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Dissecting the Differences between the α and β Anomers of the Oxidative DNA Lesion FaPydG

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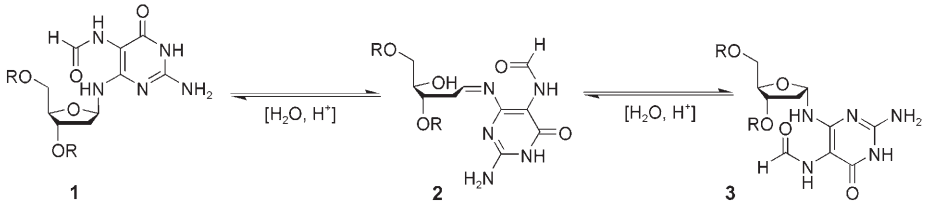
Abstract: The oxidative DNA lesion, FaPydG rapidly anomerizes to form a mixture of the α and β anomer. To investigate the mutagenic potential of both forms, we prepared stabilized bioisosteric analogues of both configurational isomers and incorporated them into oligonucleotides. These were subsequently used for thermodynamic melting-point studies and for primer-extension experiments. While the β compound, in agreement with earlier data, prefers cytidine as the pairing partner, the α compound is not able form a stable base pair with any natural base. In primer-extension studies with the high-fidelity polymerase *Bst* Pol I, the polymerase was able to read through the lesion. The β compound showed no strong mutagenic potential. The α compound, in contrast, strongly destabilized DNA duplexes and also blocked all of the tested DNA polymerases, including two low-fidelity polymerases of the Y-family.

Keywords: DNA lesions · DNA repair · DNA · DNA · mutagenesis · oxidative stress

Introduction

One of the most common DNA lesions that is found in cells after oxidative stress or γ radiation[1,2] is the 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPydG).[3] This lesion **1** is a hydrolysis product of guanine that features an opened imidazole ring, which strongly increases the reactivity and flexibility of the compound. This structural element also leads to rapid anomerization of **1** via the ring-opened intermediate **2**, as depicted in Scheme 1, to give a mixture of the α and β anomers **1** and **3**.[4] Additionally, hydrolysis of the glycosidic bond can occur at elevated temperatures. So far, it is neither known if such an anomerization occurs in DNA, nor has the reactivity of the lesion been fully explored. Because repair of α-configured nucleobases is a well-known fact, it is essential to study the mutagenic effect of the maybe rare but important α versions of lesions.



Scheme 1. Anomerization of the β-FaPydG lesion **1** via the open intermediate **2** to its configurational α-isomer **3**.

To clarify the mutagenic potential of the FaPydG lesion, several attempts to prepare the compound have been reported. Cadet et al. obtained the formamidopyrimidine lesion by direct irradiation of an oxygen-free aqueous solution that contained the nucleotide dG with gamma rays.[5] They obtained an α/β mixture of the pyranosyl-FaPydG in low yields, which was the first direct proof of the instability of the compound. Greenberg et al. reported an elegant syn-

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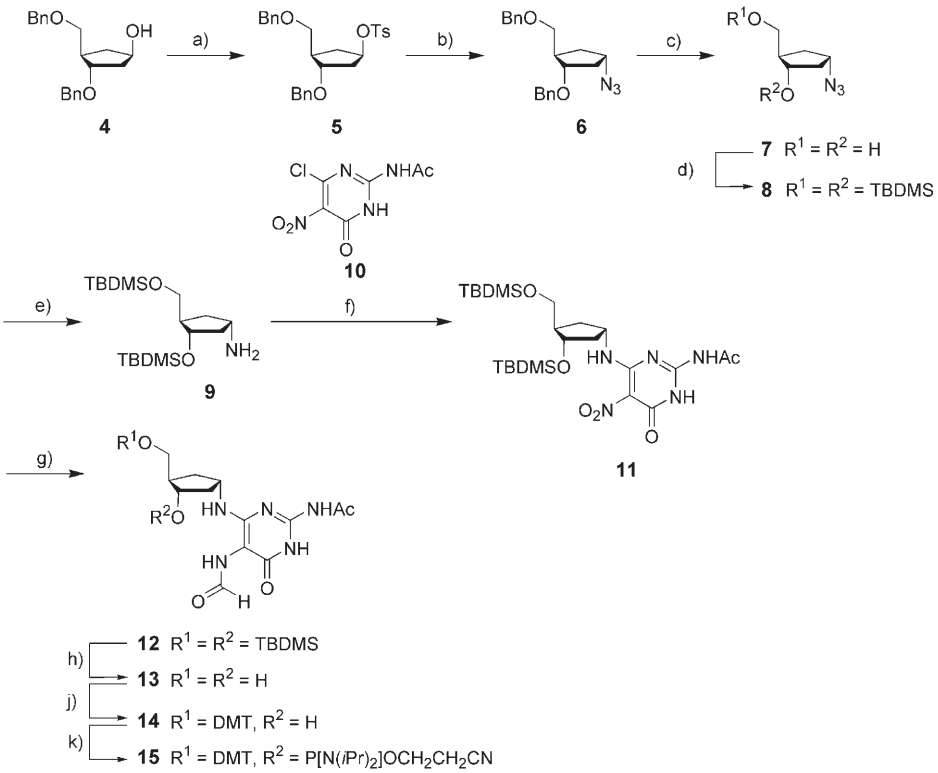
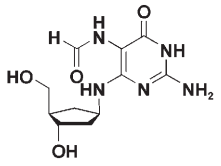
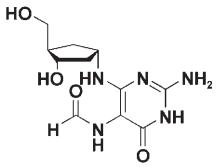
thesis of the lesion, and this group was also able to incorporate it into DNA.[6–8] To address the question of which anomer is finally present in DNA, a biochemical repair study was performed, which suggested that the lesion exists in DNA mainly as the β anomer.[7,8] Experiments by our group to introduce the lesion into DNA by solid-phase synthesis were thwarted by the rapid anomerization of the compound during its synthesis or handling. In all cases, we obtained a mixture of both anomers, particularly in DNA single strands.[9] To circumvent this problem, we[10] and others[11,12] developed different non-hydrolyzable derivatives of the FaPy-lesion. To minimize the influence of the modification on the base-pairing properties of the lesion, we designed a stabilized isosteric analogue of the β anomer (β-*c*FapydG), in which the oxygen atom in the ribose ring is replaced by a methylene unit. This eliminates any anomerization reaction. The strategy also allows the synthesis of both "anomers" in a pure form for a direct comparison, as reported here. In recent studies, β-*c*FapydG was incorporated into DNA and its influence on DNA structure was evaluated.[10] In addition, a crystal structure of the stabilized lesion in DNA in complex with the FPG repair enzyme could be obtained; this shows that the preparation of bioisosteric lesion analogues, such as β-cFaPydG and α-cFaPydG, provides detailed information about the biological and biophysical properties of fragile DNA lesions.[13]



Scheme 2. Synthesis of the α-*c*FaPydG phosphoramidite building block **15**. a) *p*-TsCl, pyridine, RT, 18 h, 79%; b) NaN_3, DMF, 60°C, 16 h, 94%; c) BCl_3, CH_2Cl_2, −78°C→RT, 99%; d) TBDMSCl, imidazole, DMF, RT, 16 h, 86%; e) 10% Pd/C, H_2, EtOH, 15 h, quant.; f) DIEA, **10**, 30 min RT, then 45 min at 60°C, 71%; g) 10% Pd/C, H_2, EtOH/CH_2Cl_2 6:1, EDC·HCl, formic acid, THF/pyridine 5:3, RT, 10 h, 79%; h) HF–pyridine complex, pyridine, THF, RT, 15 h, quant.; j) DMTCl, pyridine, RT, 3.5 h, 74%; k) 2-cyanoethyl-tetraisopropylphosphordiamidite, tetrazolate, CH_3CN, RT, 6 h, 84%. DIEA = diisopropylethylamine.



b-cFaPydG



a-cFaPydG

Here, we report the synthesis of the α anomer of the FaPydG lesion as its stabilized carbacyclic analogue, α-*c*FaPydG. We report the pairing properties of the α analogue and primer-extension studies to decipher the mutagenic potential of the α-configured analogue by using high and low-fidelity polymerases.

