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Synthetic Access to the Chemical Diversity of DNA and RNA 5′-Aldehyde Lesions

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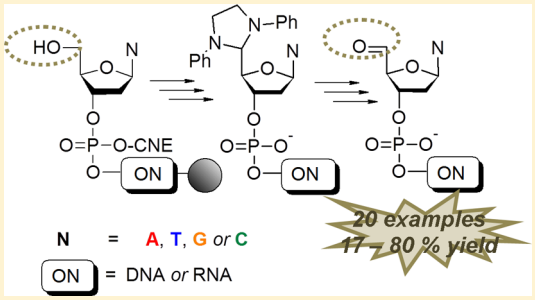
Supporting Information

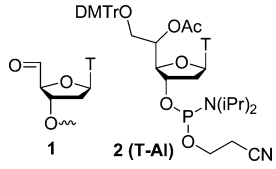
ABSTRACT: Hydrogen atom abstraction from the C5′-position of nucleotides in DNA results in direct strand scission by generating alkali-labile fragments from the oxidized nucleotide. The major damage consists in a terminus containing a 5′-aldehyde as part of an otherwise undamaged nucleotide. Moreover it is considered as a polymorphic DNA strand break lesion since it can be borne by any of the four nucleosides encountered in DNA. Here we propose an expeditious synthesis of oligonucleotides (ON) ending with this 5′-aldehyde group (5′-AODN). This straightforward and cheap strategy relies on Pfitzner−Moffatt oxidation performed on solid support followed by a transient protection of the resulting aldehyde function. This method is irrespective of the 5′-terminal nucleobase and most interestingly can be directly extended to RNA to produce the corresponding 5′-AORN. We also report preliminary results on recognition of 5′-AODN by base excision repair (BER) enzymes.



INTRODUCTION

DNA is continuously exposed to various damaging conditions (oxidizing agents, ionizing radiations or endogenous species) that produce strand breaks, cross-links or damaged nucleotides through radical abstraction of hydrogen atoms.[1] DNA strand breaks terminated by a 5′-aldehyde **1** (hereafter called 5′-AODN, cf. Scheme 1) is one common 5′-end damage produced from either 3′ (internucleotidic) or 5′ hydrogen abstraction.[2]

Scheme 1. Structure of the 5′-Aldehyde 1 and of T-Al Phosphoramidite 2[9]



Generation of a 5′-aldehyde strand break was obtained by many molecular systems, such as enediyne,[3,4] porphyrinic rings[5] or copper complexes.[6] Analytical methods were also developed in order to quantify this lesion.[7] This strand break, which retains a nucleotide fragment terminated by a non-natural 5′-aldehyde extremity is most likely cytotoxic for cells if not removed by DNA repair process.[8]

A precise study of the biochemistry of this lesion requires the development of a versatile synthetic route giving access to DNA fragments and taking into account its polymorphism since eight different nucleotides are expected (A, T, G, C and their RNA counterparts).

The first method was based on reaction of a self-complementary duplex ODN with an enediyne drug such as neocarzinostatin under aerobic conditions, followed by extensive RP-HPLC purification. The yields were generally low and highly sequence-dependent.[4]

More recently, Greenberg et al. described their synthesis by automated incorporation of the thymidine phosphoramidite **2** (**T-Al**) at the 5′ end of an ODN (Scheme 1).[9] After complete deprotection of the modified ODN, the aldehyde function was released upon oxidation by sodium periodate. However, this approach suffered from three drawbacks: (i) synthesis from thymidine requires 10 steps with an overall 24% yield,[9,10] (ii) the synthesis of the other phosphoramidites (A, C and G derivatives) is not described and would require supplementary protection steps, (iii) the synthesis of 3′-unmodified RNA fragments ending with a 5′-aldehyde (5′-AORN) is not possible, since the final treatment with periodate ring-opens the ribose moiety. As a consequence, the precise investigation of the biochemistry of this lesion still requires the design of a versatile and user-friendly synthetic pathway giving access to its polymorphism.

In previous studies Greenberg et al. have shown that the lesion formed from oxidation of thymidine is not a substrate for nucleotide excision repair pathway but that its excision can be achieved by strand displacement synthesis by DNA polymerase β (Pol β) in the presence or absence of flap endonuclease 1 (FEN1).[8] In the present study we decided to test a collection of enzymes representative of the base excision repair (BER) pathway. Because of the fact that the 5′-aldehyde lesion is produced opposite its cognate nucleotide, it is not expected to be a specific substrate for a DNA glycosylase, the first step in

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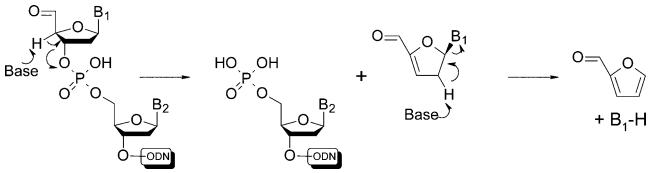
BER. Nevertheless, because of the propensity of the 4′ hydrogen to be abstracted through β-elimination reaction (Scheme 2), we considered judicious to test a series of mono- and bifunctional glycosylases.

