SUPPORTING INFORMATION

Direct and Topoisomerase II mediated DNA damage by Bis-3-chloropiperidines: the importance of being an earnest G

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Figure S1. Time- and concentration-dependence of the reaction between bis-3-chloropiperidine **1** and oligodeoxynucleotide **ODN1**. The single-stranded construct included a 5'-FAM label to enable visualization. 2 μM aliquots of **ODN1** were treated with either 5 or 50 μM final concentrations of compound **1** at 37 °C in BPE buffer, pH 7.4, and incubated for the time indicated (see ***Experimental*** in the main text). The reaction mixtures were analyzed by denaturing polyacrylamide gel electrophoresis (PAA 20 %, 7M urea, TBE 1X). Arrows indicate the position of cleavage products. **C** (control) indicates an untreated sample of oligodeoxynucleotide **ODN1**.

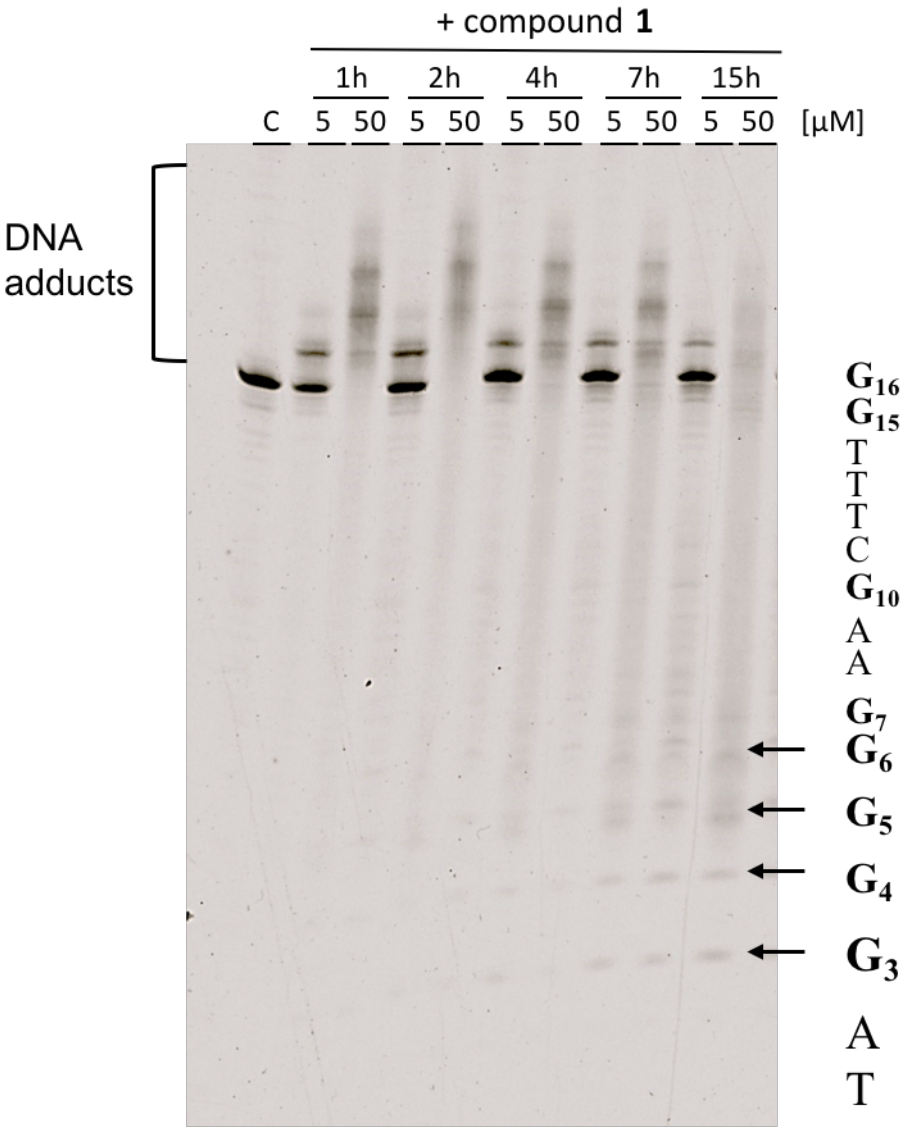
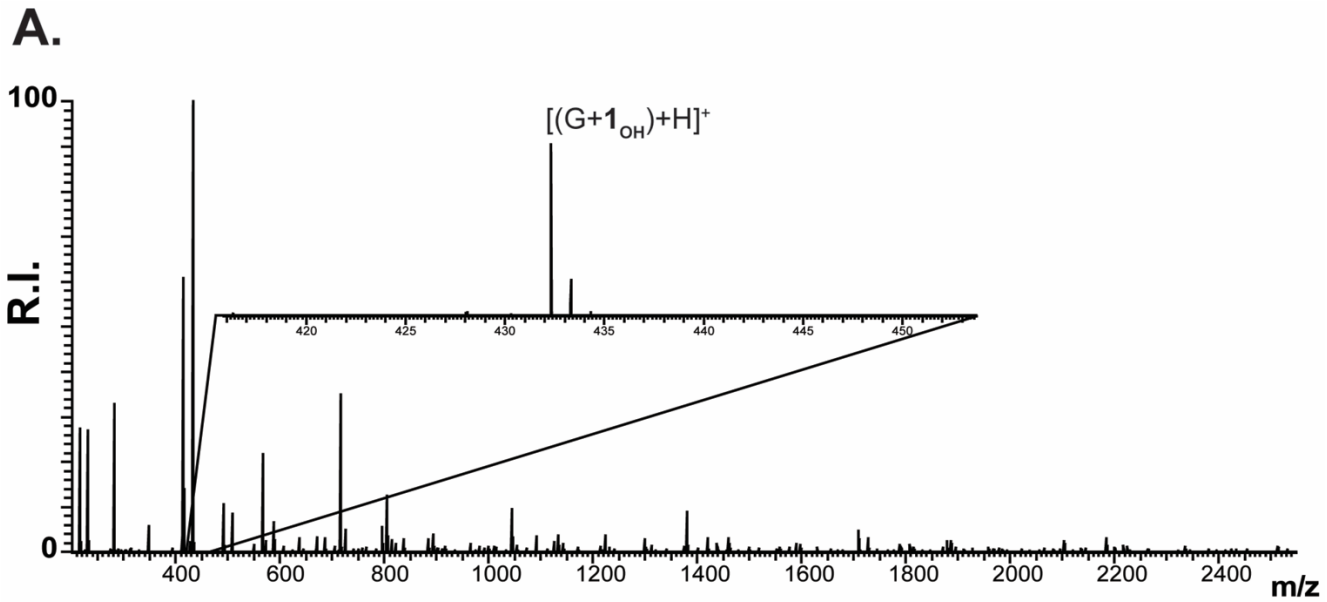


