DOI: 10.1002/cbic.200700690

Synthesis of a Stabilized Version of the Imidazolone DNA Lesion

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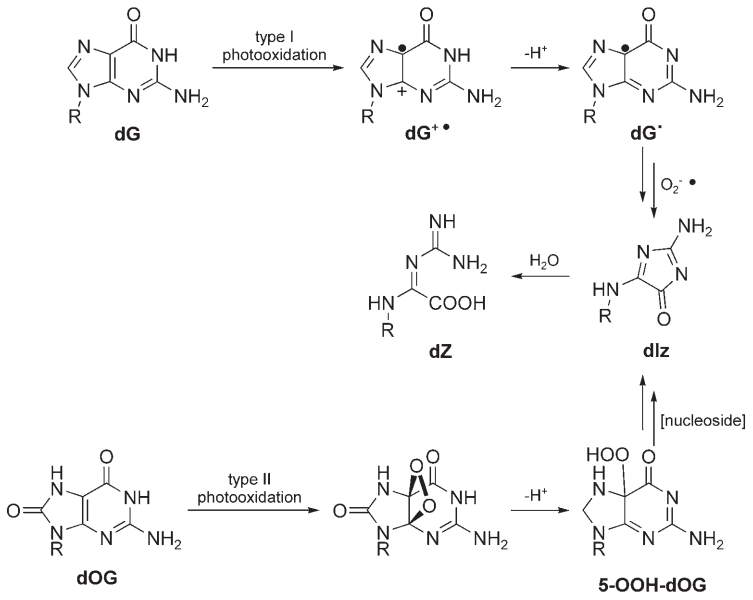
Imidazolone (dIz) is an abundant, highly mutagenic, and rather unstable DNA lesion that can cause dG→dC transversion mutations. dIz is generated in DNA by a variety of oxidative processes such as type I photooxidation. Herein we report the synthesis of a carbocyclic nucleoside analogue of dIz and of DNA containing this stabilized lesion analogue. The carbocyclic modification protects this lesion analogue from anomerization. As the repair of the lesion analogue by DNA glycosylases is not possible, this analogue should allow cocrystallization studies together with wild-type repair enzymes. Characterization of the lesion analogue was performed by using spectroscopic methods and enzymatic digestion experiments of the oligonucleotides.

Introduction

Oxidation processes induce the formation of more than 50 known DNA lesions, which are partially highly mutagenic.[1] Many of these lesions are derived from the oxidation of the guanine base because it has the lowest oxidation potential of all four canonical bases.[2,3] Imidazolone (dIz) is one of the major reaction products formed during one-electron oxidation of dG in either DNA or of the nucleoside itself.[4a] dIz is, for example, very efficiently formed when DNA is irradiated with UV-A light in the presence of riboflavin as a photosensitizer and oxygen (type I photooxidation).[4b,c] Under these conditions, 8-oxo-7,8-dihydro-2′-deoxyguanosine (dOG) is only a minor reaction product (Scheme 1).[5] The dIz lesion itself is rather unstable and today it is well established that dIz hydrolyzes to give the oxazolone (dZ) lesion. Although the exact structure of dZ is still a matter of debate, the most likely open-ring structure is depicted in Scheme 1.[4c,6]



Scheme 1. One electron oxidation of dG initially forms the guanine radical cation, which can be deprotonated. The neutral radical reacts with $O_2^{\bullet-}$. Subsequent degradation leads to the imidazolon lesion (dIz) and its hydrolysis product oxazolone (dZ) as the major products. Singlet oxygen oxidation of dOG nucleoside (type II photooxidation) gives rise to several DNA lesions, predominantly dIz and dZ. R = DNA or 2-deoxy-β-D-ribofuranosyl.

Another major pathway towards formation of the dIz lesion starts with the dOG lesion, which has an oxidation potential even lower than that of dG.[7,8] The singlet oxygen treatment of dOG nucleoside was found to lead predominantly to dIz and dZ (Scheme 1).[9] dIz is likely to be formed in cells because its hydrolysis product was quantified in liver tissue of diabetic rats.[10] The half-life of dIz in double-stranded DNA in neutral media and at 37 °C is only slightly greater than 20 h,[11] which is, however, long enough for the lesion to be replicated by polymerases. The half-life for the dIz nucleoside is even shorter with only 2.5 h.[6] It was established that the lesion codes like a dC in accordance with theoretical and melting point studies.[3,12] In *E. coli* dIz induces mainly dG→dC transversion mutations (88%).[13] The data about the repair of dIz is rather limited.[14] Its hydrolysis product dZ, however, was found to be a substrate for the bacterial DNA repair glycosylase MutM/FPG.[15]