Scheme 2. β- and γ-Elimination Pathways from 5′-AODN



Both types of enzymes use amino acid residues as nucleophiles to eliminate abnormal nucleobases leaving a 2′-deoxyribose residue (abasic or AP site). Furthermore, bifunctional glycosylases perform a β-elimination reaction on 2′ position of deoxyribose following the modified nucleobase removal. Even if it is not very likely that the 5′-aldehyde lesion is a specific substrate for these enzymes, we cannot rule out a favorable interaction leading to a significant β-elimination reaction producing a 5′-phosphate end with the loss of a nucleoside. The bifunctional glycosylases considered were hOGG1 (human 7,8-dihydro-8-oxoguanine-DNA glycosylase), Nth (*Escherichia coli* endonuclease III), Fpg (*E. coli* formamidopyrimidine-DNA glycosylase) and the monofunctional glycosylases are MUG (*E. coli* mismatch specific uracil-DNA glycosylase), TagI (*E. coli* 3-methyladenine-DNA-glycosylase I) and ANPG 40 and 60 (truncated human alkylpurine-DNA N-glycosylase).

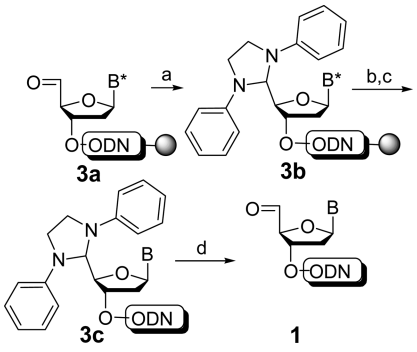
RESULTS AND DISCUSSION

Synthesis of 5′AODN. We previously developed a method to master the oxidation of the 5′- primary alcohol of a controlled-pore glass (CPG) linked ODN just prior to the final alkaline deprotection step.[11] Under mild on-support Pfitzner–Moffatt conditions, roughly 70–90% of the primary alcohol are converted into aldehyde, whatever the nature of the last nucleobase. Such modified ODNs are known to be quantitatively converted to truncated 5′-phosphorylated ODNs through β-elimination during the final alkaline deprotection step (Scheme 2). Nevertheless, we believed that a suitable protection of the aldehyde prior to the final deprotection step could give an easy access to 5′-AODN *in solution*.

Ideally, CPG-linked 5′-AODN **3a** (Scheme 3) have to be suitably derivatized to resist the strong alkaline deprotection conditions and then be easily separated from unreacted and truncated ODNs. Moreover, the 5′-aldehyde group has to be rapidly recovered by a simple treatment. Keeping these criteria in mind, we retained the *N*,*N*′-diphenylimidazoline (DPI) group as an ideal candidate for transient protection of the aldehyde function in the CPG-linked 5′-AODN (Scheme 3) . DPI group is known to withstand ammonia deprotection and is easily removed by mild acidic treatment.[12,13]

Model ODNs were treated by *N*,*N*′-diphenylethylenediamine in the presence of PTSA (*para*-toluene sulfonic acid, method A) or DDQ (2,3-dichloro-5,6-dicyano-*para*-benzoquinone, method B).[14] The length of the ODNs and the nature of the final nucleobase were both screened. Whatever the reaction conditions, good overall reaction yields (comprising automated synthesis, 5′-oxidation and protection by DPI) were obtained as demonstrated by RP-HPLC analysis (see Table 1).

Scheme 3. General Synthetic Pathway from CPG-Linked 5′-AODN to Deprotected 5′-AODN[a]



[a]Structures of CPG-linked 5′-AODN **3a**, of CPG-linked DPI-protected 5′-AODN **3b**, of DPI-protected 5′-AODN **3c** and of 5′-AODN **1**. B* = ^{Bz}A, T, ^{iBu}G or ^{Ac}C; B = A, T, G or C; gray ball stands for the CPG support. (a) *N*,*N*′-diphenylethylenediamine, PTSA or DDQ (see text); (b) 40% aq. methylamine 65 °C, 10 min; (c) RP-HPLC purification; (d) AcOH 80%, 5 min.