Results and Discussion

Synthesis: Synthesis of α-*c*FaPydG was achieved in about 15 steps as depicted in Scheme 2. The cyclopentane core **4** was available in only four steps in good yields.[14,15] This intermediate was subsequently transformed into azide **6** in two steps, which included the tosylation of the hydroxyl group to give **5**, followed by a S_N2 reaction with sodium azide. Deprotection of the benzyl ethers with boron trichloride in dichloromethane furnished compound **7**.

After protecting the free hydroxyl groups as *tert*-butyldimethylsilanes (TBDMS) to afford compound **8**, the azide function was reduced to the amine **9**. Compound **9** was subsequently coupled with heterocycle **10**[9,16] to give the lesion precursor **11**. The absolute configuration of this compound was verified by using NOE NMR spectroscopy. Reduction of the nitro group of **11** and subsequent formylation of the amine intermediate to **12** required stringently anhydrous and anaerobic conditions. The best yields were obtained

with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and formic acid. After completing the synthesis of the silyl-protected lesion nucleotide analogue, compound **12** had to be transformed into the required solid-phase synthesis building block. This involved deprotection of the hydroxyl groups in **12** with HF–pyridine complex in THF to yield diol **13**. Additional pyridine was needed for the transformation to protect the acid-labile formyl group from cleavage. Compound **13** was finally converted into the dimethoxytrityl (DMT)-protected phosphoramidite **15** by using standard procedures. With this building block in hand, the stage was set to perform solid-phase synthesis. The lability of the formyl group, however, required us to change the standard procedures. The coupling time of building block **15** was increased, and the typical capping reagents, phenoxyacetyl or acetic anhydride, were changed to isobutyryl anhydride to avoid any transamidation of the formamidopyrimidine. Cleavage of the DNA from the solid support was performed with concentrated ammonia in water/ethanol 3:1 at 15 °C overnight. All oligonucleotides were purified by reverse-phase HPLC and characterized by ESI-FTICR mass spectrometry. The sequences of the oligonucleotides that were prepared for this study are listed in Table 1. DNA that contained β-*c*FaPydG was prepared by following previously published procedures.[9,10]

Table 1. DNA sequences that were prepared for melting-point studies and primer-extension investigations. **X** = α-*c*FaPydG; **Y** = β-*c*FaPydG.

Name	Sequence
ODN 1	5′-CTC TTT **X** TTT CTC G
ODN 2	5′-GCG AT**X** TAG CG
ODN 3	5′-TGC AGT **X**AC AGC
ODN 4	5′-TAC **X**CC TGG TCA TT
ODN 5	5′-TAG **Y**CC TGG TCA TT

Enzymatic digest: To clarify that the correct incorporation of α-*c*FaPydG into oligonucleotides has taken place, an enzymatic digest of the DNA strand followed by HPLC-MS/MS characterization is needed. For this experiment, DNA (ODN 1) was treated with four different enzymes, which are able to cleave the DNA into the corresponding nucleosides. The obtained mixture was subsequently separated by HPLC on a reversed-phase column that was coupled to a FTICR mass spectrometer. In the HPLC chromatogram that is depicted in Figure 1, three sharp signals are seen, which could be assigned, based on the molecular weights, to the three normal nucleosides (dC, dG and dT) that are present in the strand. The signal at 10.1 min possessed the same molecular weight and retention time as the deacetylated derivative of compound **13**, which is the compound that is expected to be the product from the enzymatic digest of an α-*c*FaPydG-containing oligonucleotide. This result proves that α-*c*FaPydG was not modified during DNA synthesis or purification. The *cis*/*trans*-isomerization of the formamide on the column is responsible for the rather broad appearance of the signal.

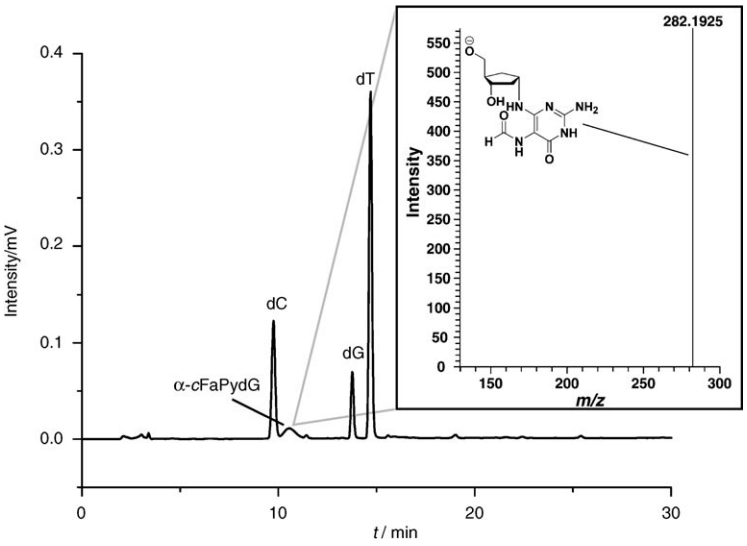


Figure 1. HPLC chromatogram and mass spectrum of an enzymatic digest of ODN 1. The peak at 10.1 min was assigned to α-*c*FaPydG by co-injection and by mass spectrometry.

Thermodynamic measurements: After successful incorporation into oligonucleotides, the influence of the pseudo-α anomer on duplex stability and its base-pairing properties were addressed. Two strands (ODN 2 and ODN 3) were hybridized with four different counter strands. The counter strands contained all four of the canonical nucleobases opposite the lesion to form all possible base pairs. UV melting points of these duplexes were measured with DNA concentrations ranging from 0.3 to 27 μM. The thermodynamic parameters $\Delta H°$, $\Delta S°$ and $\Delta G°_{298\,K}$ of the duplex dissociation process were determined by using van′t Hoff plots (see the Supporting Information).[17] For a better comparison, Table 2 also contains data for β-*c*FaPydG, which were taken from previous work.[10]

From the data listed in Table 2, it is evident that the presence of either of the two *c*FaPydG lesion anomers in DNA induces large duplex destabilizations, but while β-*c*FaPydG still favours base pairing with dC ($\Delta G°_{298\,K} = -10.2$ kcal mol^{-1}), no preferred pairing partner was observed in the case of α-*c*FaPydG. The absolute value of $\Delta G°_{298\,K}$ for the melting process of duplexes that contain a α-*c*FaPydG:dN base pair is strongly reduced compared even to a dG:dA mismatch;[9] α-*c*FaPydG induces a large destabilization of the DNA duplex regardless of the opposing base, and is not able to form any stabilizing interaction with any of the canonical bases.

