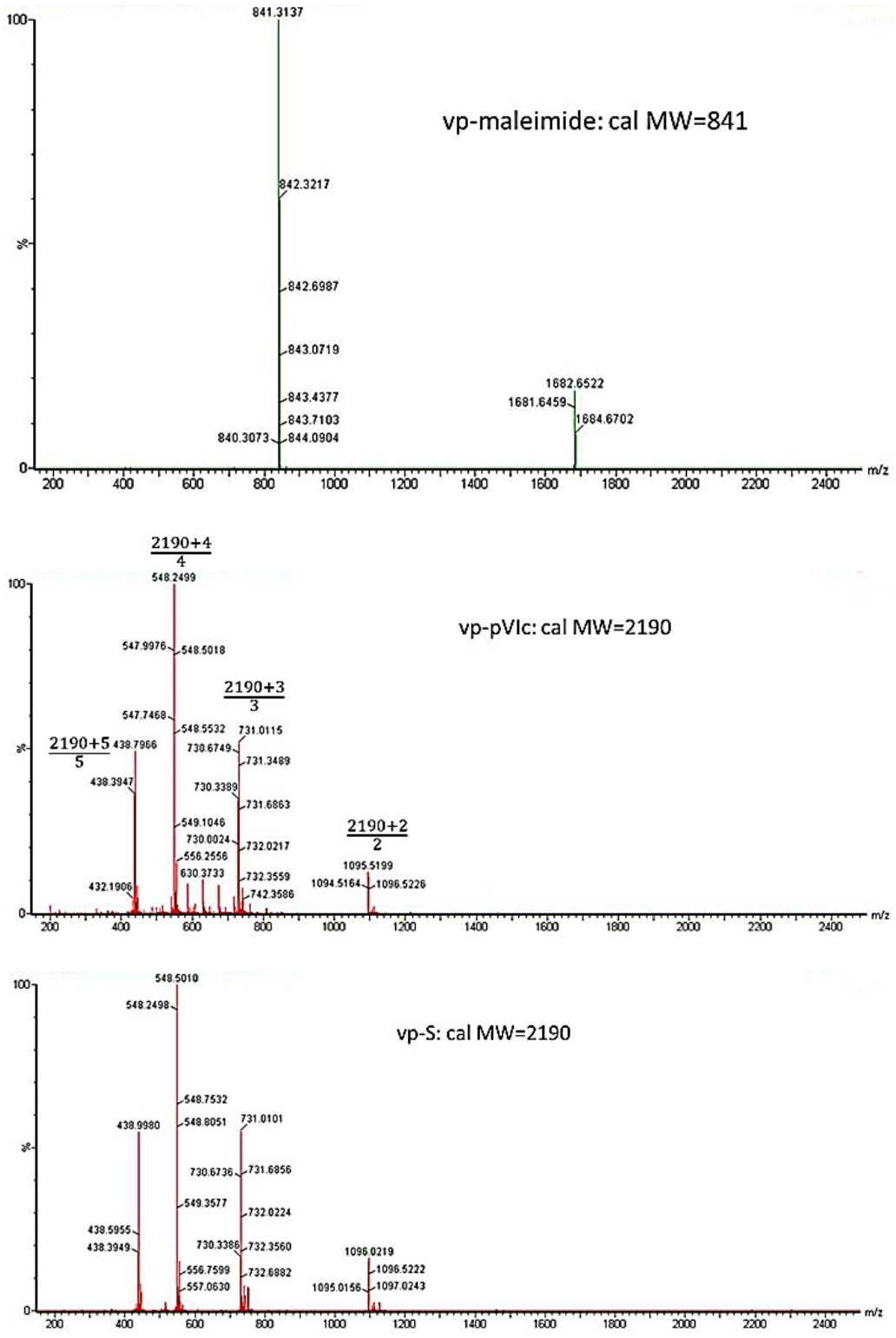


Fig. S7. *ESI-MS spectra of vp-maleimide, vp-pVIc and vp-S conjugates.*

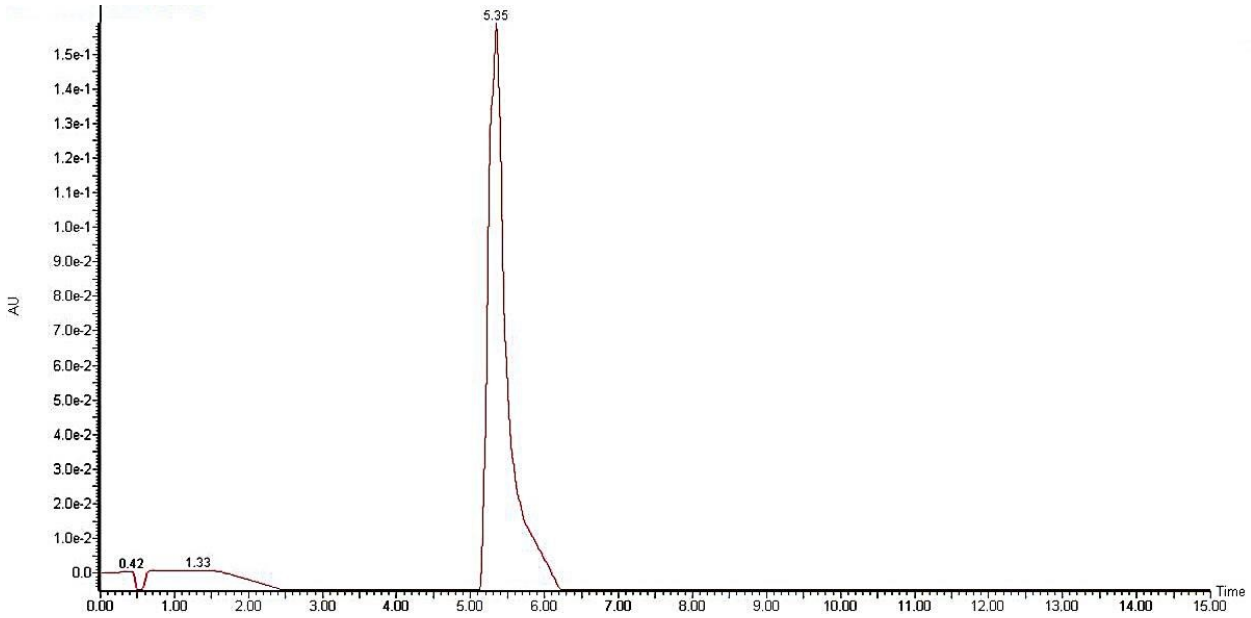


Fig. S8: *HPLC elugram of vp-pVIc documenting the purity of the compound. (HPLC column: Phenomenex Aeris Peptide, 2.1 × 150mm; Detection wavelength: 434 nm; Buffer A: 0.1% TFA in H_2O; Buffer B: 0.1% TFA in CH_3CN; Flow rate: 0.3 mL/min).*