Table 1. Protection of a Model CPG-Linked 5′-AODN by *N*,*N*′-Diphenylethylenediamine Following Method A (PTSA Activation) or Method B (DDQ Activation)

	overall reaction yields (%)			
	method A		method B	
model CPG-linked 5′-AODN ($^{5'}$NT$_n^{3'}$)	n = 4	n = 11	n = 4	n = 11
N = A	65	63	74	65
N = T	66	57	80	69
N = G	67	71	71	50
N = C	73	73	77	68

The strong liphophilicity of the DPI group enabled RP-HPLC purification: the DPI-protected 5′-AODN was easily separated from failure sequences, unreacted 5′-AODN, β-elimination product and methylthiomethyl adduct[11] (see Figure 1). DPI-protected 5′-AODN was then deprotected by treatment with 80% aqueous AcOH, which is commonly used in ODN chemistry. Full deprotection was achieved in less than 5 min at rt. Altogether these elements show that 5′-AODN can be synthesized and purified as routinely as nonmodified ODNs, following procedures similar to the well-known "DMT-On" and "DMT-Off" strategies.

We next extended our reaction conditions to various ODNs sequences. Whatever their length, ODNs were efficiently converted into 5′-AODNs. Slightly lower yields are observed for longer sequences, most likely due to overall elongation chain yield (see Table 2).

Notably, the reaction properly worked whatever the nature of the 5′-nucleobase (entries 2–5), although for a given length slightly better yields were obtained with pyrimidines (entries 2 and 4 vs 3 and 5).

We further extended our method to oligoribonucleotides (ORNs) oxidation. To the best of our knowledge, this is the first method allowing the chemical modification of the 5′ end of an ORN without using specifically designed phosphoramidite synthons.

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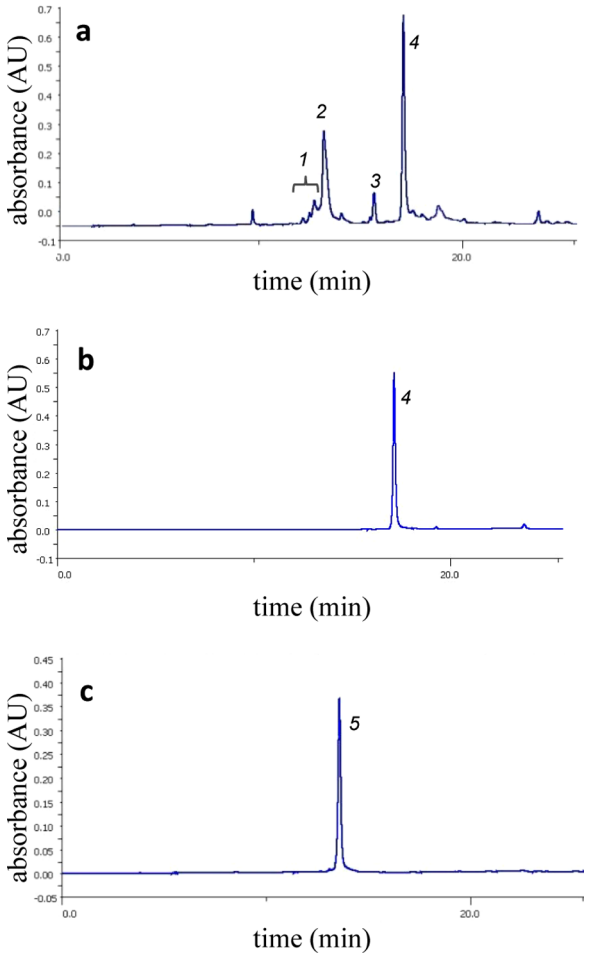

Figure 1. RP-HPLC profiles of 5′-AODN **13**. (a) Protected as DPI derivative (crude deprotection mixture); (b) protected as DPI derivative (after RP-HPLC purification); (c) unprotected (after 5 min 80% AcOH treatment). Peak 1: failure sequence and 5′-unoxidized ODN (natural ODN); peak 2: β-elimination product; peak 3: methylthiomethyl adduct;[11] peak 4: DPI-protected 5′-AODN and peak 5:5′-AODN **13**.

CPG linked 5′-AORN were synthesized according to our previously described method.[11] Unfortunately, when *N,N′*-diphenylethylenediamine was used as protecting group, no DPI-protected 5′-AORN was recovered. We suspected that the conditions classically used for deprotecting the 2′-OtBDMS group of tritylated RNA (i.e., TEA·3HF in DMSO/TEA 2:1 at 65 °C, 60 min; called method C) were still too harsh for the DPI group. Using milder conditions[16] (NH_4F in a DMSO/water/CH_3NH_2 mixture at pH = 8.5; method D), 21% of the model DPI-protected 5′-AORN U_8 were recovered (see Table 3).