Primer-extension experiments: To measure the thermodynamic properties of the lesion in double-stranded DNA alone is not sufficient to evaluate how it influences DNA replication, because the basis for the replication fidelity is the base-pairing situation inside the polymerase. This, however, is influenced by the steric constraints that are established by the active site of the DNA polymerase. To investigate the mutagenic potential, we prepared a DNA template/

Table 2. Thermodynamic data for two different double strands, containing either α- or β-*c*FaPydG with all natural bases as pairing partners.[a] For β-*c*FaPydG, see also reference [10].

base pair[a] **X**:**Y**	5′-GCGAT**X**TAGCG 3′-CGCTA**Y**ATCGC			5′-TGCAGT**X**ACGC 3′-ACGTCA**Y**TGCG		
	$\Delta G^{\circ}_{298\,K}$ [kcal mol^{-1}]	ΔH° [kcal mol^{-1}][b]	ΔS° [cal mol^{-1} K^{-1}][b]	$\Delta G^{\circ}_{298\,K}$ [kcal mol^{-1}]	ΔH° [kcal mol^{-1}][b]	ΔS° [cal mol^{-1} K^{-1}][b]
α-*c*FaPydG:dA	−7.0	−44±1.1	−125±7.8	−10.4	−70±1.3	−201±8.0
α-*c*FaPydG:dC	−7.7	−41±1.7	−111±4.7	−10.7	−64±1.9	−180±6.5
α-*c*FaPydG:dG	−7.7	−40±2.0	−109±3.2	−11.1	−74±1.0	−211±7.2
α-*c*FaPydG:dT	−7.8	−38±1.6	−100±6.1	−11.4	−77±1.1	−219±3.7
β-*c*FaPydG:dA	−7.0	−42±2.0	−118±7.0	−10.4	−64±1.6	−181±5.0
β-*c*FaPydG:dC	−10.2	−51±1.0	−137±3.2	−13.4	−80±1.8	−222±5.7
β-*c*FaPydG:dG	−6.9	−43±2.0	−121±6.8	−9.9	−61±1.1	−173±3.7
β-*c*FaPydG:dT	−8.3	−47±1.0	−129±3.4	−10.8	−68±1.1	−193±3.7

[a] Conditions: 150 mM NaCl, 10 mM Tris-HCl (pH 7.4, c_{oligo} = 0.3–27 μM). [b] The error was calculated by propagation of the standard error of the linear regression.

primer complex (Figure 2) that contains one of the two compounds, β-*c*FaPydG (ODN 5) or α-*c*FaPydG (ODN 4), at position X, respectively.

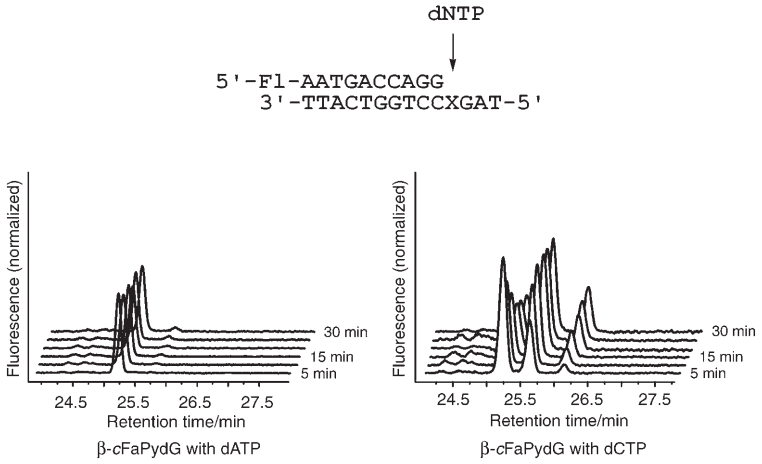


Figure 2. Capillary electrophoresis coupled to laser-induced fluorescence detection of the elongation reaction of a fluorescent-labelled primer with a β-*c*FaPydG that contains a template by using *Bst* Pol I as the polymerase (conditions: 0.2 μM primer, template 0.4 μM ODN 5, 3 nM *Bst* Pol I, 0.2 mM dATP or dCTP).

The template was hybridized to the fluorescently (Fl) labelled primer so that the first base that is read by the polymerase for replication is the DNA lesion analogue (standing start conditions).[18,19] For the analysis of the fluorescence-labelled primer and its extension products, capillary electrophoresis that was coupled to a laser-induced fluorescence detector was employed. This allows exact quantification of the extension products with single-base resolution.

In the first experiments, we studied how the lesion analogues influence the fidelity of the polymerase *Bst* Pol I from *Geobacillus stearothermophilus*. The experiment was conducted by using 0.2 μM primer, 0.4 μM template, 3 nM *Bst* Pol I and 0.2 mM dNTP at 25 °C. Every 5 min, aliquots from the assay were quenched and subsequently analyzed by using capillary electrophoresis. Figure 2 depicts the chromatograms of the primer extension reaction with the template that contains β-cFaPydG with dATP and dCTP. The primer strand was detected with a retention time of 25.2 min, while the elongation products appear at a slightly longer retention time. An efficient elongation is clearly taking place in the presence of dCTP, which is nicely inserted opposite β-*c*FaPydG. This is in full agreement with our earlier data.[10] The increasing $n+2$ peak in the right part of Figure 2 is caused by the sequence of the template strand ODN 5. At the position after the lesion analogue, a dG base is instructing the incorporation of a second dCTP, so that in our case, the primer is elongated stepwise by two bases: First the incorporation opposite the β-*c*FaPydG lesion is observed, then a second dC is incorporated in a regular extension step. To avoid the $n+2$ peak, we changed the base that follows the lesion to dC, and indeed only one incorporation step opposite the lesion analogue is observed (data not shown). In experiments in which dATP was used instead of dCTP, one observes only very inefficient incorporation of dATP opposite the β-*c*FaPydG lesion. These results are in agreement with a recent primer-extension study with a FaPydG triphosphate, which showed preferred incorporation of the FaPydG triphosphate opposite dC.[20] The observation is also in agreement with our own primer-extension data,[10] and with recent in vivo studies.[21] In summary, β-*c*FaPydG has very low mutagenic potential in the replication process.

To our surprise, when the primer extension was conducted under the same conditions by using the DNA template that contains α-*c*FaPydG (ODN 4), no primer extension could be observed. Even extensive screening of different enzyme concentrations (1, 5, 10, 100 nM) at 50 μM dNTP revealed no

elongation within 3.0 min of the assay time regardless of the dNTP that was used. We also studied the low-fidelity polymerases Pol η and DinB to investigate if the blocking character of α-*c*FaPydG is limited to high-fidelity polymerase. These polymerases are able read through certain DNA lesions, and belong to the recently discovered Y-family of polymerases.[22] To our surprise, the same blocking effect was also observed with *S. cerevisiae* Pol η and with DinB from *Geobacillus stearothermophilus*. Even after increasing the enzyme concentration by a factor of 100, no primer extension was obtained; this shows that the α anomer is a very efficient replication blocker for high- and low-fidelity polymerases.