Figure S2. Positive ion mode MS/MS spectra of (**A**) triply charged mono-functional adduct **ODN1+1$_{OH}$** and of (**B**) triply charged bi-functional adduct **ODN1+1$_X$**. The enlarged insets show the detected products corresponding to modified nucleobases.



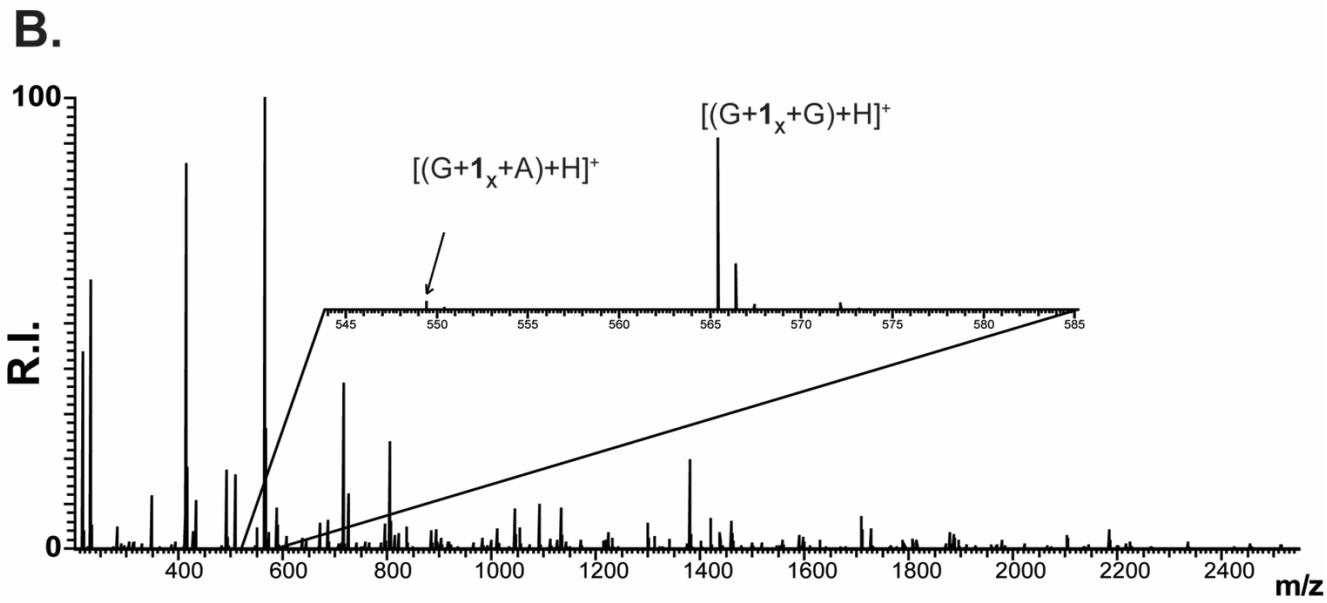


Table S1. Name, description, experimental and calculated mass for species detected in the ESI-MS spectrum of reaction mixture obtained by incubating 2 μM of oligodeoxynucleotide **ODN1** with 50 μM of bis-3-chloropiperidine **1** at 37 °C for 2 h. Monoisotopic masses are reported in mass units. The nomenclature $\mathbf{1_{OH}}$ corresponds to the mono-alkylation adduct; $\mathbf{1_X}$ crosslinking adduct; ▽ base hydrolysis (modified G formally replaced by OH); ⊓ base elimination (elimination of alkylated G nucleobase).

Name	Description	Experimental mass (u)	Calculated mass (u)
ODN1(▽;⊓;⊓)	Loss of 3 G nucleobases (1 base hydrolysis, 2 base elimination) from **ODN1**	4579.72	4579.72
ODN1(▽;▽;⊓)	Loss of 3 G nucleobases (2 base hydrolysis, 1 base elimination) from **ODN1**	4597.73	4597.73
ODN1(⊓;⊓)	Loss of 2 G nucleobases (2 base elimination) from **ODN1**	4712.76	4712.76
ODN1(▽;⊓)	Loss of 2 G nucleobases (1 base hydrolysis; 1 base elimination) from **ODN1**	4730.77	4730.77
ODN1(▽;▽)	Loss of 2 G nucleobases (2 base hydrolysis) from **ODN1**	4748.78	4748.78
