Supporting Information

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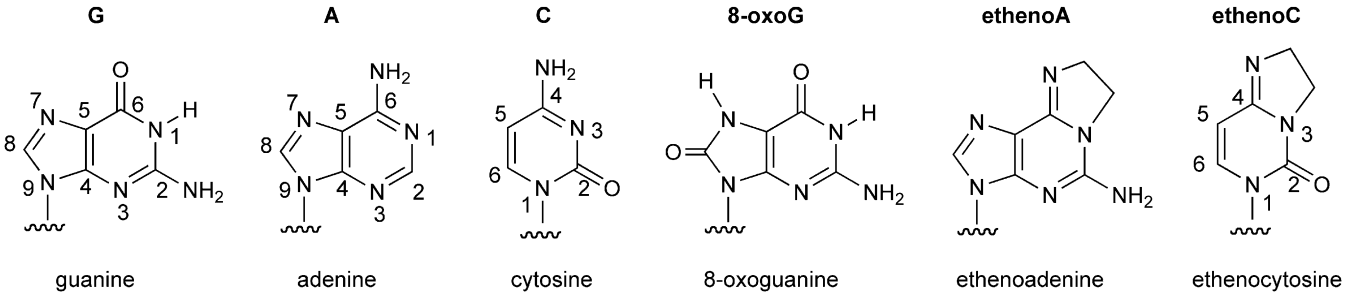

Fig. S1. Structures of selected natural and damaged bases in DNA.

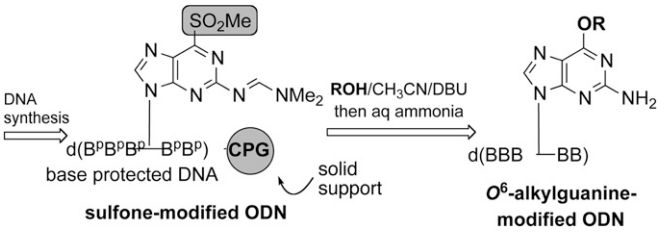

Fig. S2. Post-DNA synthesis modification of oligodeoxyribonucleotides (ODNs) to incorporate O^6-alkylguanine analogs [replacement of alcohol (ROH) with an amine gives a 2,6-diaminopurine analog].

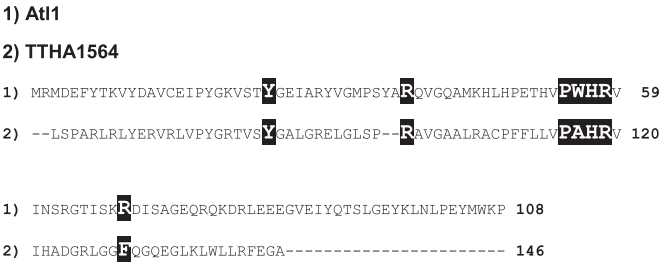

Fig. S3. Sequence alignment of alkyltransferase-like (ATL) protein homologs Atl1 from *Schizosaccharomyces pombe* and TTHA1564 from *Thermus thermophilus* generated using Sequoia (1). The highlighted amino acids correspond to the key residues discussed in the main text.

1. Bruns CM, Hubatsch I, Ridderström M, Mannervik B, Tainer JA (1999) Human glutathione transferase A4-4 crystal structures and mutagenesis reveal the basis of high catalytic efficiency with toxic lipid peroxidation products. *J Mol Biol* 288(3):427–439.

Table S1. Binding data for complexes of ATL proteins with SIMA(HEX)-d(GCCATGXCTAGTA) determined using fluorescence emission intensity measurements

	Atl1		
Modified base (X)	K_D (nM)	ΔΔG (kJ/mol)	TTHA1564 K_D (nM)
O^6-benzylguanine (BnG)	0.3 ± 0.01	−19.4	1.6 ± 0.15
O^6-propylguanine (PrG)	0.4 ± 0.02	−18.7	1.8 ± 0.14
O^6-methyladamantylguanine (MAG)	0.6 ± 0.03	−17.9	1.9 ± 0.18
O^6-hydroxyethylguanine (HOEtG)	0.7 ± 0.04	−17.2	1.9 ± 0.09
O^6-aminoethylguanine (AEG)	1.0 ± 0.02	−16.4	3.6 ± 0.13
O^6-ethylguanine (EtG)	1.1 ± 0.12	−16.2	1.7 ± 0.17
O^6-pyridyloxobutylguanine (PobG)	1.1 ± 0.02	−16.1	1.8 ± 0.10
N^6-hydroxypropyl-2,6-diaminopurine (HOPr-DAP)	1.1 ± 0.14	−16.1	3.0 ± 0.22
O^6-carboxymethylguanine (CMG)	2.2 ± 0.06	−14.4	3.6 ± 0.03
O^6-methylguanine (MeG)	2.4 ± 0.12	−14.2	1.2 ± 0.13
2,6-diaminopurine (DAP)	4.9 ± 0.25	−12.5	8.4 ± 0.65
2-aminopurine (2-AP)	9.3 ± 0.09	−10.9	7.5 ± 0.46
O^6-methylhypoxanthine (MeHx)	370 ± 13	−1.7	21 ± 2.2
Guanine (G)	740 ± 91	0	103 ± 2.0

