

Electronic Supplementary Information

5-Bromo-2'-deoxycytidine – a Potential DNA Photosensitizer

*Magdalena Zdrowowicz, Paweł Wityk, Barbara Michalska, Janusz Rak**

Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland

Corresponding Author

*E-mail: janusz.rak@ug.edu.pl. Phone: +4858 523 5118. Fax: +4858 523 5771

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PCR Reaction with BrdCTP

The double-stranded oligonucleotide labeled with BrdC was obtained in PCR reaction. Amplification was performed using an Eppendorf thermocycler in 100 µl of a reaction mixture containing 50 mM Tris-HCl (pH 9.0), 20 mM (NH₄)₂SO₄, 2.5mM MgCl₂, 5% DMSO, 7 ng of single-stranded DNA template (5'-ACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAG-3'), 1 µM of each primer (the forward primer sequence: 5'-ACGACAGGTTTCCCGAC-3' the reverse primer sequence: 5'-CTGGGGTGCCTAATGAGTG-3'), 200 µM of each dNTP (either dATP, dGTP, dCTP, TTP or dATP, dGTP, BrdCTP, TTP) 5% ethylene glycol and 1 unit of thermostable Marathon DNA polymerase. An optimized PCR protocol was used in order to increase the reaction yield and eliminate the formation of non-specific products. Cycle parameters for the first thermocycle were 95 °C for 30 sec (initial denaturation), 85 °C for 60 sec (Hot Start addition of the polymerase), 95 °C for 30 sec (denaturation), 55 °C for 1 sec (annealing of the forward primer) and 72 °C for 2 sec (elongation). The product created in the first PCR cycle was a double-stranded DNA template. Then thirty additional cycles were performed as follows: 95 °C for 30 sec (denaturation), 56 °C for 1 sec (annealing), and 72 °C for 2 sec (elongation).

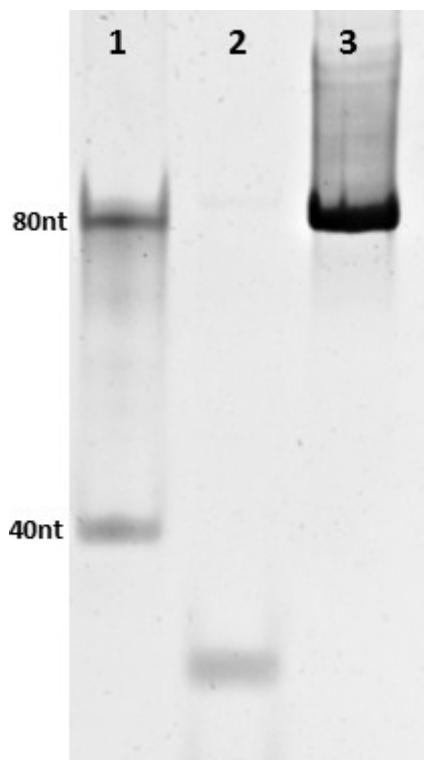


Figure S1. Non-denaturing PAGE of the studied DNA fragment: lane 1- mass standard containing two single stranded oligonucleotides: 40 and 80 nt in length; lane 2 –reaction mixture before PCR reaction; lane 3 – PCR product - double-stranded oligonucleotide labeled with BrdC 80 bp in length. 15% non-denaturing polyacrylamide gel was prepared in 1xTBE buffer¹. The electrophoresis was performed for 1,5 h with 100 V. The gels were visualized after staining (30 min.) with GelRed (Biotum) using a Fusion FX imaging system (Vilber Lourmat, Germany).

(1) Ausubel, F. M.; Brent, R.; Kingston, R. E.; Moore, D. D.; Seidman, j. G.; Smith, J. A.; Struhl, K. Short Protocols in Molecular Biology, fifth ed., John Wiley & Sons, New York, **2002**.