Table 3. Protection Reaction Yields of CPG-Linked 5′-AODN and 5′-AORN by *N,N′*-Diphenylethylenediamine Derivatives Following Method A

	5′- DPI-protected AODN[a]		5′- DPI-protected AORN[b] (yield[c])	
	yield[c] (%)	t_R (min)	method C	method D
N,N′-diphenyl ethylenediamine	65	15.1	traces	21
15 (4-Br)	86	16.5	34	58
16 (4-CN)	42	14.6	28	29
17 (3,5-bis Cl)	66	17.1	34	57

[a]Sequence of the model CPG-linked 5′-AODN was AT_4. [b]Sequence of the model CPG-linked 5′-AORN was U_8. [c]Overall reaction yields (ODN chain elongation, 5′-alcohol oxidation, protection by *N,N′*-diphenylethylenediamine derivatives and deprotection) were given. Estimated by RP-HPLC.

In order to improve the reaction yields, other protecting groups were screened. The DPI acid-sensitivity can be indeed lowered by introducing electron-withdrawing groups on phenyl rings and thus *N,N′*-bis(4-bromophenyl)-ethylenediamine **15**, *N,N′*-bis(4-cyanophenyl)-ethylenediamine **16** and *N,N′*-bis-(3,5-dichlorophenyl)-ethylenediamine **17** were synthesized. First of all, we evaluated their efficiency on a CPG-linked 5′-AODN model (sequence AT_4) and reagents **15**−**17** gave good yields (from 42 to 86%, see Table 3, column 2) and compared favorably with their unsubstituted parent reagent (65% yield). We then used reagents **15**−**17** to protect CPG-linked 5′-AORN (sequence U_8), and the reaction yields were enhanced

Table 2. ESI-MS and HPLC Data of Protected 5′-AODN (4−13) and Derivatized 5′-AODN

		DPI-protected 5′-AODN				5′-AODN		
		MW					MW[c]	
entries	sequence of the 5′-AODN[a]	theor.	exp.	t_R (min)	yield[b] (%)	t_R (min)	theor.	exp.
1	TTG TT (**4**)	1675.5	1675.2	15.40	76	12.55	1636.6	1636.3
2	ATT TTT TTT TTT (**5**)	3787.8	3787.2	16.83	74	13.30	3748.9	3749.1
3	TTT TTT TTT TTT (**6**)	3778.8	3779.3	18.18	80	13.37	3739.9	3740.3
4	GTT TTT TTT TTT (**7**)	3803.8	3803.3	16.85	71	13.27	3764.9	3765.1
5	CTT TTT TTT TTT (**8**)	3763.8	3764.3	17.43	77	13.32	3724.9	3725.3
6	TCA GCT AGA CCA TGC A (**9**)	5040.0	5039.6	17.10	49	13.14	5001.2	5002.3
7	CCA CGC ATC GCT GGT A (**10**)	5032.0	5033.0	17.33	54	13.21	4992.6	4994.1
8	TTC AGA TAC TTA GCA TGG ACA ACA (**11**)	7533.5	7533.7	17.50	49	13.50	7494.6	7497.0
9	TCG ACA TCG ACA TCG CA (**12**)	5329.1	5328.9	17.00	42	13.30	5290.2	5290.2
10	TCC ATA TCC AAT TCA CAT ACT C (**13**)	6762.3	6762.2	17.20	52	13.50	6724.3	6725.0
11	TAG CTT GAC CG (**14**)	3522.8	3523.3	17.51	50	13.40	3483.9	3483.9

[a]For clarity purpose, the 5′-oxidized nucleoside is underlined. [b]Global yields of synthesis are given (ODN elongation, on-support oxidation and protection as DPI derivatives) and were determined by UV monitoring on crude reaction mixture (see SI for consideration on UV absorbance of the DPI group). [c]5′-AODNs were derivatized and analyzed by ESI-MS as *n*-decyloxime ethers.[15]

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even when method C was used for the 2′-OtBDMS groups removal (from 0 to 34%). However, better yields were obtained with deprotection method D (see Table 3). Interestingly, the presence of halogen atoms in **15** and **17** facilitates the purification step by increasing the retention times of the resulting 5′-DPI-protected AODN (Table 3, column 3).

With these conditions in hand, we were able to synthesize and purify 5′-AORN (see Table 4).

Table 4. ESI-MS and HPLC Data of DPI-Protected 5′-AORN (Method A)

		MW			
entries	sequence[a]	theor.[c]	exp.	t_R (min)	yield[b] (%)
1	UUU UUU UU (**18a**)[d]	2736.2	2736.2	21.00	57
2	UUU UUU UU (**18b**)[e]	2718.2	2715.8	21.80	58
3	UGA CGA UCG UUA (**19b**)[e]	4144.5	4145.0	17.90	41
4	AAU CCA AUC UGA UUC CCA AG (**20a**)[d]	6643.8	6644.2	19.56	47
5	AAU CCA AUC UGA UUC CCA AG (**20b**)[e]	6623.8	6624.4	20.72	17
6	UAG CUU GAC CG (**21a**)[d]	3816.4	3815.0	20.52	55
7	GUU UCU UAU GU (**22a**)[d]	3756.3	3755.1	19.42	56
8	GUU UCU UAU GU (**22b**)[e]	3736.4	3735.1	20.72	32

[a]For clarity purpose, the 5′-oxidized nucleoside is underlined. [b]Global yields of synthesis are given (ODN elongation, on-support oxidation and protection as DPI derivatives) and were determined by UV monitoring on crude reaction mixture. [c]Theoretical mass of protected 5′-AORN containing the most abundant combination of isotopes was given. [d]Protected by **15** as 4,4′-dibromo DPI. [e]Protected by **17** as 3,3′,5,5′-tetrachloro DPI.