We finally decided to drastically increase the reaction time and the triphosphate concentration. Indeed at a final concentration of 6.5 μM *Bst* Pol I (about 2100 times higher than in case of the β-*c*FaPydG), 2 mM dNTPs and 1.5 h reaction time we could measure an elongation past the lesion (see Figure 3). After 90 min reaction time, 55% of the primer starting material was elongated under these enforced conditions with dCTP, but only 28% was elongated with dATP. We observed 10% elongation if we used dGTP, and only 4% elongation was observed with dTTP. To our surprise, dCTP remained the "best" triphosphate, followed by dATP. Under these extremely harsh conditions, the high-fidelity polymerase is able to read through the α-*c*FaPydG compound. dCTP, and to a lesser extent dATP, are preferentially inserted.

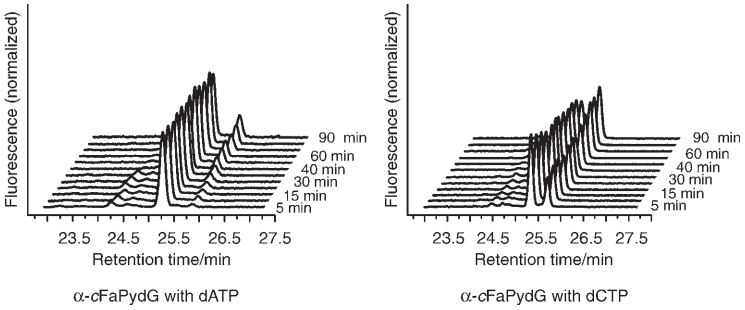


Figure 3. Capillary electrophoresis with laser-induced fluorescence detection of the elongation reaction of a fluorescent-labelled primer with α-*c*FaPydG that contained template by using *Bst* Pol I (conditions: 0.2 μM primer, 0.4 μM template ODN 4, 6.5 μM *Bst* Pol I, 2 mM dNTP).

Outlook and Conclusions

In this publication, we report the synthesis of the α-*c*FaPydG cyclopentane analogue of the FaPydG lesion, its incorporation into DNA strands, base-pairing properties and finally primer-extension data. As shown by previous studies from our group and other laboratories, the favoured base pair of the β-*c*FaPydG lesion is formed with dC. The β isomer destabilizes the DNA, but it does not stop the polymerase, and it fully retains the coding potential of dG. The β version of the lesion is, therefore, not strongly mutagenic. In the case of α-*c*FaPydG, we observed different properties: The thermodynamic data show that this anomer strongly destabilizes the duplex irrespective of the counter base. One possible explanation for this behaviour is that the α-configured base might be outside the helix, and, therefore, unable to form a base pair inside the base stack. In primer extension studies, α-*c*FaPydG turned out to be a strong blocking unit for high- (*Bst* Pol I) and low-fidelity polymerases (DinB and Pol η), but if the enzyme, triphosphate concentrations and the reaction time are strongly increased, dCTP is incoporated by *Bst* Pol I.

The presented data allow a direct comparison between the α and β version of a lesion that is based on the studies of appropriate model compounds for the first time. From these analogue studies, it is clear that the β version of the lesion is neither blocking nor mutagenic, but the α version is mainly blocking. Because the role of the anomerization of the natural FaPydG lesion is still unknown in biological systems, the results presented here indicate that the rate of anomerization is an important parameter that needs to be investigated in more detail in duplex DNA. This is in accordance with recent in vivo experiments in which a mixture of both anomers was employed.

Experimental Section

Chemicals were purchased from Sigma–Aldrich, Acros or Lancaster and were used without further purification. Solvents were of reagent grade and were purified by standard methods. The reactions were monitored on Merck Silica 60 F_{254} TLC plates. Detection was carried out by irradiation with UV light (254 nm) and staining with 0.5% $KMnO_4$ in aq. NaOH (1 N) or acidic anisaldehyde solution. Flash chromatography was performed on Silica 60 (Merck, 230–400 mesh). NMR spectra were recorded on the following spectrometers: Varian Oxford 200, Bruker AC 300, Varian XL 400 and Bruker AMX 600. Chemical shifts (δ) are given in ppm, the coupling constants (J) in Hz. Mass spectra were recorded on the following machines: Finnigan MAT 95 (EI), Bruker Autoflex II (MALDI-TOF) and Thermo Finnigan LTQ-FT (ESI-ICR). IR spectra were measured on a Nicolet 510 FTIR spectrometer in a KBr matrix or with a diamond-ATR (attenuated total reflection) setup. DNA synthesis was performed on a PerSeptive Biosystems Expedite 8900 Synthesizer and an Äkta Oligopilot 10 (Amersham Biosciences). Analysis and purification of the oligonucleotides was performed on Merck LaChrome HPLC systems with UV and diode array detectors by using 5 μ silica-C_{18} RP columns and 0.1 M NEt_3/AcOH in H_2O/MeCN as eluent. UV spectra and melting points were measured on a Cary 100 UV/Vis spectrometer by using 1 mL quartz cuvettes with 1 cm pathlength. The samples contained NaCl (150 mM), Tris-HCl (10 mM, pH 7.4) and 0.3–27 μM of each oligonucleotide. For every strand, five temperature cycles from 85 to 0 °C were recorded. The melting point was calculated computationally by using Microcal Origin. ESI spectra of DNA strands were measured in flow injection analysis mode or coupled to chromatographic separation (eluent: 2 mM NEt_3/AcOH in H_2O/MeCN). In flow injection mode, a 2 μL sample (30 μM DNA, 100 mM NH_4OAc) was injected in a steady flow of H_2O/MeCN (8:2; 200 μL min^{-1}). The capillary temperature was 300 °C, spray voltage 4–5 kV (negative mode).

(1*R*,3*S*,4*R*)-3-(Benzyloxy)-4-[(benzyloxy)methyl]cyclopentane-1-tosylate (5): (1*R*,3*S*,4*R*)-3-Benzyloxy-4-benzyloxymethylcyclopentan-1-ol **4** (7 g, 22.4 mmol) was dissolved in dry pyridine (30 mL) and cooled to 0 °C. *p*-

Toluenesulfonyl chloride (6.4 g, 33.6 mmol) was added in portions over a period of 30 min. After the addition, the reaction was stirred at room temperature for 18 h. The suspension was diluted with ethyl acetate (300 mL) and H_2O (200 mL). The organic phase was separated, washed with sat. NH_4Cl solution (3×200 mL), brine (100 mL) and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography (silica gel (500 mL), hexane/ethyl acetate 5:1) to give **5** (8.2 g, 17.6 mmol, 79%) as a colourless oil. R_f=0.26 (20% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.79 (m, 2H), 7.38–7.27 (m, 12H), 5.05–4.99 (m, 1H), 4.50 (s, 2H), 4.45 (s, 2H), 3.96–3.92 (m, 1H), 3.48–3.40 (td, 2H, J=6.3 Hz), 2.45 (s, 3H), 2.33–2.20 (m, 2H), 2.14–2.01 (m, 2H), 1.69–1.62 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=144.5, 138.2 (2C), 129.7, 128.3 (2C), 127.7 (4C), 127.5 (4C), 127.4 (4C), 82.1, 80.1, 73.0, 71.6, 71.2, 44.0, 38.8, 34.0, 21.5 ppm; IR (ATR): $\tilde{\nu}$= 3436 (br), 3063 (s), 3031 (m), 2927 (m), 2862 (m), 1598 (m), 1454 (m), 1360 (s), 1189 (s), 1175 (vs), 1096 (s), 988 (m), 894 (s), 668 cm^{-1}; HRMS (ESI$^+$): m/z: calcd for $C_{27}H_{30}O_5S$: 466.1814; found: 466.1799 $[M]^+$.