ODN1(▽;⊓)+$\mathbf{1_X}$	Loss of 2 G nucleobases (1 base hydrolysis; 1 base elimination) from crosslinked **ODN1**	4993.01	4993.01
ODN1(▽;⊓)+$\mathbf{1_{OH}}$	Loss of 2 G nucleobases (1 base hydrolysis; 1 base elimination) from mono-alkylated **ODN1**	5011.02	5011.01
ODN1(⊓)+$\mathbf{1_X}$	Loss of 1 G nucleobase (1 base elimination) from crosslinked **ODN1**	5126.05	5126.05
ODN1(▽)+$\mathbf{1_X}$	Loss of 1 G nucleobase (1 base hydrolysis) from crosslinked **ODN1**	5144.06	5144.06
ODN1(▽)+$\mathbf{1_{OH}}$	Loss of 1 G nucleobase (1 base hydrolysis) from mono-alkylated **ODN1**	5162.07	5162.06
ODN1+$\mathbf{1_X}$	crosslinking adduct of **1** on **ODN1**	5277.10	5277.10
ODN1+$\mathbf{1_{OH}}$	mono-alkylation adduct of **1** on **ODN1**	5295.10	5295.11
ODN1(▽)+2($\mathbf{1_X}$)	Loss of 1 G nucleobase (1 base hydrolysis) from double crosslinked **ODN1**	5406.30	5406.30
ODN1(▽)+$\mathbf{1_X}$+$\mathbf{1_{OH}}$	Loss of 1 G nucleobase (1 base hydrolysis) from mono-alkylated and crosslinked **ODN1**	5424.31	5424.30
ODN1(▽)+2($\mathbf{1_{OH}}$)	Loss of 1 G nucleobase (1 base hydrolysis) from double mono-alkylated **ODN1**	5442.32	5442.30
ODN1+2($\mathbf{1_X}$)	2 crosslinking adducts of **1** on **ODN1**	5539.34	5539.34
ODN1+$\mathbf{1_X}$+$\mathbf{1_{OH}}$	Combination of one crosslinking adduct and one mono-alkylation adduct of **1** on **ODN1**	5557.35	5557.34
ODN1+2($\mathbf{1_{OH}}$)	2 mono-alkylation adducts of **1** on **ODN1**	5575.36	5575.34