In order to get more insight into the lesion recognition process,[16] we report herein, within the framework of our research program to decipher DNA repair and mutagenesis pathways, the synthesis of a carbocyclic imidazolone lesion analogue (cdIz)[17] that will allow the crystallization of repair enzymes in complex with DNA containing this compound. We recently introduced the concept of replacing the 2′-deoxyribose moiety of DNA lesions by a cyclopentane skeleton. The lack of a glyco-

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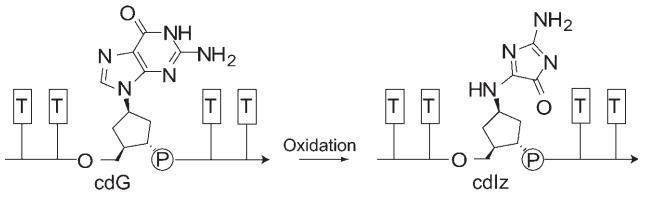
sidic bond stabilizes lesions against anomerization by eliminating the mechanistic pathway for this reaction.[18] This is important for lesions such as dIz which are known to anomerize under certain conditions.[19] It is even more important that carbocyclic modified lesions cannot be repaired by DNA glycosylases, again due to the lack of the glycosidic bond. For the dIz lesion however, this alone does not stabilize the lesion to an extend that would allow its incorporation into DNA by solid-phase DNA synthesis.[4b] We therefore developed a new strategy which involves the incorporation of a carbocyclic guanosine (cdG) precursor into DNA which allowed us to create cdIz in a subsequent oxidation reaction directly in DNA (Scheme 2).



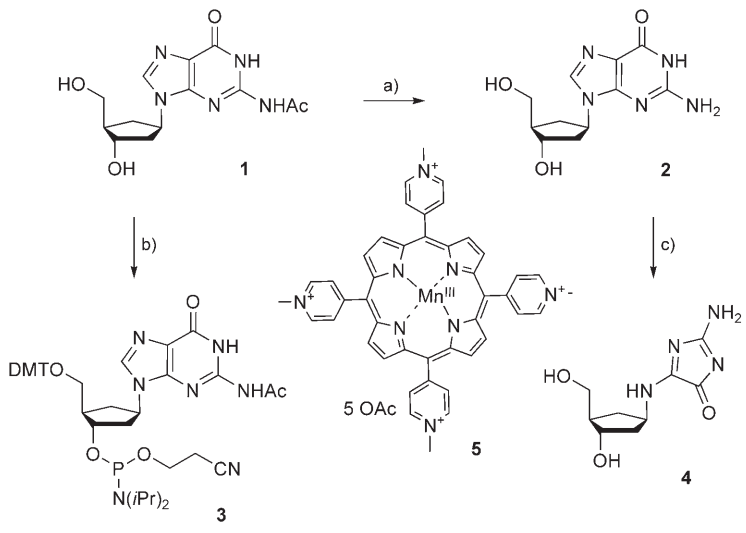
Scheme 2. Carbocyclic guanosine (cdG) can be used as a precursor for the synthesis of carbocyclic imidazolone (cdIz) in DNA.

Results and Discussion

The most promising synthesis of cdIz was thought to involve the oxidation of the carbocyclic analogue of guanosine (cdG). To this end we initially prepared the acetyl-protected cdG derivative **1** according to a procedure recently published by us.[18b] This building block was fully deprotected to give **2** by using a mixture of ethanol and concentrated aqueous ammonia (99% yield after 18 h at 35 °C) as depicted in Scheme 3.

For the oxidation we treated **2**, according to a procedure published by Meunier et al., with a mixture of MnTMPyP **5** and $KHSO_5$ in triethylammonium acetate buffer.[20] HPLC analysis of the reaction mixture proved that the reaction was completed within one minute. The oxidation was stopped by addition of HEPES buffer to avoid overoxidation. Almost quantitative conversion to a new product was observed, as depicted in the HPLC profiles shown in Figure 1 A and B. The obtained product was purified by reversed-phase HPLC by using a gradient of 0.1 M triethylammonium acetate pH 7.0 in water (buffer A) and 0.1 M triethylammonium acetate pH 7.0 in 80% acetonitrile (buffer B) of 0→15% buffer B over 45 min. The fraction eluting at 13 min was collected and analyzed by UV spectroscopy and ESI mass spectrometry. The compound indeed featured the molecular weight of *m/z* 225.0995 expected for compound **4** (calculated for $[M-H]^-$: *m/z* 225.0993). In addition we found that the fragmentation pattern (Figure 1 D) of the compound is in accordance with the literature data for dIz.[4a] In order to compare its stability with the natural lesion, we followed the hydrolysis of this lesion analogue in neutral aqueous phosphate buffer at 37 °C. Under these conditions, we found a half-life for this lesion of 304.1 min, which is in perfect accordance with literature data for dIz hydrolysis under similar conditions (147 min) and demonstrates that the carbocyclic modification does not influence the hydrolysis process.[6] Furthermore, no anomerization product has been observed during this process. The only observed hydrolysis product of **4** had a molecular weight of *m/z* 243.1098, which is in perfect agreement with the molecular weight of the expected hydrolysis product of **4**, a carbocyclic oxazolone cdZ (calculated for $[M-H]^-$: *m/z* 243.1099)