(1*S*,3*S*,4*R*)-3-(Benzyloxy)-4-[(benzyloxy)methyl]cyclopentane-1-azide (6): Compound **5** (8.0 g, 17.1 mmol) was dissolved in dry DMF (30 mL) and NaN_3 (12.2 g, 18.8 mmol) was added. The mixture was stirred at 60°C for 14 h. After the addition of ethyl acetate (300 mL), the organic layer was washed with sat. $NaHCO_3$ solution (2×100 mL) and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel (100 mL), hexane/ethyl acetate/$CHCl_3$ 10:1:0.5) to give **6** (5.4 g, 16 mmol, 94%) as a colourless oil. R_f=0.54 (20% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.39–7.25 (m, 10H), 4.52 (dd, J=26.0, 18.6 Hz, 2H), 4.49 (s, 2H), 3.97–3.89 (m, 1H), 3.86 (td, J=7.0, 5.1 Hz, 1H), 3.44 (d, J=5.5 Hz, 2H), 2.54–2.43 (m, 1H), 2.24 (td, J=13.8, 6.8 Hz, 1H), 1.99 (dddd, J=13.4, 8.7, 4.7, 1.3 Hz, 1H), 1.84 ppm (dddd, J=28.2, 20.8, 9.5, 4.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=138.2 (2C), 129.7 (2C), 128.3 (2C), 127.7 (2C), 127.5 (3C), 127.4, 80.1, 73.0, 71.6, 71.2, 44.0, 38.8, 34.0, 21.5 ppm; IR (ATR): $\tilde{\nu}$=3064 (w), 3030 (w), 2931 (m), 2858 (m), 2097 (vs), 1730 (w), 1491 (w), 1454 (m), 1360 (m), 1263 (m), 1206 (w), 1097 (s), 1028 (m), 736 (s), 697 cm^{-1}; HRMS (ESI$^+$): m/z: calcd for $C_{20}H_{23}N_3O_2$: 337.1790; found: 337.1591 $[M]^+$.

(1*S*,3*S*,4*R*)-3-(Hydroxy)-4-[(hydroxy)methyl]cyclopentan-1-azide (7): Compound **6** (5.2 g, 15.4 mmol) was dissolved in dry CH_2Cl_2 (100 mL) and cooled to −78°C. A solution of 1 M BCl_3 (200 mL) in CH_2Cl_2 was added by means of a dropping funnel over a period of 45 min and the mixture was stirred at −78°C for 3 h, then warmed up to room temperature. The reaction was quenched with dry MeOH (80 mL) at −78°C and was allowed to warm up to room temperature overnight. The solvents were evaporated and the residue was purified on silica gel (10 cm pad of 350 mL silica gel, 10% MeOH in $CHCl_3$). Product **7** (2.4 g, 15.3 mmol, 99%) was obtained as a yellow oil. R_f=0.27 (10% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.07 (dd, J=13.3, 6.0 Hz, 1H), 4.02–3.95 (m, 1H), 3.79 (ddd, J=10.4, 5.2, 3.0 Hz, 1H), 3.56 (dd, J=10.4, 8.0 Hz, 1H), 2.36–2.20 (m, 4H), 1.99–1.92 (m, 1H), 1.77 (dddd, J=14.0, 6.0, 4.5, 1.6 Hz, 1H), 1.58 ppm (ddd, J=13.9, 9.8, 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=75.8, 65.0, 60.2, 48.4, 40.7, 33.3 ppm; IR (ATR): $\tilde{\nu}$=3351 (vs), 2935 (s), 2499 (w), 2103 (vs), 1651 (w), 1442 (m), 1338 (m), 1261 (s), 1165 (m), 1076 (s), 1048 (s), 941 (m), 878 (w), 687 (m), 559 cm^{-1}; HRMS (ESI$^-$): m/z: calcd for $C_6H_{11}N_3O_2Cl$: 192.0545; found: 192.0544 $[M+Cl]^-$.

(1*S*,3*S*,4*R*)-3-(*tert*-Butyldimethylsilanyloxy)-4-[(*tert*-butyldimethylsilanyloxy)methyl]cyclopentane-1-azide (8): Compound **7** (2.4 g, 15.3 mmol) was dissolved in dry DMF (20 mL), and imidazole (2.61 g, 38.3 mmol) was added at room temperature. After the portionwise addition of TBDMSCl (5.77 g, 38.3 mmol) at 0°C, the mixture was stirred at room temperature for 16 h. The reaction was diluted with CH_2Cl_2 (200 mL), washed once with sat. NH_4Cl (150 mL) and with brine (100 mL) and dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by flash chromatography (300 mL silica gel, 5% ethyl acetate in hexane) to give compound **8** (5.1 g, 13.2 mmol, 86%) as colourless oil. R_f=0.59 (ethyl acetate/hexane 20:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.04 (q, J=6.5, 6.5, 6.5 Hz, 1H), 3.87–3.77 (m, 1H), 3.60–3.52 (m, 2H), 2.23 (td, J=13.7, 6.9, 6.9 Hz, 1H), 2.10 (tdt, J=8.6, 8.6, 6.2, 4.4, 4.4 Hz, 1H), 1.90–1.74 (m, 2H), 1.72–1.63 (m, 1H), 0.88 (s, 18H), 0.05 (s, 6H), 0.03 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=73.1, 62.6, 59.5, 49.1, 41.2, 32.7, 25.9 (3C), 25.8 (3C), 18.3, 18.0, −4.6, −4.8, −5.4, −5.5 ppm; IR (ATR): $\tilde{\nu}$=2956 (s), 2930 (s), 2896 (m), 2858 (s), 2098 (s), 1472 (m), 1464 (m), 1389 (w), 1361 (w), 1257 (s), 1108 (s), 1006 (m), 939 (w), 874 (m), 836 (vs), 813 (m), 776 (s), 669 cm^{-1} (w); HRMS (ESI$^+$): m/z: calcd for $C_{18}H_{39}N_3O_2Si_2$: 385.2581; found: 385.2579 $[M]^+$.