Both reagents **15** and **17** gave consistently good results, although 4,4′-bis-bromoDPI derivatives seem to slightly better withstand the 2′-OtBDMS deprotection conditions, especially for longer sequences (compare entries 4 and 6 to 5 and 7). Both protecting groups were removed by treating with 80% AcOH during less than 5 min.

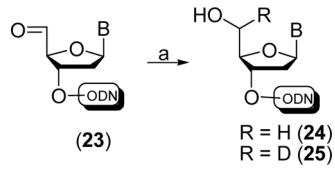
It should be noted that method A (PTSA) was used to achieve the aldehyde protection of the CPG-linked 5′-AORN by substituted DPI **15**–**17**. Little 5′-AORN was recovered when method B (DDQ) was used (<10%).

For both 5′-AODN and 5′-AORN (DPI-protected or not), it should be mentioned that they cannot be stored for long periods, even frozen at −80 °C, and should be used in the days following their synthesis (see Experimental Section for more details).

To further assess the presence of the aldehyde moiety in our synthesized 5′-AODN, the model 5′-AODN **23** (MW = 3267.6 amu) was subjected to $NaBH_4$ and $NaBD_4$ reductions[17] affording products **24** and **25** with respectively +2.1 and +3.0 amu. (Scheme 4).

5′-AODN's are stable in aqueous solutions. Half-life of ca. 100 h was reported by Greenberg for hybridized 5′-AODN in PBS buffer (pH 7.2 at 37 °C).[9] In our hands, nonhybridized 5′-AODNs were also extremely stable whatever the nature of the last nucleobase: only 15% of 5′-AODN **5**–**8** decomposed by β-elimination after 10 days in PBS buffer (pH 8.0 at rt). On the other hand, heating at 60 °C dramatically increased the decomposition rate (half-life 15.1 h, data not shown)

Scheme 4. Reduction of 5′-AODN[a]



[a]ODN sequence was $^{5'}$CGCAGAGACGC$^{3'}$. (a) $NaBR_4$, R_2O.

Action of BER Enzymes. The β-elimination activity of various DNA glycosylases was evaluated on ternary complexes containing a 3′-end labeled 5′-AODN (17 mer, the 3′ end labeling adds an adenosin residue) and a 5′ phosphate ODN (15 mer) hybridized with a 32 mer complementary strand. Despite the fact that it is well-known that MUG, Tag1 and ANPG are not able to cleave an abasic site (2′-deoxyribose or AP site), their effect on the 5′-aldehyde lesion was investigated since this lesion was shown to be very sensitive to β-elimination reaction.[8]

As can be seen in Figure 2, the 5′-aldehyde lesion is resistant to the AP lyase activity of Fpg, Nth and hOGG1 and is not either substrate for the monofunctional glycosylases Mug, Tag1, ANPG 40 and 80.

The absence of cleavage does not exclude the formation of tight complexes, and the intrinsic reactivity of the lesion may provoke a covalent cross-link with the interacting protein. In our case, when the DNA/protein complex is formed, the aldehyde group may react with close amino groups forming unstable Schiff base, which can be chemically reduced. Even in the presence of a large excess (50 mM) of sodium triacetoxyborohydride, no covalent trapping could be detected for the seven tested enzymes (data not shown).

■ CONCLUSION

5′-AODN, as well as the unprecedented 5′-AORN, can now be accessed in two working days irrespective of the nature of the final nucleobase, using cheap and simple procedure. The versatility and the efficiency of this method compares favorably with previously described chemical method, which required long and tedious synthetic work and was limited to the access of 5′-AODN ending with a thymidine. In this paper we show that the 5′-aldehyde lesion is not substrate for several major BER enzymes. The user-friendly method developed here can be a milestone, as it gives an easy access to all possible 5′-aldehyde containing nucleic acids and should help in understanding the enzymatic reparation mechanisms of this lesion.

Works are currently on progress in our lab on the 5′-aldehyde chemistry and on its biochemistry in RNA and DNA (notably in a context of radio-induced clusters of lesions).