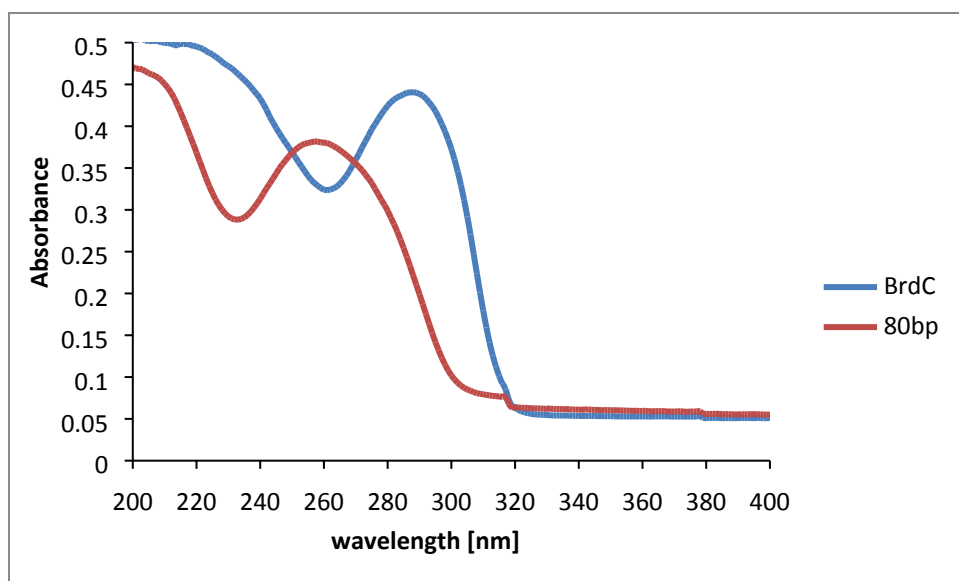


Figure S2. Comparison of BrdC and native DNA UV spectra.

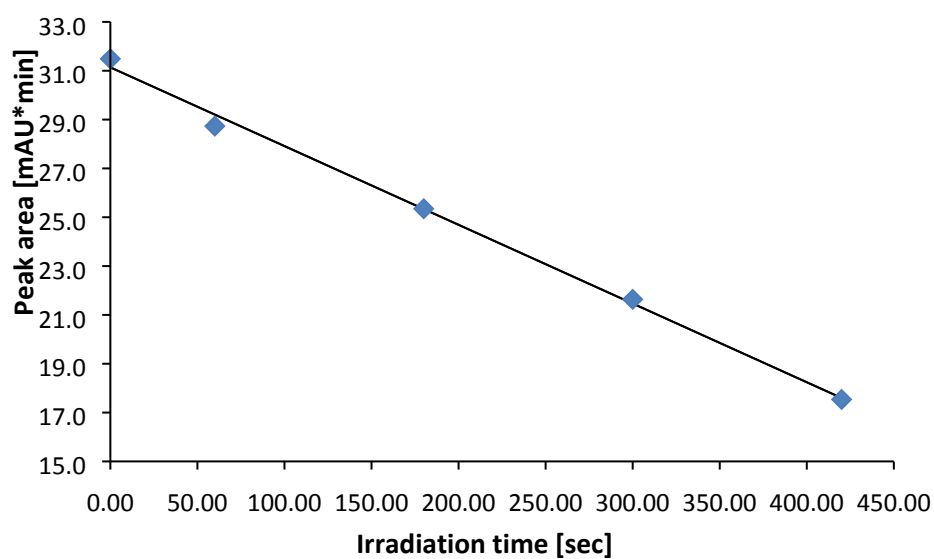


Figure S3. Decay of the total DHPLC signal of substrate (80bp oligonucleotide labeled with BrdC) vs irradiation time.