(1*S*,3*S*,4*R*)-3-(*tert*-Butyldimethylsilanyloxy)-4-[(*tert*-butyldimethylsilanyloxy)methyl]cyclopentane-1-amine (9): 10% Pd/C (300 mg) was added to a suspension of compound **8** (2.94 g, 7.6 mmol) in dry EtOH (20 mL). The reaction was evacuated twice to exchange the inert gas atmosphere, and then connected to two balloons that were filled with H_2. The suspension was vigorously stirred at room temperature for 15 h. The palladium catalyst was removed by using a PTFE-Filter (Whatman Puradisc) and the solvent was removed under reduced pressure. The amine **9** (2.73 g, 6.0 mmol, quant.) was obtained as a colourless liquid, and was used without further purification. R_f=0.31 (10% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.04 (dd, J=10.6, 5.1 Hz, 1H), 3.47 (ddd, J=28.0, 10.0, 5.4 Hz, 2H), 3.29 (ddd, J=11.7, 6.8, 4.9 Hz, 1H), 2.21–2.12 (m, 1H), 2.03 (td, J=12.8, 6.3, 6.3 Hz, 1H), 1.75–1.67 (m, 3H), 1.61 (ddd, J=13.5, 9.1, 4.4 Hz, 1H), 1.44 (td, J=13.0, 5.2, 5.2 Hz, 1H), 0.87 (s, 9H), 0.86 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H), 0.02 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=74.9, 64.0, 51.2, 49.6, 45.1, 37.7, 25.9 (3C), 25.8 (3C), 18.3, 18.0, −4.6, −4.8, −5.4, −5.5 ppm; IR (ATR): $\tilde{\nu}$=2956 (s), 2930 (s), 2887 (m), 2858 (s), 1472 (m), 1464 (m), 1389 (w), 1361 (w), 1257 (s), 1103 (s), 1006 (m), 939 (w), 874 (m), 836 (vs), 813 (m), 775 (s), 668 cm^{-1} (w); HRMS (ESI$^+$): m/z: calcd for $C_{18}H_{41}NO_2Si_2$: 359.2676; found: 359.2679 $[M]^+$.

***N*-{4-[3-(*tert*-Butyldimethylsilanyloxy)-4-[(*tert*-butyldimethylsilanyloxy)-methyl]cyclopentylamino]-5-nitro-6-oxo-1,6-dihydropyrimidin-2-yl}acetamide (11)**: The amine **9** (2.73 g, 7.6 mmol) was dissolved in dry DMF (50 mL) and treated with diisopropylethylamine (1.5 mL, 8.4 mmol) and the heterocycle chloride **10** (1.77 g, 7.6 mmol, 1 equiv). This reaction was first stirred at room temperature for 30 min then at 60°C for 45 min. TLC analysis with ninhydrin staining indicated the completion of the reaction. The mixture was diluted with ethyl acetate (300 mL) and the organic phase was washed with brine (3×). After drying over $MgSO_4$, the solvent was evaporated and the residue was dried overnight under high vacuum. Purification by flash chromatography (silica gel (500 mL), 5% MeOH in $CHCl_3$) gave **11** (3.0 g, 5.4 mmol, 71%) as a yellow powder. R_f=0.38 (10% MeOH in $CHCl_3$); m.p. 220–225°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=11.43 (brs, 1H), 10.36 (brs, 1H), 10.00 (d, J=7.8 Hz, 1H), 4.62–4.52 (m, 1H), 4.18 (td, J=5.5, 2.8, 2.8 Hz, 1H), 3.51 (dd, J=10.1, 5.8 Hz, 1H), 3.38 (dd, J=10.1, 6.1 Hz, 1H), 2.29 (s, 3H), 2.22 (m, 1H), 2.00 (ddd, J=13.7, 6.7, 5.6 Hz, 1H), 1.90–1.82 (m, 1H), 1.71 (m, 2H), 0.87 (s, 18H), 0.07 (s, 3H), 0.06 (s, 3H), 0.03 (s, 3H), 0.02 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.6, 157.4, 155.2, 149.8, 112.9, 75.6, 64.0, 52.4, 49.9, 41.7, 35.0, 25.9 (6C), 18.3, 18.2, −4.6 (2C), −5.4, −5.5 ppm; IR (ATR): $\tilde{\nu}$=3436 (m), 3214 (m), 2955 (s), 2931 (s), 2887 (m), 2858 (s), 1669 (vs), 1623 (vs), 1589 (vs), 1537 (s), 1472 (m), 1427 (m), 1373 (m), 1330 (m), 1232 (s), 1151 (w), 1108 (m), 1034 (w), 1005 (w), 939 (vw), 837 (s), 776 (m), 699 cm^{-1} (w); HRMS (ESI$^+$): m/z: calcd for $C_{24}H_{45}N_5O_6Si_2$: 556.2987; found: 556.2993 $[M+H]^+$.

***N*-{4-[3-(*tert*-Butyldimethylsilanyloxy)-4-[(*tert*-butyldimethylsilanyloxy)-methyl]cyclopentylamino]-5-formylamino-6-oxo-1,6-dihydropyrimidin-2-yl}acetamide (12)**: Compound **11** (1.9 g, 3.4 mmol) was dissolved in dry EtOH (30 mL) and CH_2Cl_2 (5 mL). The reaction was evacuated twice to exchange the inert gas atmosphere and then connected to two balloons that were filled with H_2. The suspension was vigorously stirred at room temperature for 15 h. The palladium catalyst was removed by using a PTFE-Filter (Whatman Puradisc) and the solvent was removed under reduced pressure. The amine was obtained as a colourless powder, which was directly converted into the formamide; the residue was dissolved in dry THF (50 mL) and dry pyridine (30 mL). EDC·HCl (1.7 g, 8.8 mmol) was added in one portion and after the majority of the suspension was dissolved, formic acid (162 μL, 4.3 mmol) was introduced. The reaction was stirred for 10 h, and then the solvent was removed under reduced pressure. The obtained residue was dissolved in ethyl acetate (200 mL)

and washed with H_2O (2×100 mL), sat. $NaHCO_3$ solution (2×100 mL) and brine (100 mL). After drying over $MgSO_4$, the solvent was evaporated and the residue was purified by flash chromatography (silica gel (350 mL), 5% MeOH in $CHCl_3$). Compound **12** (1.5 g, 2.7 mmol, 79%) was isolated as yellow powder after recrystallization from heptane. R_f= 0.22 (10% MeOH in $CHCl_3$); m.p. 115–119°C; ^{1}H NMR (400 MHz, DMSO): δ=11.27 (brs, 2H), 8.73 (d, J=1.6 Hz, 0.61H), 8.04 (d, J= 1.6 Hz, 0.39H), 8.04 (d, J=11.3 Hz, 0.61H), 7.80 (d, J=11.5 Hz, 0.39H), 6.55 (d, J=8.2 Hz, 1H), 6.18 (d, J=8.3 Hz, 1H), 4.37 (qd, J=14.3, 7.17, 7.17, 6.86 Hz, 1H), 3.98–3.90 (m, 1H), 2.15 (s, 3H), 2.18–2.02 (m, 2H), 1.77–1.61 (m, 2H), 1.53 (ddd, J=25.82, 12.87, 6.47 Hz, 1H), 0.86 (s, 9H), 0.85 (s, 9H), 0.03 (s, 3H), 0.03 (s, 3H), 0.03 (s, 3H), 0.02 ppm (s, 3H); ^{13}C NMR (100 MHz, DMSO): δ=174.1, 174.0, 166.8, 161.3, 158.5, 157.5, 157.4, 148.9, 148.8, 74.4, 63.8, 49.4, 49.1, 42.2, 34.6, 26.2 (3C), 24.3, 18.4, 18.2, −4.2, −4.4, −5.0 ppm (2C); IR (ATR): $\tilde{\nu}$=3436 (s), 2956 (m), 2950 (m), 2858 (m), 1652 (s), 1472 (w), 1254 (m), 1088 (w), 837 (m), 777 cm^{-1} (m); HRMS (ESI$^+$): m/z: calcd for $C_{25}H_{47}N_5O_5Si_2$: 554.3194; found: 554.3190 $[M+H^+]^+$. The *cis*/*trans* isomers of the formyl group are responsible for the signals at δ=8.67/8.04 and 8.04/7.8 ppm.