■ EXPERIMENTAL SECTION

Oligonucleotides Synthesis and Purification. Oligodeoxyribonucleotides synthesis was performed following the phosphoramidite strategy on 1 μmol scale, by using classical conditions. Briefly, 0.2 M 4,5-dicyanoimidazole in MeCN was used as activator, 0.02 M iodine solution in THF/water/pyridine as oxidizer, acetic anhydride in THF and 16% *N*-methylimidazole in THF as cap A and B reagents, 3% TCA in DCM as deblock reagent and acetonitrile (<30 ppm water) as washing solvent. Preloaded 500 Å CPG (controlled pore-glass support) was used. Oligoribonucleotides were synthesized by using the same protocol except that 0.25 M ETT solution was used as activator, nucleosides were introduced as 2′-tBDMS derivatives, and coupling times were extended from 20 to 300 s.

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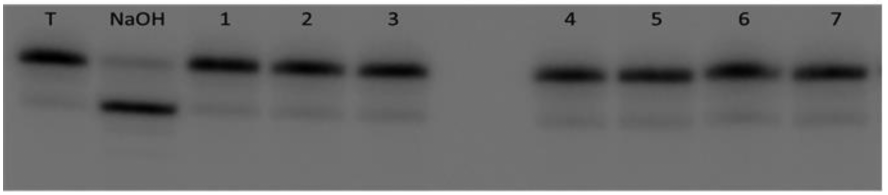

Figure 2. Cleavage assay of 5′ AODN by various BER proteins. 500 nM ternary complex containing a 3′-^{32}P-labeled AODN were incubated with 100 nM DNA repair enzyme at 37 °C for 30 min. Lanes: T, no enzyme; NaOH, treated with NaOH; 1, incubated in the presence of hOGG1; 2, Fpg; 3, Nth; 4, MUG; 5, TagI; 6, ANPG 40; 7, ANPG 80. The products of the reaction were analyzed as described under Experimental Section.

Oxidation of the 5′-Alcohol of CPG-Linked 5′-ODN/ORN to CPG-Linked 5′-AODN/5′-AORN. CPG-linked ODNs were oxidized according to a published protocol.[11] Briefly, CPG-linked ODN were gently stirred in a diisopropylcarbodiimide/dichloroacetic acid/DMSO (19:1.7:200; *v*/*v*) mixture for 30 min at rt.

Protection from CPG-Linked 5′-AODN/5′-AORN to DPI-Protected CPG-Linked 5′-AODN/5′-AORN. Supported 5′-AODN/5′-AORN was protected either by (i) PTSA or (ii) DDQ.

PTSA (Method A). PTSA solution was prepared by dissolving 2 mg *p*-toluenesulfonic acid (10 μmol) in 1 mL of DMF. CPG-linked 5′-AODN (ca. 4 mg, initial loading 30−40 μmol/g) was reacted with 200 μL of a *N*,*N*′-diphenylethylendiamine solution (23 mg; 110 μmol in 1 mL of the aforementioned PTSA in DMF solution, or 32 mg (87 μmol) of **15** or 30.4 mg (87 μmol) of **17**). The suspension was vortexed and centrifuged to ensure a complete wetting of the resin. The mixture was left overnight at rt without stirring. The colorless supernatant was discarded, and the resin was thoroughly washed by MeCN (2 × 1 mL) and by Et_2O (2 × 1 mL). CPG-linked protected 5′-AODN was then air-dried.

DDQ (Method B). CPG-linked 5′-AODN (ca. 4 mg) was reacted with 200 μL of a *N*,*N*′-diphenylethylendiamine solution (23 mg, 110 μmol in 1 mL 96% EtOH solution). Then, 17 μL of a DDQ solution (26 mg, 114 μmol in 1 mL 96% EtOH solution) were added. The initially yellow solution instantaneously turned to dark blue and faded to greenish yellow. The suspension was vortexed and centrifuged to ensure a complete wetting of the resin. The mixture was left overnight at rt without stirring. The dark yellow supernatant was discarded and the resin was thoroughly washed by MeCN until removal of a dark powder formed during the reaction and by Et_2O (2 × 1 mL). CPG-linked protected 5′-AODN was then air-dried.

Cleavage of CPG-Linked DPI-Protected 5′-AODN/5′-AORN to DPI-Protected 5′-AODN/5′-AORN. DPI group is very acid sensitive, and it should be noted that evaporation to dryness or prolonged heating can lead to partial or full deprotection of the DPI-protected 5′-AODN. In all cases, pH have to be maintained above 8 before NH_4OH or methylamine evaporation. It is advisible to add a slightly volatile base, such as diisopropylethylamine (DIEA).