Figure S3. ESI-MS spectrum of reaction mixture obtained by incubating 2 μM of oligodeoxynucleotide **ODN1** with 50 μM of bis-3-chloropiperidine **1** at 37 °C for 15 h. The spectrum shows the time-dependent reactions induced by compound **1** on **ODN1**. In the spectrum are labelled the 4- charged depurination products from the initial **ODN1** and the triply charged species corresponding to loss of TAG, TAGG, TAGGG, etc. from the initial **ODN1**. Spectra were recorded in 150 mM ammonium acetate (see **Experimental Section** in the main text for conditions). Lower intensity signals near free/bound species consist of typical sodium and ammonium adducts. To facilitate the interpretation, we included in the spectrum the graphical representation of identified reaction products (see inset for symbols legend). The ⎯ corresponds to the unmodified oligodeoxynucleotide **ODN1**; ⎾⏌ base hydrolysis (modified G formally replaced by OH); * fragment of **ODN1**.

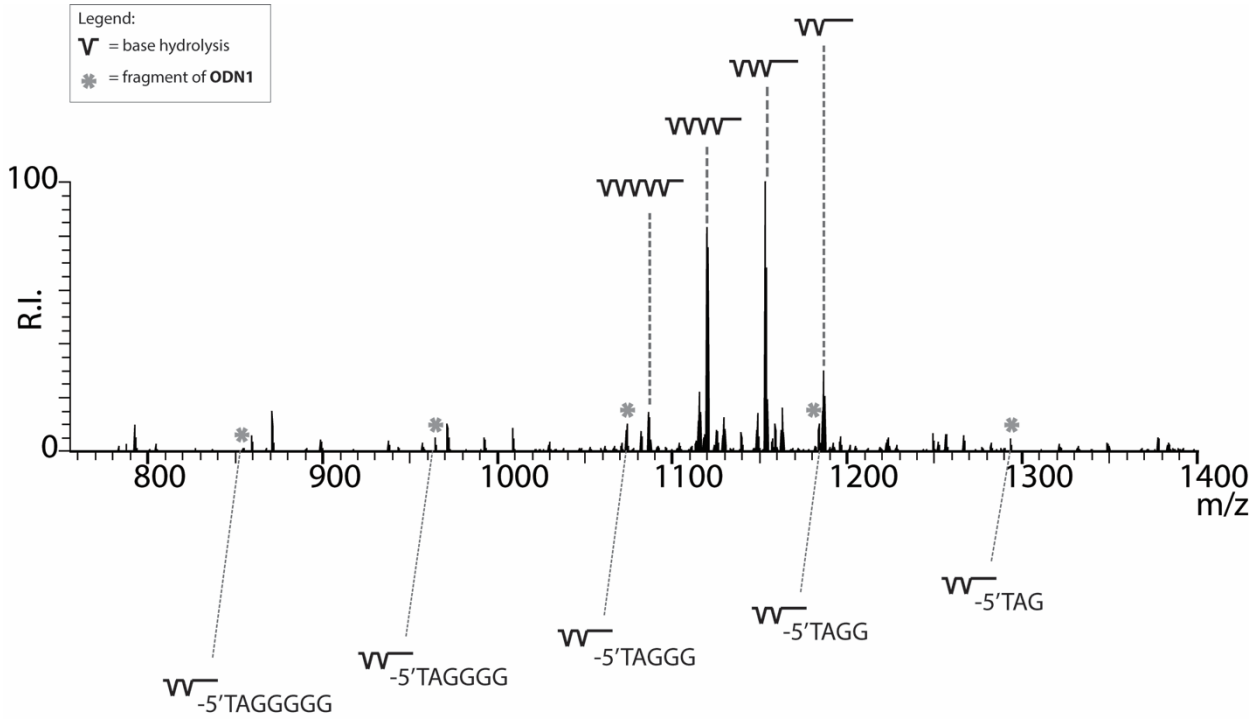


Table S2. Name, description, experimental and calculated mass for species detected in the ESI-MS spectrum obtained by incubating 2 μM of double-stranded oligodeoxynucleotide **dsDNA** with 5 μM of bis-3-chloropiperidine **1** at 37 °C for 2 h. Monoisotopic masses are reported in mass units. The nomenclature $\mathbf{1_{OH}}$ corresponds to the mono-alkylation adduct; $\mathbf{1_X}$ crosslinking adduct.

Name	Description	Experimental mass (u)	Calculated mass (u)
ODN1	single-stranded oligodeoxynucleotide **ODN1**	5014.86	5014.86
ODN1+$\mathbf{1_X}$	crosslinking adduct of **1** on **ODN1**	5277.10	5277.10
ODN1+$\mathbf{1_{OH}}$	mono-alkylation adduct of **1** on **ODN1**	5295.11	5295.11
dsDNA	double-stranded oligodeoxynucleotide **dsDNA**	10759.80	10759.80
dsDNA+$\mathbf{1_X}$	crosslinking adduct of **1** on **dsDNA**	11022.03	11022.05
dsDNA+$\mathbf{1_{OH}}$	mono-alkylation adduct of **1** on **dsDNA**	11040.04	11040.05
ODN2	single-stranded oligodeoxynucleotide **ODN2**	5744.94	5744.95

Figure S4. ESI-MS spectrum of a 2 μM sample of the initial double-stranded oligodeoxynucleotide **dsDNA** obtained by annealing **ODN1** with its complementary oligodeoxynucleotide **ODN2**.