***N*-{4-[3-(hydroxy)-4-[(hydroxy)methyl]cyclopentylamino]-5-formylamino-6-oxo-1,6-dihydropyrimidin-2-yl}acetamide (13)**: Compound **12** (250 mg, 0.45 mmol) was dissolved in dry THF (4.5 mL) and pyridine (125 μL) in a 10 mL plastic tube. After addition of HF–pyridine complex (125 μL), the reaction was stirred overnight. The resulting orange precipitate was centrifuged, and the supernatant was decanted off. The solvent was discarded. Traces of HF–pyridine in the precipitate were quenched by adding methoxytrimethylsilane (0.6 mL). The crude product was washed twice with dry THF, and dried completely for 2 d under high vacuum. Compound **13** (145 mg, 0.9 mmol, quant.) was obtained as a yellow powder. R_f=0.67 (C18-TLC, MeOH/H_2O 1:1); m.p. 152–155°C; ^{1}H NMR (400 MHz, DMSO): δ=11.34 (brs, 1H), 11.31 (brs, 1H), 8.75 (d, J= 1.4 Hz, 0.61H), 8.12 (d, J=11.5 Hz, 0.37H), 8.06 (d, J=1.5 Hz, 0.65H), 7.81 (d, J=11.5 Hz, 0.37H), 6.57 (d, J=8.2 Hz, 0.33H), 6.27 (d, J= 8.2 Hz, 0.62H), 4.76 (dd, J=11.5, 4.2 Hz, 1H), 4.49 (brs, 1H), 4.33 (sept., J=7.4, 7.4, 7.4, 7.4, 7.4 Hz, 1H), 3.83–3.74 (m, 1H), 3.43–3.23 (m, 2H), 2.15 (s, 3H), 2.07 (td, J=12.8, 6.4, 6.4 Hz, 1H), 2.01–1.91 (m, 1H), 1.73–1.62 (m, 2H), 1.50 ppm (ddd, J=26.6, 13.1, 6.7 Hz, 1H); ^{13}C NMR (75 MHz, DMSO): δ=202.7, 173.9, 161.1, 157.2, 113.6, 92.8, 67.4, 62.9, 49.6, 48.8, 35.0 (2C), 25.5, 24.2 ppm; IR (ATR): $\tilde{\nu}$=3420 (s), 2929 (w), 1645 (vs), 1587 (s), 1505 (m), 1440 (w), 1422 (m), 1375 (w), 1241 (s), 1152 (w), 1044 (m), 1013 (w), 879 (w), 710 (w), 563 cm^{-1} (w); HRMS (ESI$^-$): m/z: calcd for $C_{13}H_{18}N_5O_5$: 324.1308; found: 324.1315 $[M-H]^-$.

***N*-{4-[3-(hydroxy)-4-[(dimethoxytrityloxy)methyl]cyclopentylamino]-5-formylamino-6-oxo-1,6-dihydropyrimidin-2-yl}acetamide (14)**: Compound **13** (352 mg, 1.08 mmol) was dissolved in pyridine (15 mL) at room temperature. DMTCl (477 mg, 1.41 mmol) was added in 3 portions (2× 0.5 equiv + 1×0.3 equiv) every 30 min. After 3.5 h stirring, TLC analysis indicated that the reaction was complete and the mixture was quenched with MeOH (3 mL). The solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography (silica gel, 10% MeOH and 5% pyridine in $CHCl_3$. The DMT-protected carbocycle **14** (500 mg, 7.97 mmol, 74%) was obtained as a powder. R_f=0.16 (10% MeOH in $CHCl_3$); m.p. 145–148°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 11.34 (brs, 1H), 11.31 (brs, 1H), 8.75 (d, J=1.30 Hz, 0.61H), 8.12 (d, J= 11.5 Hz, 0.39H), 8.06 (d, J=1.5 Hz, 0.61H), 7.98 (d, J=11.5 Hz, 0.39H), 7.24–7.17 (m, 9H), 6.75–6.71 (m, 4H), 6.14 (d, J=8.2 Hz, 0.33H), 6.05 (d, J=8.2 Hz, 0.62H), 4.46–4.30 (m, 1H), 3.99–3.89 (m, 1H), 3.70 (s, 6H), 3.00–2.89 (m, 2H), 2.10 (s, 3H), 2.35–2.25 (m, 1H), 2.13–1.98 (m, 2H), 1.83 (ddd, J=13.0, 8.9, 4.1 Hz, 1H), 1.63–1.52 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.6, 159.8, 157.8 (2C), 157.1, 155.2, 149.2, 144.6, 135.6, 135.4, 129.4 (4C), 127.5, 127.2, 126.1 (2C), 123.2, 12.5 (4C), 94.1, 85.2, 74.6, 64.9, 54.6, 49.8, 46.2, 41.4, 35.3, 23.5 ppm; IR (ATR): $\tilde{\nu}$=3436 (s), 2920 (s), 1732 (m), 1650 (m), 1445 (w), 1384 (m), 1249 (m), 1156 (w), 1070 (m), 612 cm^{-1} (w); HRMS (ESI$^+$): m/z: calcd for $C_{34}H_{38}N_5O_7$: 628.2771; found: 628.2776 $[M+H]^+$.