In order to prepare the cdIz lesion inside DNA we next incorporated cdG into two different oligonucleotides. We prepared the phosphoramidite building block **3** and synthesized the 19-mer and hexamer oligonucleotides ODN 1 and ODN 2 (Table 1), respectively. Besides the cdG base, these strands were designed to contain no other guanine residues to avoid oxidation at these alternative sites. The sequence of ODN2 contained two adjacent cdG bases because it is known that this further reduces the oxidation potential.[5] The automated DNA synthesis was performed on controlled pore glass (cpg) support under standard conditions for the coupling of **3**. Benzylthiotetrazole (BTT) was utilized as the coupling reagent. The oligonucleotides were purified on a Dionex DNA Pac-PA 100 9×250 semipreparative column by using a buffer system of 20 mM TRIS/HCl in water (buffer A) and 1 M NaCl (buffer B) with a gradient of either 25–50% B in 45 min (ODN1) or 0–50% B in 45 min (ODN2).



Scheme 3. a) conc. aq. ammonia, ethanol, 18 h, 35 °C, 99%; b) 1.2 equiv dimethoxytritylchloride, pyridine, molecular sieve, 2.5 h, RT; 1.5 equiv cyanoethyldi*iso*propylchlorophosphoramidite, di*iso*propylethylamine, THF, 2 h, RT; c) triethylammonium acetate, pH 6.5, $KHSO_5$, MnTMPyP (cat.) **5**, 1 min, RT.

Despite the efficient oxidation of cdG to cdIz on the nucleoside level, preparation of cdIz inside DNA with $KHSO_5$ and MnTMPyP **5** turned out to be problematic. We observed significant degradation of the oligonucleotides. These difficulties forced us to search for alternative oxidizing reagents and we turned our attention finally to photooxidation.[4c,5] Both oligonucleotides ODN1 and ODN2 were irradiated with UV-A light in the presence of riboflavin. The best results were obtained with an irradiation