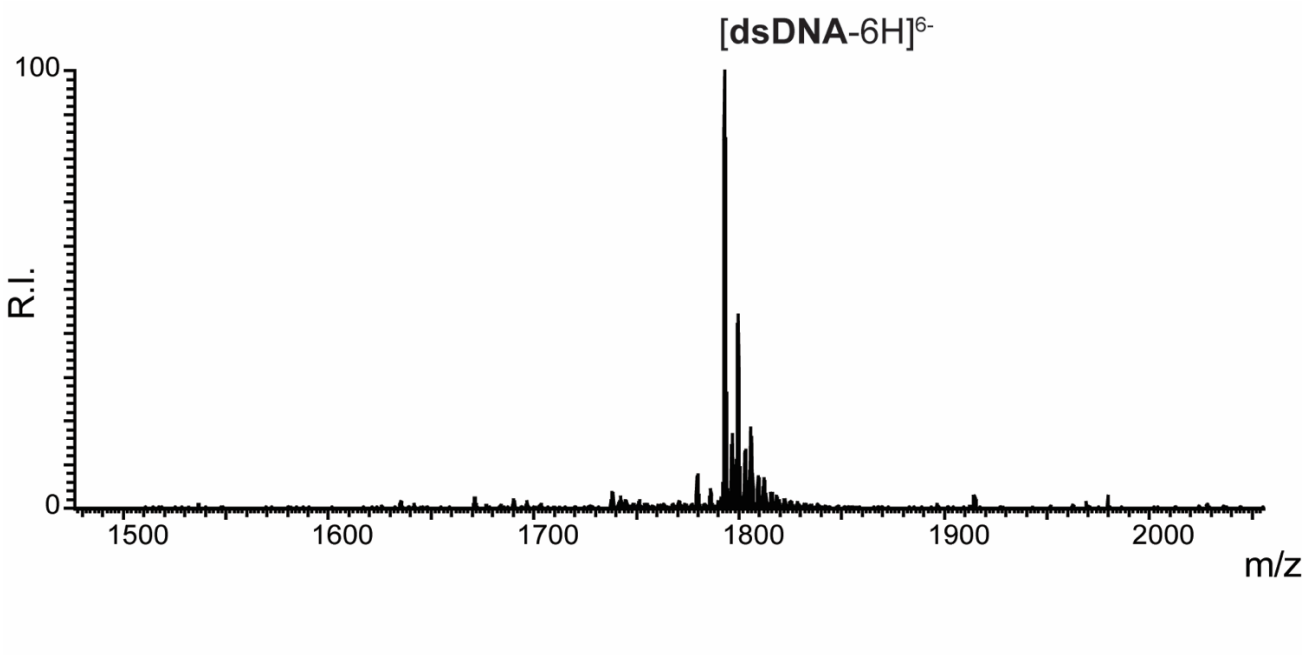


Figure S5. ESI-MS spectrum of reaction mixture obtained by incubating 2 μM of double-stranded oligodeoxynucleotide **dsDNA** with 50 μM of bis-3-chloropiperidine **1** at 37 °C for 2 h. The spectrum shows the concentration-dependent reactions induced by compound **1** on **dsDNA**. Spectra were recorded in 150 mM ammonium acetate (see **Experimental Section** in the main text for conditions). Lower intensity signals near free/bound species consist of typical sodium and ammonium adducts. To facilitate the interpretation, we included in the spectrum the graphical representation of identified reaction products (see inset for symbols legend). The ▬ corresponds to the unmodified single-stranded oligodeoxynucleotide **ODN1**; ▬ single-stranded oligodeoxynucleotide **ODN2**; ═ double-stranded oligodeoxynucleotide **dsDNA**; ⌐ mono-alkylation adduct in which the remaining 3-chloropiperidine function was hydrolyzed to a 3-hydroxyl; ⊓ crosslinking adduct; ▽ base hydrolysis (modified G formally replaced by OH).