Final deprotection was performed as follows. (i) For ODN: dried resin was treated with 30% aqueous NH_4OH (55 °C, 16 h) or 40% aqueous methylamine (MA, 65 °C, 15 min). Ammoniac or MA was removed with a speedvac evaporator; DPI-protected 5′-AODN were then purified by RP-HPLC. (ii) For ORN, following method C: by treatment with 500 μL 40% methylamine solution at 65 °C for 15 min. Sample was cooled down and placed in a speedvac unit until complete evaporation of methylamine. Sample was next frozen and lyophilized. ODNs were taken up in 115 μL DMSO and treated with 60 μL TEA and then by 75 μL TEA·3HF. The solution was heated for 90 min at 65 °C. (iii) For ORN, following method D: by treating with 200 μL of a 40% methylamine solution at 65 °C for ca. 10 min. Sample was cooled down, centrifuged and the supernatant was transferred in a plastic vial. 600 μL DMSO were added and the resulting solution was cooled to −10 °C before addition of 100 μL of 70% aqueous glycolic acid solution. Solution was vortexed, 200 μL of NH_4F solution (40% aqueous) were added and solution was vortexed again. To avoid aldehyde deprotection or phosphate migration, the pH value was kept between 8 and 9 (tested on pH paper) by adding either aqueous methylamine solution or glycolic acid solution. Sample was heated at 65 °C over 1 h.

In method C or D, the sample was diluted with water to a final volume of 1.5 mL and purified by size exclusion column (NAP 25) before RP-HPLC analysis and purification.

RP-HPLC analyses were achieved by using C18 column (length 250 mm, diam 4.6 mm; particle size 5 μm, porosity 100 Å) at 1 mL/min flow. Eluent A was a 50 mM TEAA solution pH = 7 in water/MeCN (95:5, v/v) and eluent B a MeCN/water (9:1, v/v) solution. Absorbance was monitored at 260 nm. For purification, a 10 mm diameter column was used with a 4 mL/min flow. Protected 5′-AODN and 5′-AORN were analyzed and purified using the following gradient: 0% B during 2 min, then 0% to 45% B in 23 min.

Purified DPI-protected 5′-AODN/5′-AORN fully decomposes in a few weeks (deprotection of the 5′-aldehyde) even stored at −20 °C.

Deprotection of DPI-Protected 5′-AODN into 5′-AODN. DPI-protected 5′-AODN was treated with 400 μL 80% aqueous AcOH for 5 min at rt. 5′-AODN solution was diluted with 400 μL water and was concentrated to 400 μL final volume in a speedvac evaporator (it is advisible to not evaporate sample to dryness since heat released by the apparatus favors the β-elimination reaction[11]). Water was then added (ca. 1 mL), and the 5′-AODN was purified on steric exclusion column (NAP 25) and lyophilized. 5′-AODN was stored at −20 °C. Lyophilized 5′-AODN slowly decompose even stored at −80 °C upon few weeks and should be preferably keep in 0.1% AcOH solution.

Derivatization of 5′-AODN to Their *n*-Decyloxime Ether. 5′-AODNs were analyzed by ESI-MS as *n*-decyloxime ether derivative obtained by reacting with *n*-decyloxiamine synthesized as previously described.[18] 5′-AODN (ca. 50 nmol) was dissolved in 50 μL 0.4 M $AcONH_4$ buffer (pH = 4.6) and 25 μL of a solution of *n*-decyloxiamine (10 equiv) in MeCN were added and stirred overnight. Resulting conjugates were purified by RP-HPLC

Synthesis of Substituted *N*,*N*′-Diphenylethylendiamine. *N*,*N*′-*Bis(4-bromophenyl)ethylenediamine (**15**).* *N*,*N*′-Diphenylethylenediamine (500 mg; 2.36 mmol) was dissolved in 20 mL of $CHCl_3$. Tetraethylammonium tribromide (2.27 g; 4.72 mmol; 2 equiv) was then added portionwise. After ca. 20 min, crude reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (CH_2Cl_2/pentane mixture, from 1:2 to 1:1, then CH_2Cl_2) to afford a pale yellow powder (585 mg, 67%): ^{13}C NMR (75 MHz, $CDCl_3$) 146.9; 132.1; 114.7; 109.6; 43.2; ^{1}H NMR (300 MHz, $CDCl_3$) 7.16 (d, 2H, J = 8.9 Hz, H_{Ar}); 6.40 (d, 2H, J = 8.9 Hz, H_{Ar}); 3.58 (br, 2H, NH); 3.24 (s, 4H, CH_2); MS (IE+, CH_2Cl_2) m/z 369.0 (51%), $[M + H]^+$; 371.0 (100%) ; 373.0 (49%); mp 110.0 °C ± 0.1 °C (lit.[19] mp 108 °C).