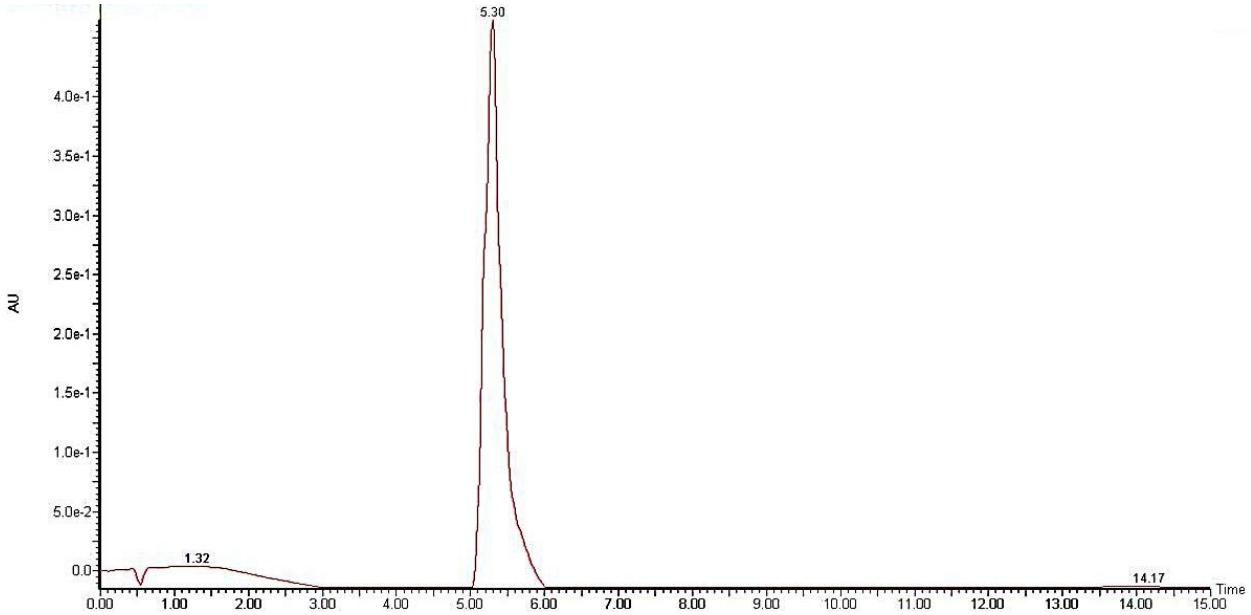


Fig. S9: *HPLC elugram of vp-s documenting the purity of the compound. (HPLC column: Phenomenex Aeris Peptide, 2.1 × 150mm; Detection wavelength: 434 nm; Buffer A: 0.1% TFA in H_2O; Buffer B: 0.1% TFA in CH_3CN; Flow rate: 0.3 mL/min).*

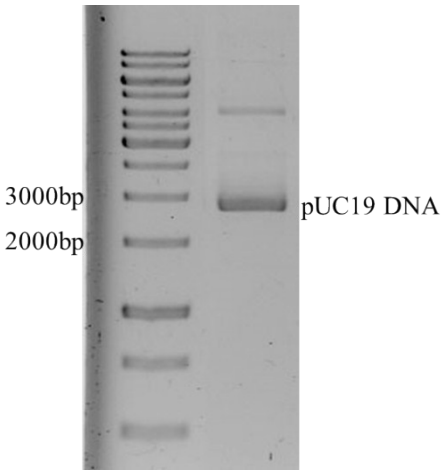


Fig. S10. *Gel electrophoretic analysis of prepared pUC 19 DNA. The top faint band on the right is attributed to nicked plasmid DNA.*

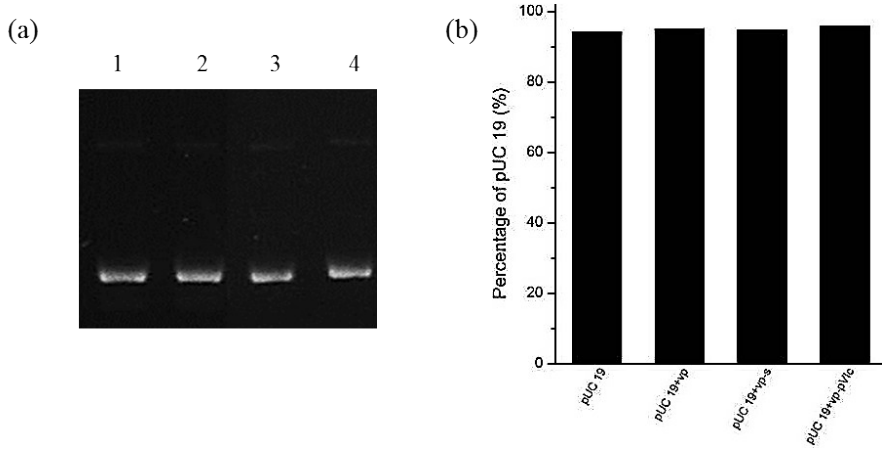


Fig. S11. *Incubation of verteporfin-conjugates with DNA overnight in dark. a) Agarose gel electrophoresis analysis; lane 1: pUC 19 DNA, lane 2: DNA+vp, lane 3: DNA+vp-s, lane 4: DNA+vp-pVIc. b) Recovery of pUC19 DNA as determined from gel electrophoretic analysis.*