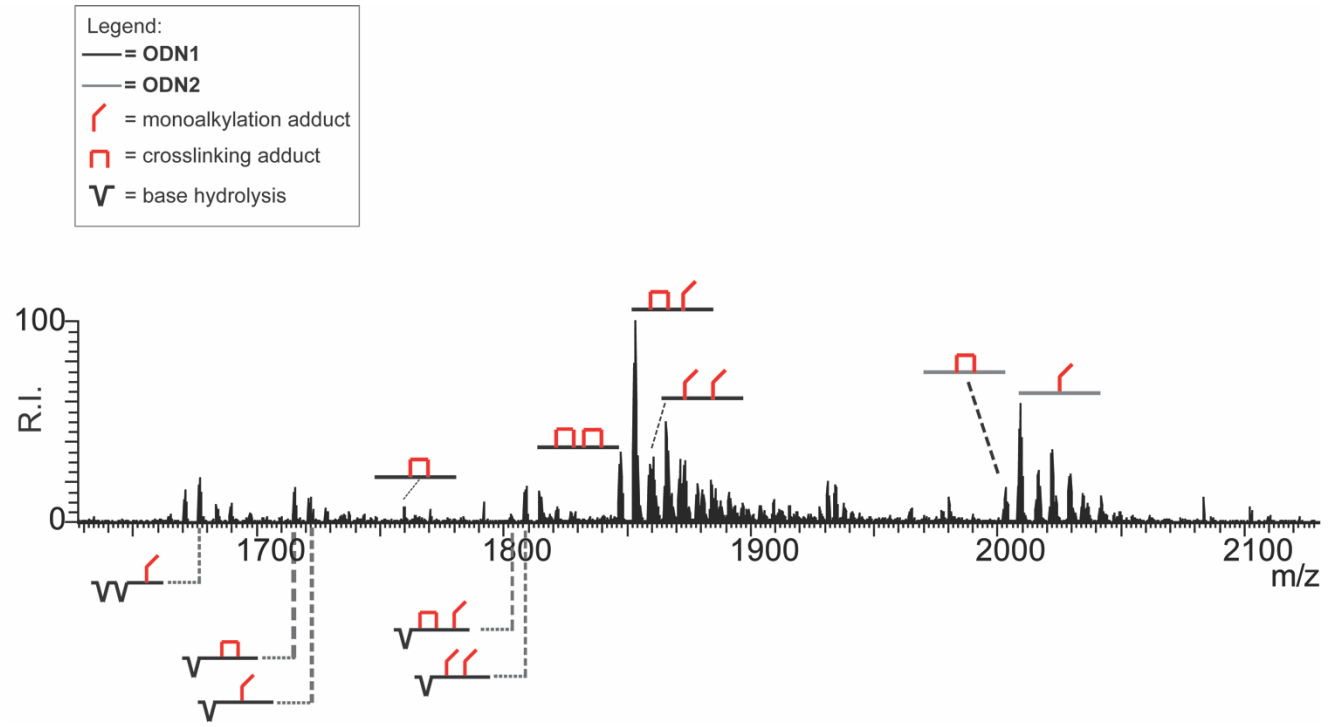


Table S3. Name, description, experimental and calculated mass for species detected in the ESI-MS spectrum obtained by incubating 2 μM of double-stranded oligodeoxynucleotide **dsDNA** with 50 μM of bis-3-chloropiperidine **1** at 37 °C for 2 h. Monoisotopic masses are reported in mass units. The nomenclature $\mathbf{1_{OH}}$ corresponds to the mono-alkylation adduct; $\mathbf{1_X}$ crosslinking adduct; ▽ base hydrolysis (modified G formally replaced by OH); ▽ base elimination (elimination of alkylated G nucleobase).

Name	Description	Experimental mass (u)	Calculated mass (u)
ODN1(▽;▽;▽)	Loss of 3 G nucleobases (2 base hydrolysis, 1 base elimination) from **ODN1**	4597.73	4597.73
ODN1(▽;▽)+$\mathbf{1_{OH}}$	Loss of 2 G nucleobases (1 base hydrolysis, 1 base elimination) from mono-alkylated **ODN1**	5011.02	5011.01
ODN1(▽)+$\mathbf{1_X}$	Loss of G nucleobase (1 base hydrolysis) from crosslinked **ODN1**	5144.06	5144.06
ODN1(▽)+$\mathbf{1_{OH}}$	Loss of G nucleobase (1 base hydrolysis) from mono-alkylated **ODN1**	5162.07	5162.06
ODN1+$\mathbf{1_X}$	crosslinking adduct of **1** on **ODN1**	5277.10	5277.10
ODN1(▽)+$\mathbf{1_X}$+$\mathbf{1_{OH}}$	Loss of G nucleobase (1 base hydrolysis) from mono-alkylated and crosslinked **ODN1**	5424.31	5424.30
ODN1(▽)+2($\mathbf{1_{OH}}$)	Loss of G nucleobase (1 base hydrolysis) from double mono-alkylated **ODN1**	5442.32	5442.30
ODN1+2($\mathbf{1_X}$)	2 crosslinking adducts of **1** on **ODN1**	5539.34	5539.34
ODN1+$\mathbf{1_X}$+$\mathbf{1_{OH}}$	Combination of one crosslinking adduct and one mono-alkylation adduct of **1** on **ODN1**	5557.35	5557.34
ODN1+2($\mathbf{1_{OH}}$)	2 mono-alkylation adducts of **1** on **ODN1**	5575.36	5575.34
ODN2+$\mathbf{1_X}$	crosslinking adduct of **1** on **ODN2**	6007.18	6007.19
ODN2+$\mathbf{1_{OH}}$	mono-alkylation adduct of **1** on **ODN2**	6025.19	6025.19