N,*N*′-*Bis(4-cyanophenyl)ethylenediamine (**16**).*[20] Ethylenediamine (167 μL; 2.50 mmol), 4-fluorobenzonitrile (667 mg; 5.51 mmol; 2.2 equiv) and potassium carbonate (690 mg; 5.00 mmol; 2 equiv) were refluxed in 2.5 mL of dimethylacetamide overnight. The crude reaction mixture was then cooled to room temperature, diluted with 10 mL AcOEt/THF (1:1) and washed by a 5% $NaHCO_3$ solution, brine and water. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The resulting oil was purified by silica gel column chromatography (cyclohexane/AcOEt mixture, from 5:1 to 1:1) to afford a white powder (420 mg, 64%): ^{13}C NMR (75 MHz, DMSO-d_6) 152.0; 133.4; 120.5; 111.8; 95.8; 41.1; ^{1}H NMR (300 MHz, DMSO-d_6) 7.50 (d, 2H, J = 8.9 Hz, H_{Ar}); 5.80 (br, 2H, NH); 6.71 (d, 2H, J = 8.9 Hz, H_{Ar}); 3.35 (br, 4H, CH_2); MS (IE+, CH_2Cl_2) m/z

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263.1 (100%), $[M + H]^+$; mp 198.5 °C ± 1.3 °C (lit.[19] mp 205−206 °C).

N,N′-Bis(3,5-dichlorophenyl)ethylenediamine (17).[13] 1,2-Dibromoethane (1.05 g; 5.59 mmol) and 3,5-dichloroaniline (5.5 g; 33.9 mmol; 6 equiv) were dissolved in 5 mL of dimethylacetamide and refluxed for 10 h. The crude reaction mixture was evaporated to dryness, taken up in 20 mL of AcOEt and washed by a 5% $NaHCO_3$ solution, brine and water. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The resulting oil was purified by silica gel column chromatography (cyclohexane/AcOEt 9:1) to afford a yellow powder (948 mg, 48%): ^{13}C NMR (75 MHz, DMSO-d_6) 150.8; 134.4; 114.3; 110.0; 41.4; ^1H NMR (300 MHz, DMSO-d_6) 6.56 (br, 6H, H_{Ar}); 6.30 (br, 2H, NH); 3.20 (br, 4H, CH_2); MS (IE+, CH_2Cl_2) m/z 348.0 (78%), $[M + H]^+$; 350.0 (100%); 352.0 (48%); mp 125.8 ± 1.0 °C (lit.[13] mp 127−129 °C).

Preparation of Ternary Complex Containing 5′AODN. AODN fragment ($^{5'}$CCACGCATCGCTGGTA$^{3'}$) was 3′-end labeled by Terminal Deoxynucleotidyl Transferase (Sigma-Aldrich, France) in the presence of [α-^{32}P]ATP (3000 Ci/mmol, PerkinElmer). The radiolabeled oligonucleotide was hybridized with 10% molar excess of the template strand and of the flanking oligonucleotide. Hybridization was conducted at 90 °C (5 min) followed by slow cooling to room temperature in the enzyme incubation buffer. For most enzymes, the buffer was 70 mM Hepes-KOH (pH 7), 100 mM KCl, 1 mM EDTA except for hOGG1 (commercial buffer: 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT).

Enzymes. hOGG1 (human 8-oxoG-DNA glycosylase) was from New England Biolabs; TagI (3-methyladenine-DNA glycosylase I), MUG (mismatch-specific uracil-DNA glycosylase), Fpg (formamidopyrimidine-DNA glycosylase), Nth (endonuclease III) and ANPG 40 and 80 proteins (human truncated alkyl-N-purine-DNA glycosylase missing 63 amino acids at C terminal end and 73 amino acids at N terminal end respectively) were generous gifts from M. Saparbaev (Institut Gustave Roussy, Villejuif, France)

Cleavage Assay. Enzymatic activities were measured in the appropriate incubation buffer. Typically, 10 nM 3′-^{32}P-labeled of the ternary complex oligonucleotides were incubated with 10 nM DNA repair enzyme at 37 °C for 30 min (10 μL). The reaction was stopped by adding 10 μL of loading buffer (formamide containing 0.1% TFA) and analyzed by 20% denaturing PAGE. Strand 1 (complementary strand): $^{5'}$TTA CCA GCG ATG CGT GGG AGC GTG AAT TCA TC$^{3'}$; strand 2:3′ phosphate ODN: $^{5'}$GAT GAA TTC ACG CTCp$^{3'}$; strand 3:5′ AODN: $^{5'}$CHO−CCA CGC ATC GCT GGT A$^{3'}$.

Analysis of radiolabeled oligonucleotides was carried out using a Typhoon 9410 Phosphorimager and ImageQuant TL software. Experiments were carried out at least three times.

To attempt to trap DNA−protein complexes, the reaction was performed in the same conditions, in the presence of triacetoxyborohydride (50 mM final concentration), quenched with loading buffer, and analyzed as described above.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of compounds **15−17**, RP-HPLC chromatograms of 5′-AODN **4−14** both protected and derivatized and 5′-AORN **18a−22b** and ESI-MS analysis of 5′-AODN **4−25** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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