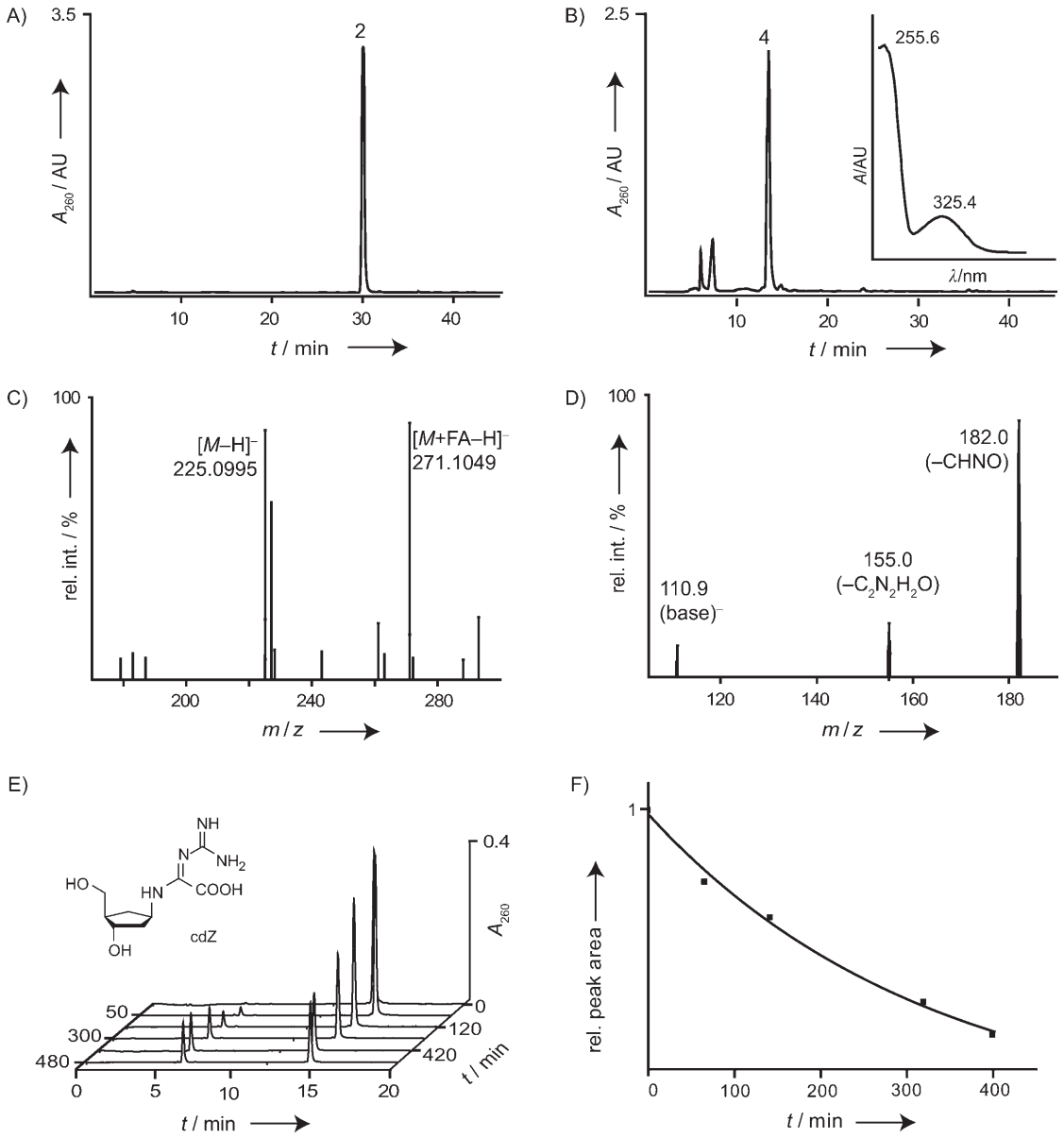


Figure 1. A) HPLC profile of purified cdG **2** and B) of the crude reaction mixture after oxidation of **2** with $KHSO_5$ in the presence of **5**. Nearly full conversion of the starting material was observed The inset shows the UV spectrum of the peak eluting at 13 min, the characteristic absorption is similar with the absorption of dIz;[6] C) negative-mode ESI-MS spectrum of **4**, FA = formic acid; D) MS/MS spectrum of the $[M-H]^-$ peak at 35 eV, *M* = cdIz; E) HPLC profile of the product mixture after incubation of **4** in aqueous phosphate buffer at pH 7 gives rise to only one new peak at a retention time of 7 min with a molecular weight of *m/z* 243.1098; F) exponential fit demonstrating the decrease of the peak areas of **4** after 0, 1, 2, 5, 7, and 8 h of hydrolysis.

Table 1. Synthesized, modified ODNs.

ODN	Sequence[a]	M_{calcd}	M_{found}[b]	Yield[c]
ODN1	5′-d(TTT[cG]TCATTCTTTTCTTTC)	5689.8	5689.0	–
ODN2	5′-d(TT[cGcG]TA)	1818.4	1818.1	–
ODN3	5′-d(TTT[cIz]TCATTCTTTTCTTTC)	5650.7	5649.4	18
ODN4	5′-d(TT[cIz]cGTA)/	1779.3	1779.4	16/8
ODN4′	5′-d(TTcG[cIz]TA)			

[a] Nucleotides in brackets indicate the carbocyclic analogues [b] MALDI-TOF analysis; [c] in % after 5 min of irradiation time, ε of cdIz at 260 nm estimated as 5 $mM^{-1}cm^{-1}$.[20a]

time of 5 min with a 365 nm cut-off filter in a 1 mm quartz cuvette with 2.6 μL of a solution containing 0.6 mM of corresponding oligonucleotides in 9 mM cacodylate buffer at pH 7.0. Irradiation was performed at room temperature. The obtained crude reaction mixtures (see Figure 2A and C for the HPLC profiles) were subjected to HPLC separation, which allowed us to isolate the cdIz-containing 8888oligonucleotides ODN3 and ODN4, ODN4′ in a respectable yield of 15–20%. The HPLC fractions containing these product oligonucleotides were collected and dialyzed for 60 min against water prior to MALDI-TOF analysis (Figure 2). All cdIz-containing oligonucleotides had to be kept cold in order to avoid further degradation