K_D values (nM) determined in triplicate ± SEM. All binding data were determined at 298K. ΔΔG values were calculated using the following equation: $\Delta\Delta G = -RT\ln(K_D \text{ (guanine)}/K_D \text{ (purine analog)}$. Structures of all analogs are shown in Fig. 1*A*.

Table S2. Crystallographic diffraction data collection and refinement statistics

Parameter	Atl1-DAP	Atl1-2-AP
Data collection		
Space group	$P6_122$	$P6_122$
Cell dimensions		
a, *b*, *c* (Å)	59.50, 59.50, 237.35	59.98, 59.98, 235.49
α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Resolution (Å)	50.0–2.7 (2.8–2.7)*	50.0–2.85 (2.95–2.85)
R_{sym} or R_{merge}	5.6 (40.5)	4.8 (36.9)
$I/\sigma I$	74.4 (9.6)	83.6 (12.2)
Completeness (%)	99.9 (100.0)	99.8 (100.0)
Redundancy	26.2 (27.6)	19.9 (20.9)
Protein Data Bank code	4HDV	4HDU
Refinement		
Resolution (Å)	43.2–2.7	34.9–2.85
No. reflections	7353	6352
R_{work}/R_{free}	21.1/26.6	20.5/27.6
No. atoms		
Protein	897	900
Ligand/ion	527	526
Water	1	0
B-factors		
Protein	51.3	66.5
Ligand/ion	56.4	67.8
Water	35.3	N/A
rmsds		
Bond lengths (Å)	0.012	0.012
Bond angles (°)	1.294	1.346

Data were collected using a single crystal.
*Values in parentheses are for highest-resolution shell.

Table S3. K_D values for complexes of Atl1 wild-type and mutant proteins with SIMA(HEX)-d(GCCATGXCTAGTA) determined using fluorescence emission intensity measurements

Modified base (X)	Atl1 wild type K_D (nM)	Atl1 R69F K_D (nM)	Atl1 R69A K_D (nM)
O^6-benzylguanine (BnG)	0.3 ± 0.01	1.6 ± 0.12	14 ± 2.3
O^6-methyladamantylguanine (MAG)	0.6 ± 0.03	2.3 ± 0.10	16 ± 2.8
O^6-methylguanine (MeG)	2.4 ± 0.12	35 ± 6	96 ± 1
2,6-diaminopurine (DAP)	4.9 ± 0.25	79 ± 15	240 ± 5
2-aminopurine (2-AP)	9.3 ± 0.09	196 ± 3	560 ± 47
Guanine (G)	740 ± 91	755 ± 12	135 ± 16

K_D values (nM) determined in triplicate ± SEM. All binding data were determined at 298K. Structures of all analogs are shown in Fig. 1*A*.

Table S4. Calculated and observed mass ions for the modified ODNs SIMA(HEX)-d(GCCATGXCTAGTA) used in this study determined using ESI-mass spectrometry

O^6-modification/purine	Calculated mass (Da)	Determined mass (Da)
None (G)	4,733	4,733
Methyl	4,747	4,748
Ethyl	4,761	4,761
Propyl	4,775	4,775
Benzyl	4,823	4,823
Hydroxyethyl	4,777	4,777
N^6-Hydroxypropyl-DAP	4,790	4,790
Pyridyloxobutyl	4,880	4,880
Carboxymethyl	4,791	4,791
Aminoethyl	4,776	4,776
Methyladamantyl	4,881	4,881
DAP	4,732	4,732

Table S5. List of *S. pombe* strains used in study

Name	Genotypes
GM1	*h+ leu1-32 ura4-D18 his7-366 ade6-M210*
GM3	*h+ leu1-32 ura4-D18 his7-366 ade6-M210 atl1Δ::ura4*
GM177	*h+ leu1-32 ura4-D18 his7-366 ade6-M210 atl1-R69A*
GM178	*h+ leu1-32 ura4-D18 his7-366 ade6-M210 atl1-R69F*