***N*-{4-[3-(hydroxy)-4-[(dimethoxytrityloxy)methyl]cyclopentylamino]-5-formylamino-6-oxo-1,6-dihydropyrimidin-2-yl}acetamide phosphoramidite (15)**: Compound **14** (420 mg, 0.67 mmol), tetrazolate (20 mg, 0.12 mmol) and 2-cyanoethyl-tetraisopropylphosphordiamidite (636 μL, 2.0 mmol) were suspended in acetonitrile (15 mL) and stirred for 6 h under an argon atmosphere at room temperature. The acetonitrile was evaporated under reduced pressure. The crude product was purified by flash chromatography (50 mL of deactivated silica gel (the silica gel was suspended in 1% pyridine in dichloromethane, stirred for 1 h, evaporated and dried in an oven overnight) 8 cm pad, 5% MeOH in $CHCl_3$ + 5% pyridine). The phosphoramidite **15** (468 mg, 0.57 mmol, 84%) was obtained as a colourless film. R_f=0.27 (10% MeOH in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.64–8.60 (m, 1H), 8.22 (s, 1H), 7.71 (m, 1H), 7.67 (tt, J=7.6, 7.6, 1.8, 1.8 Hz, 1H), 7.49–7.40 (m, 2H), 7.36–7.25 (m, 6H), 7.21 (t, J=7.4, 7.4 Hz, 1H), 6.86–6.76 (m, 3H), 6.33 (t, J=7.1, 7.1 Hz, 1H), 4.40–4.08 (m, 2H), 3.83–3.71 (m, 5H), 3.70–3.46 (m, 2H), 3.20–3.01 (m, 2H), 2.63 (m, 2H), 2.52 (t, J=6.0, 6.0 Hz, 1H), 2.39–2.30 (m, 2H), 2.21–2.05 (m, 3H), 2.05–1.80 (m, 2H), 1.75–1.60 (m, 1H), 1.53–1.28 (m, 2H), 1.28–1.23 (m, 2H), 1.24–1.18 (m, 2H), 1.18–1.06 (m, 6H), 1.04 (d, J=6.8 Hz, 2H), 0.90 ppm (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=172.1, 171.8, 159.9, 168.6 (2C), 154.8, 154.7, 150.0, 145.5, 136.6, 136.2, 131.1, 130.4 (2C), 130.3, 128.5, 128.4 (2C), 126.9, 123.9, 118.2, 118.0, 113.4, 113.2, 86.0 (2C), 75.6, 75.5, 64.3, 64.1, 58.0, 57.9, 57.4, 57.3, 55.4, 50.7, 50.4, 50.3, 45.8, 45.5, 43.3 (2C), 43.2 (2C), 41.1, 41.0, 35.0, 34.7, 24.9 (2C), 24.8 (2C), 24.7 (2C), 24.6 (2C), 20.8, 20.6 ppm; ^{31}P NMR (81 MHz, $CDCl_3$): δ=147.5, 148,4 ppm; IR (ATR): $\tilde{\nu}$=3423 (s), 3242 (s), 2962 (m), 1655 (s), 1584 (s), 1510 (s), 1444 (m), 1415 (m), 1365 (w), 1250 (s), 977 (w), 728 cm^{-1} (w); HRMS (ESI$^-$): m/z: calcd for $C_{43}H_{53}N_7O_8P$: 828.3693; found: 828.3696 $[M-H]^-$.

DNA synthesis: Oligonucleotide synthesis was performed on an ÄKTA Oligopilot 10 from Amersham Biosciences by using standard DNA synthesis conditions (deblocking solution: 2.5% dichloroacetic acid in toluene; activator: 0.25 M 5-benzylmercaptotetrazole in acetonitrile; oxidation: 150 mM I_2 in 2,6-lutidine/MeCN/H_2O 1:11:5; capping solutions: 1 M Ac_2O, 2,6-lutidin (11%) in acetonitrile and *N*-methylimidazole (16%) in MeCN. Ultra-mild phosphoramidites for dA, dC, dG, dT and CPG carriers were obtained from Amersham, Glen Research or PE Biosystems. Because oligonucleotides that contained the *c*FaPydG building block were not compatible with the regular capping procedure, the standard capping solutions were replaced by 2,6-lutidine/isobutyrylanhydride/THF 1:1:8, and *N*-methylimidazole (16%) in acetonitrile, and were used for all couplings after the modification. The coupling of α-cFaPydG itself was carried out by using an eight-fold excess of phosphoramidite in a double-coupling protocol with 15 min for each step. After the modification, all the phosphoramidites were also coupled with a double-coupling protocol by using 10 equiv for 3 min. The DNA was cleaved off of the solid support by using EtOH and sat. NH_3 solution (1:3) at 15°C overnight. The DNA was purified by RP-HPLC by using a gradient of 0.1 M triethylammoniumacetate in H_2O/0.1 M triethylammoniumacetate in MeCN/H_2O 80:20. The purity of the strands was verified by analytical RP-HPLC and MALDI-TOF or ESI-FTICR mass spectrometry.

Enzymatic digestion of the DNA: For the enzymatic digestion, the DNA (ca. 10 μg in 100 μL to clarify the mutagenic potential of the FaPydG lesion) was incubated in 10 μL of buffer (300 mM ammonium acetate, 100 mM $CaCl_2$, 1 mM $ZnSO_4$, pH 5.7), 22 units Nuclease P1 (*Penicilinum citrium*) and 0.05 units of calf spleen phosphodiesterase II. The sample was shaken at 37°C for 3 h. The digest was completed by adding 12 μL buffer (500 mM Tris-HCl, 1 mM EDTA), 10 units of alkaline phosphatase (CIP) and 0.1 units of snake venom phosphodiesterase I (*Crotalus adamanteus* venom). The sample was shaken for another 3 h at 37°C. For workup, 6 μL of 0.1 M HCl was added and the probes were centrifuged (6000 rpm, 5 min.). The digest was analyzed by HPLC (Interchim Interchrom Uptisphere 3 HDO column (150×2.1 mm), Buffer A: 2 mM NEt_3/HOAc in H_2O; Buffer B: 2 mM NEt_3/HOAc in H_2O/MeCN 20:80; 0→ 30 min; 0→30% B; 30→32 min; 30→100% B; 32→36 min; 100% B; 36→38 min; 100→0% B; 38→60 min; 0% B; flow: 0.2 mL min^{-1}). The different peaks were assigned by co-injection, UV and FTICR-HPLC-MS-MS, by using the same conditions. The incorporated *c*FaPydG could be detected by LCMS/MS and FTICR-ESI; calcd mass for α-*c*FaPydG, $C_{13}H_{20}N_5O_6^-$: m/z: 342.1419; found: 342.1416 $[M+AcOH-H^+]^-$; further signals of the lesion: FTICR-ESI$^+$: m/z: calcd for $C_{11}H_{16}N_5O_4^-$: 282.1208;

found: 282.1225 $[M-H^+]^-$; calcd for $C_{10}H_{16}N_5O_3^-$: 254.1259; found: 254.1221 $[M-CO-H^+]^-$.

Primer extensions: For primer extensions, a solution of template (4 μM) and primer (2 μM) in buffer (KCl 50 mM, 10 mM K_3PO_4/HCl pH 7.5) was heated to 80 °C and allowed to cool to 5 °C over several hours, then stored at that temperature for the experiments. For the experiments with concentrations as described in the text, primer/template solution (3 μL), enzyme solution (3 μL) and dNTP solution (3 μL) were added to 21 μL reaction buffer (Pol η: 100 mM KCl, 5 mM $MgCl_2$, 10 mM DTT, 2.5 vol% glycerin, 40 mM Tris-HCl pH 7.4, 2.5 vol% BSA (10 mg mL^{-1}); DinB: 5 mM $MgCl_2$, 10 mM DTT, 250 μg mL^{-1} BSA, 2.5 vol% glycerin, 40 mM, pH 8 Tris-HCl; *Bst* Pol I: 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, 20 mM Tris-HCl buffer pH 8.8). The reaction was quenched by adding 100 mM EDTA solution (30 μL, pH 7.4) and incubation at 95 °C for 15 min. After centrifugation at 5000 rpm, the DNA was extracted from the supernatant by using ZipTip (Millipore). The eluted probes (ca. 10 μL) were diluted with ddH_2O (40 μL) and analyzed by using capillary electrophoresis (9 kV for 30 min).

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