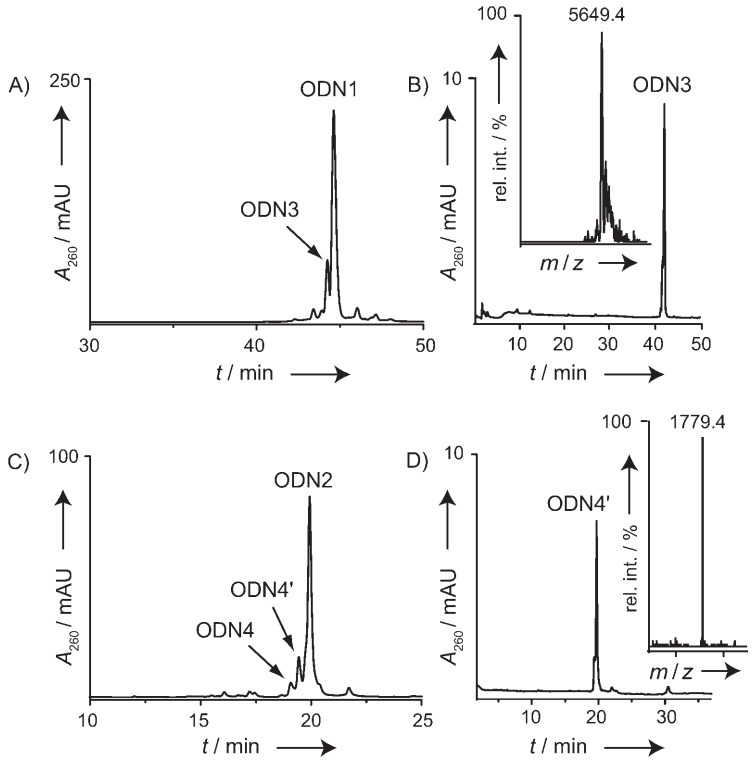


Figure 2. Photooxidation of ODN1 and ODN2. Irradiation time was 5 min for each strand. A) and C) show the HPLC profiles of the reaction mixtures after irradiation; B) and D) show the HPLC profile of the purified strands and the corresponding MALDI-TOF spectra as insets. Data for the purified ODN4 is not shown.

The obtained digested material was subjected to HPLC–MS analysis (Figure 3).

The HPLC chromatogram (Figure 3 A) shows two peaks, which were assigned to the nucleosides dT and dA, respectively. The shoulder just in front of the dT peak is caused by cdG. As a result of the relatively low extinction coefficient of cdIz and hydrolytic decay of the lesion nucleoside we expected only a very small signal for this nucleoside. In fact such a peak was detected at around 10 min. The corresponding negative mode ESI-MS spectrum of this peak is shown in Figure 3 B (top). The obtained value of *m/z* 225.0994 for $[M-H]^-$ is in full agreement with the calculated molecular weight of cdIz (*m/z* 225.0993, $[M-H]^-$). Furthermore, the MS/MS spectrum of the signal in question with a *m/z* 225.0994 showed a fragmentation pattern identical with data obtained from **4** and in accordance with the literature data.[4a]

Conclusions

In conclusion, we were able to synthesize the carbocyclic imidazolone nucleoside (cdIz), which is a stabilized version of the oxidatively generated DNA lesion imidazolone (dIz). In addition, we prepared this building block inside DNA single strands. The mass spectrum clearly identifies the presence of the

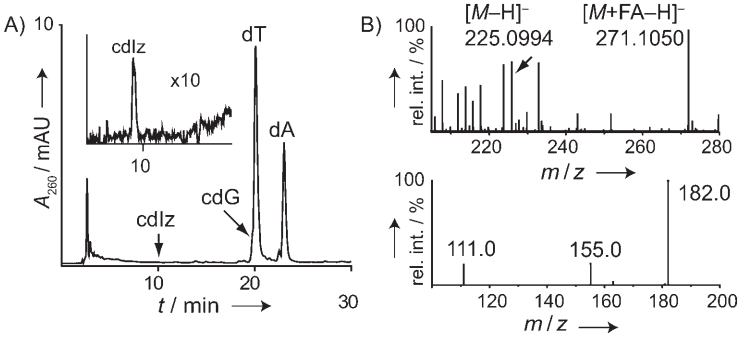


Figure 3. A) HPLC profile of the digested ODN4/4′ showing the UV trace (above) and the ion count at *m/z* 224.5–225.5 (below); B) ESI-MS (above) and MS/MS (below) of the peak eluting at 9.5 min, parent ion for the MS/MS spectrum is $[M-H]^-$ *m/z* 225.0994, *M* = cdIz, FA = formic acid. Data of the digested ODN3 are not shown.