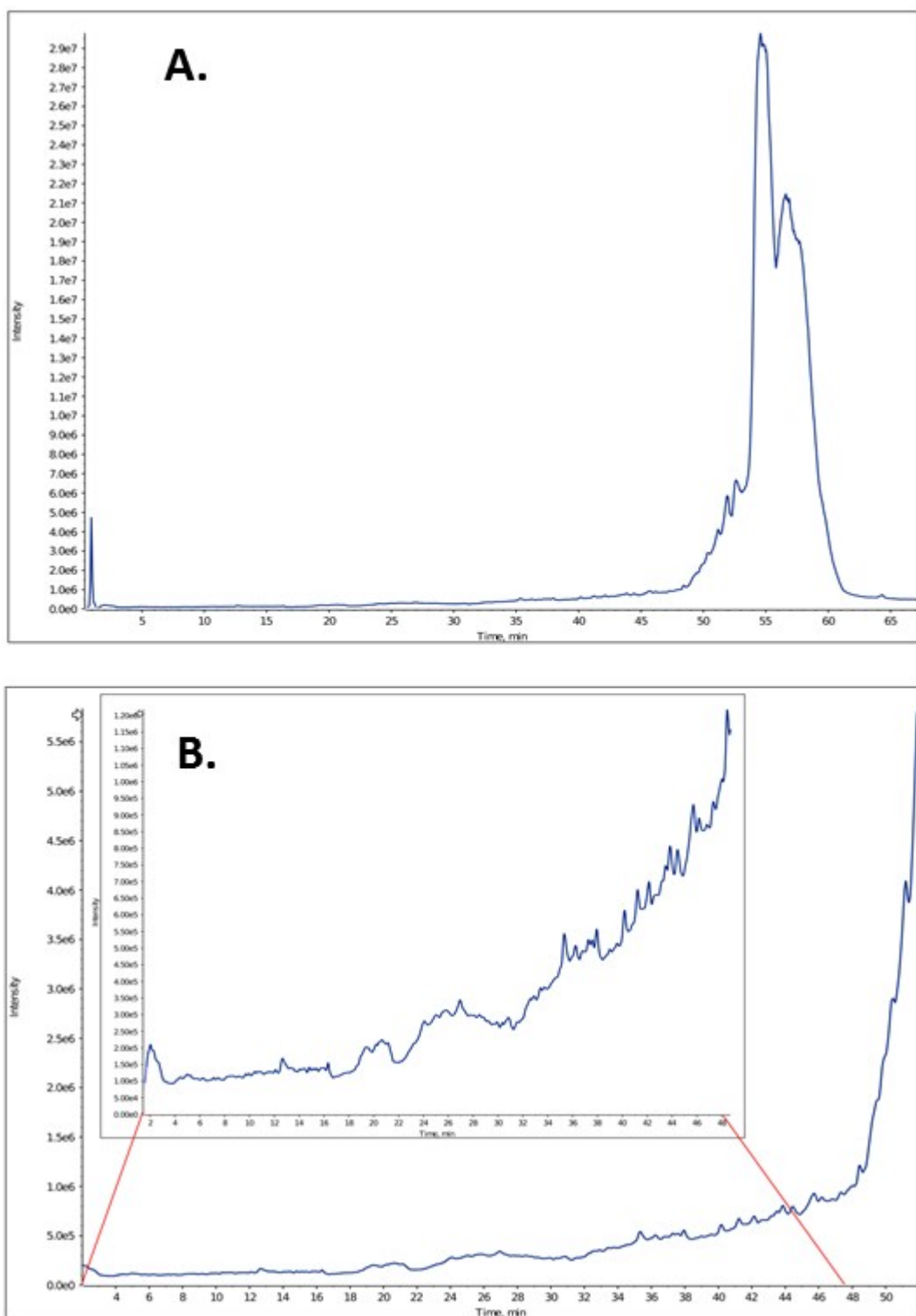


Figure S4. (A) Total ion current (TIC) for the irradiated photolyte and (B) magnification in range 4-50 min.