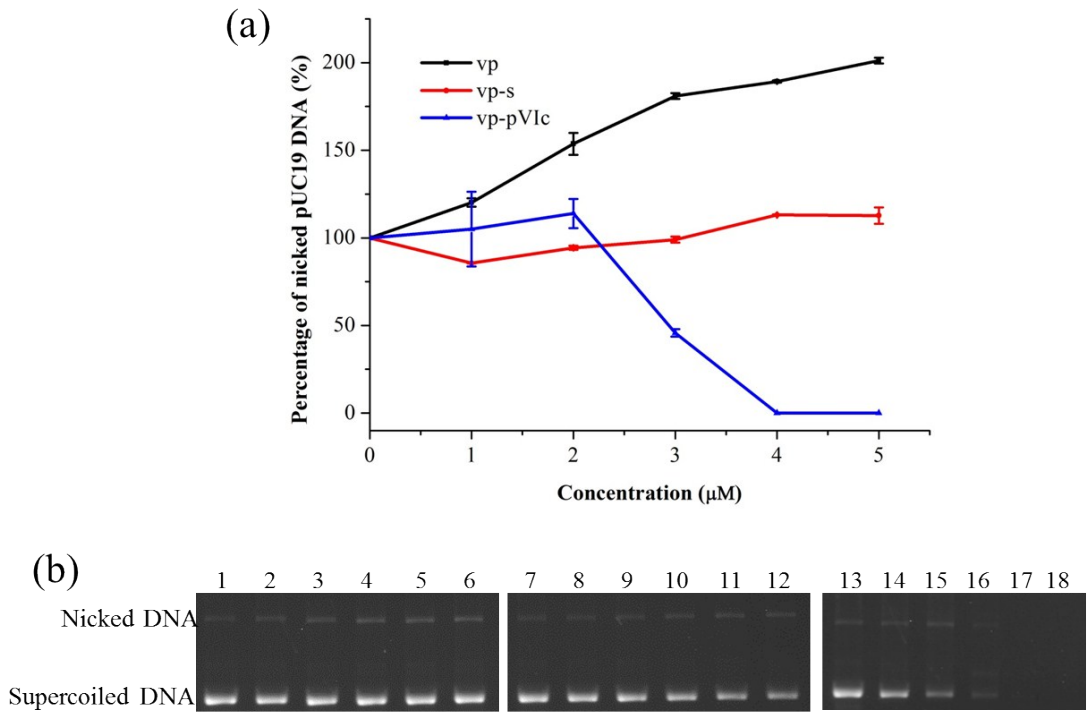


Fig. S12. *Monitoring of nicked pUC19 DNA during photodamage induced by verteporfin, verteporfin-scrambled peptide and verteporfin-pVIc conjugates at different photosensitizer concentrations. a) During the photodamage of supercoiled DNA (lower band in b), all experiments contained a smaller amount of nicked pUC19 plasmid DNA (upper band in b). The amount of nicked pUC19 DNA without photosensitizer was set to 100% and photocleavage was followed as a function of concentrations of verteporfin derivatives after 30 min light irradiation (680 nm, 0.5 mW/cm²). The increasing amounts of nicked DNA in the presence of pristine vp can be explained by the fact that the photosensitizer induces nicks in the supercoiled plasmid leading to its accumulation. This behaviour is to a lesser extend observed for reactions involving vp-s. In the case of vp-pVIc, a small increase in nicked pUC19 is detected up to concentrations of 2 μM. For higher concentrations of the photosensitizer conjugate the DNA cleavage is so efficient that smaller fragments are obtained. These observations clearly indicate that this vp conjugate also efficiently degrades non-supercoiled double stranded DNA. b) Gel electrophoretic analysis of DNA cleavage: lane 1-6, DNA control, verteporfin 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, respectively; lane 7-12 DNA control, vp-S 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, respectively; lane-13-18, DNA control, vp-pVIc 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, respectively.*

Reference

1. J. J. Sambrook, D. D. W. Russell, *Molecular Cloning: A Laboratory Manual*, **2001**, *3rd ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.