of the lesion. Although the yield of the photooxidation is only moderate, the reaction is astonishingly selective. No other oligonucleotides except the expected cdIz-containing product oligonucleotides could be detected under the conditions reported herein. In the case of ODN2, it was difficult to differentiate between the two possible products 5′-d(TTG-[cIz]TA) and 5′-d(TT[cIz]GTA) (Table 1) although separation of ODN4 and ODN4′ is possible. We could not determine which position was oxidized because the replacement of the deoxyribose unit by the cyclopentane skeleton makes the compound stable against typical Maxam–Gilbert conditions.[21] We assume however, that the product which is produced with higher yields is the 5′-d(TT[cIz]GTA) oligonucleotide, because it is known that two adjacent dG are predominantly oxidized at their 5′ end. Mass spectrometric sequencing may be a potential solution to clarify this question.

In order to prove the presence of cdIz in the irradiated ODNs, we digested the irradiated ODNs enzymatically. To this end, the photooxidation was scaled up by a factor of 5 to prepare enough material. The obtained DNA was treated with nuclease P1 (*Penicillium citrinum*), Antarctic shrimp phosphatase, and calf spleen phosphodiesterase II. The mixture was finally incubated for 2 h at 30 °C. The buffer was then set to pH 5.7. The relatively low temperature together with the slightly acidic buffer is needed to minimize hydrolytic degradation of cdIz. new lesion analogue after enzymatic digestion of the oxidized oligonucleotides. The molecular weight of the oxidized oligonucleotides is also correct; this supports the presence of the cdIz lesion. Together with the fact that we obtained clean HPLC chromatograms, we conclude that the photooxidation method allows us to prepare DNA containing the cdIz lesion analogue in excellent quality and high quantity. Replacement of the 2′-deoxyribose moiety by the cyclopentane unit stabilizes this lesion analogue against cleavage by BER enzymes because of the lack of a glycosidic bond, but this modification does not prevent degradation of the heterocycle for example,

to give the oxazolone (dZ) hydrolysis product. The lesion analogue hydrolyzes within approximately the same time as the natural lesion. Consequently, the oligonucleotides obtained have to be handled with great care (neutral pH, reduced temperature). Under these conditions the hydrolysis is slow enough so that the cdIz-containing DNA can be stored and handled. The ability to prepare the cdIz lesion analogue in DNA is a major step forward because it now allows cocrystallization studies of cdIz-containing DNA with wild-type repair enzymes such as FPG/MutM without being repaired.

Experimental Section

Materials and methods: All solvents and reagents were obtained in commercially available qualities puriss., p. a., or purum. Phosphoramidites are from Glen Research or Samchully Pharm, cpg support and benzylthiotetrazole (BTT) was from Glen Research. Dry solvents were purchased from Fluka or Acros and were used as received. Acetonitrile for DNA synthesis was from Roth in Rotidry grade with a water content of 10 ppm. Acetonitrile for HPLC and HPLC-MS was purchased from VWR in HPLC grade. Melting points were obtained with a Reichert-Jung type 65 15 01 on a Leica Galen III microscope and were not corrected. IR spectra were obtained on a Perkin–Elmer Spektrum BX connected with a Smiths Detection DuraSamp/IR II. ^{1}H NMR spectra were obtained on a Varian Mercury-200 and Varian INOVA-400. The chemical shifts were referred to DMSO in [D$_6$]DMSO. If necessary, peak assignment was carried out with the help of COSY, HMBC, or HSQC experiments. ^{13}C NMR spectra were obtained on a Varian INOVA-400 and Bruker AMX 600. ESI spectra were obtained on a Thermo Finnigan LT-FT ICR spectrometer. MALDI-TOF spectra were obtained on a Bruker Autoflex II spectrometer with a matrix consisting of a 1:1 mixture of saturated 3-hydroxypicolinic acid (HPA) in water and a solution of 50 mg HPA, 10 mg ammonium hydrogen citrate, and 10 μL of [15]crown-5 in 1 mL water. HPLC analysis was performed on a Waters 2695 with a Waters 2996 Photo Diode Array detector. HPLC separation of DNA was achieved on either a Waters 1525 with a Waters 2487 UV detector or a Merck-Hitachi L7150 with a Merck-Hitachi L7420 UV/Vis detector.

2-Amino-9-(3-hydroxy-4-hydroxymethyl-cyclopentyl)-1,9-dihydro-purin-6-one (cdG) (2): **1** (50 mg, 0.16 mmol) was dissolved in a mixture of conc. ammonia (3 mL) and ethanol (1 mL). After shaking for 18 h at 35 °C, the solvent was removed on a Savant Speed Vac. The slightly yellow product **2** (42 mg, 0.16 mmol, 99%) was used without further purification. m.p. 229–230 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.53–1.61 (m, 1H; C6′-H$_a$), 1.90–1.92 (m, 2H; C2′-H$_a$, C4′-H), 2.04–2.12 (m, 1H; C2′-H$_b$), 2.23–2.30 (m, 1H; C6′-H$_b$), 3.38–3.43 (m, 1H; C5′-H$_a$), 3.47–3.51 (m, 1H; C5′-H$_b$), 4.02–4.06 (m, 1H; C3′-H), 4.75–4.84 (m, 1H; C1′-H), 6.39 (br s, 2H; NH$_2$), 7.77 ppm (s, 1H; C8-H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 34.75 (C6′), 41.36 (C2′) 50.00 (C4′), 52.75 (C1′), 63.48 (C5′), 72.20 (C3′), 136.04 (C8), 117.44, 151.78, 154.01, 157.55 ppm (C2, C4, C5, C6); IR (film): $\tilde{\nu}$ = 3305.83 s, 3163.59 vs, 2920.43 m, 1653.29 vs, 1605.10 vs, 1572.89 s, 1533.39 s, 1478.25 m, 1405.87 s, 1378.96 s, 1170.87 w, 1042.86 w, 916.15 vw, 776.71 w, 719.10 w, 696.40 w, 670.97 w; HRMS (ESI$^+$; [M+H]$^+$): calcd for [C$_{11}$H$_{15}$N$_5$O$_3$ + H]$^+$: 266.1248, found 266.1241.

Mn^{III}-5,10,15,20-tetra(4-pyridyl)-21*H*,23*H*-porphineacetate-tetrakis(methoacetate) (MnTMPyP; 5): Mn^{III}-5,10,15,20-tetra(4-pyridyl)-21*H*,23*H*-porphinechloride-tetrakis (methochloride) (45 mg, 0.05 mmol) was dissolved in water (10 mL) and flushed three times through a column filled with Dowex 1×8 ion-exchange resin previously loaded with acetate. Small amounts of the resulting solution were diluted with distilled water and checked for remaining chloride by addition of silver nitrate solution (0.1 M). If no precipitation of silver chloride could be observed, the dark-brown solution was lyophyllized and taken up in water. Content of **5** was estimated to be approximately 7.7 mg mL^{-1}.[22]

2-Amino-5-(3-hydroxy-4-hydroxymethyl-cyclopentylamino)imidazol-4-one (cdIz) (4): A solution of **2** (50 μL, 5 mM) was added to triethylammonium acetate buffer (25 μL, 100 mM, pH 6.5) and water (25 μL). Subsequently, **5** (0.13 μL, 7.7 mg mL^{-1}) and a solution of potassium monopersulfate triple salt (25 μL, 30.7 mg mL^{-1}) was subjected to the solution. The resulting mixture was vortexed for exactly one minute at RT. The reaction was stopped by addition of HEPES buffer (50 μL, 100 mM, pH 8.0). Purification of the oxidized nucleoside was accomplished by direct injection of the solution on an analytical HPLC with a buffer system of 0.1 M triethylammonium acetate pH 7.0 in water (buffer A) and 0.1 M triethylammonium acetate pH 7.0 in 80% acetonitrile (buffer B) and a gradient of 0–15% buffer B in 45 min on a Macherey–Nagel Nucleodur 100–3 column. The pure product **2** eluted at 13 min. For NMR spectroscopy, the reaction was scaled up by a factor of 1:160. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.32 (ddd, ^2J(C6′-H$_a$,C6′-H$_b$) = 12.9 Hz, 1H; C6′-H$_a$), 1.77–1.84 (m, 3H; C2′-H$_a$, C2′-H$_b$, C4′-H), 2.11 (ddd, ^2J(C6′-H$_b$,C6′-H$_a$) = 12.9 Hz, 1H; C6′-H$_b$), 3.27–3.30 (m, 1H; C5′-H$_a$), 3.37–3.40 (m, 1H; C5′-H$_b$), 3.88–3.91 (m, 1H; C3′-H), 4.22 (m, 1H; C1′-H), 6.80 ppm (d, 1H; C1′-NH), N2-H, C3-NH, 3′-OH, 5′-OH not detected; ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 34.09 (C6′), 40.87 (C2′), 49.99 (C4′), 52.97 (C1′), 63.58 (C5′), 72.48 (C3′), 166.91 (C5), 177.30, 185.85 ppm (C1, C3); HRMS (ESI$^-$; [M−H]$^-$): calcd for [C$_9$H$_{14}$N$_4$O$_3$$M$−H]$^-$: 225.0993, found 225.0995.

Hydrolysis of cdIz: **2** was prepared and purified as previously described. Purified **2** was lyophyllized, dissolved in phosphate buffer (500 μL, 10 mM, pH 7), and incubated at 37 °C. After 50, 120, 300, 420, and 480 min, a sample of 50 μL was injected on an analytical HPLC coloumn with the same gradient and buffer system as for the purification of **2**. The area of the peak eluting at 13 min was calculated and plotted against the incubation time. The half-life was derived from first order exponential fit by using the software Microcal Origin.

Oligonucleotide synthesis: DNA synthesis was performed on an Amersham Äkta oligopilot 10 under standard conditions on controlled pore glass (cpg) support at a 1 μmol scale. The concentration of the amidite solutions was 0.1 M. Benzylthiotetrazole (BTT) was used as coupling reagent (0.25 M) and the coupling time for standard amidites was set to 3 min. Coupling time for **3** was 10 min. Syntheses were performed "DMT-off". Deprotection of the ODNs was achieved by incubation with a 1:1 mixture of conc. ammonium hydroxide and methylamine (40% in water) for 18 h at RT. The solution was evaporated to dryness by using a Savant Speed Vac®, and the crude product was resuspended in water. Purification was accomplished with a buffer system of 20 mM TRIS/HCl in water (buffer A) and 1 M NaCl (buffer B) with a gradient of eather 25–50% B in 45 min (ODN1) or 0–50% B in 45 min (ODN2) on a Dionex DNA Pac-PA 100 9×250 semipreparative column. The fractions were checked for analytical purity by HPLC with the same gradient and buffer system but a Dionex DNA Pac-PA 100 4×250 analytical column. Collected samples were desalted on a Waters Sep-Pak® cartridge according to the manufacturer protocol and analyzed by MALDI-TOF spectrometry.

Oxidation of ODN1 and ODN2: Sodium cacodylate buffer (0.24 μL, 100 mM) and a saturated solution of riboflavin in water (0.4 μL)

was added to a solution of either ODN1 or ODN2 (2 μL, 0.8 mM). The mixture was placed in a 1 mm quartz cuvette and irradiated for 5 min at RT. Subsequently, the sample was diluted with water (15 μL) and subjected directly to HPLC purification by using a Dionex DNA Pac-PA 100 4×250 analytical column with the same gradients and buffer systems as were used for the purification for ODN1 and ODN2, respectively. The corresponding fractions were collected, concentrated in vacuo, and dialyzed for 20 min against water by using a 0.2 μm Nylon membrane (Whatman Puradisc). Desalted samples were checked for purity by using the same HPLC conditions described above for the purification. Desalting procedure was repeated three times prior to MALDI-TOF analysis or enzymatic digestion. Amounts of ODN and reagents were linearly scaled up by a factor of five for enzymatic digestion experiments.

Enzymatic digestion of ODN3 and ODN4/ODN4′: Ammonium acetate buffer (5 μL, 300 mM ammonium acetate, 100 mM $CaCl_2$, 1 mM $ZnSO_4$, pH 5.7), nuclease P1 (3 units), Antarctic shrimp phosphatase (1 unit), and calf spleen phosphordiesterase II (0.05 units) were added to a solution of either ODN3 or ODN4/ODN4′ (11 μM, 45 μL), respectively. The resulting mixture was incubated for 2 h at 30 °C, followed by injection of the solution (20 μL) into a LC-MS analysis system with a buffer system of 2 mM ammonium formate pH 5.5 in water (buffer A) and in 80% acetonitrile (buffer B) and a gradient of 0–3% B in 12 min, 3–40% B in 48 min on an Uptisphere Interchrom 150×2.1. The ESI spectrometer was set to the negative mode with an ion spray voltage of 2.5 kV and a capillary temperature of 200 °C.

Acknowledgements

We thank Dr. W. Spahl for help with ESI measurements and the NMR and MS division of the LMU for support. The expense of this work was covered by the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft (DFG) in the framework of the Gottfried Wilhelm Leibniz program of the DFG, and SFB 646.

Keywords: carbocyclic analogue · DNA damage · DNA repair · imidazolone

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Received: November 13, 2007
Published online on May 28, 2008

