Synthesis, Stability, and Conformation of the Formamidopyrimidine G DNA Lesion

Lars T. Burgdorf and Thomas Carell*[a]

Abstract: The formamidopyrimidine (FapydGua) lesion, derived from the nucleobase guanine, is a major DNA lesion involved in mutagenesis and carcinogenesis. To date, the chemical information available about this main lesion is very limited. Herein, we describe a synthesis and a detailed characterization of the acetyl-protected monomer of the FapydGua lesion. Stability studies in DMSO and in water/acetonitrile show that the N-glycosidic bond, previously thought to be highly labile, is much more stable than anticipated. Decomposition of the FapydGua lesion proceeds with half-life times of 37.8 h for the β-anomer and 65.2 h for the α-anomer in water/acetonitrile. The relaxation time for the anomerization reaction was determined to $\tau = 6.5$ h at room temperature. Most important, it was found that the formamido group, which is critical for the lesion recognition process by repair enzymes, is fixed in the *cis*-conformation in apolar solvents such as chloroform. This conformation enables the formation of a hydrogen bond between the carbonyl oxygen of the formamide and the NH of the N-glycosidic bond within the framework of a seven-membered ring system. This has consequences for the recognition of the lesion by repair enzymes (hOGG1 and Fpg protein). These enzymes were so far believed to recognize the carbonyl group of the FapydGua lesion. Our investigations show that this carbonyl group is not readily accessible because it is almost buried in the dominating *cis*-conformation. In agreement with the recent X-ray structure of hOGG1 in complex with 8-oxo-7,8-dihydroguanine-containing DNA, we can conclude that repair enzymes can contact both lesions only via the N(7)-H group, which is a hydrogen-bond acceptor in guanine.

Keywords: bioorganic chemistry · DNA cleavage · DNA damage · nucleic acids · repair enzymes

Introduction

DNA damage generated by the reaction of DNA with reactive oxygen species is one of the major causes for mutations and carcinogenesis and may play role in the pathogenesis of ageing.[1–5] Reactive oxygen species (ROS) are generated by aerobic respiration, a process which involves the stepwise reduction of molecular oxygen to water.[6] The intermediate reaction products O_2-radical anion, H_2O_2 and the highly reactive OH radical are potent electrophilic oxidants, which react rapidly with cellular DNA.[7] In DNA, guanine is the major target for oxidative DNA damage because it has the lowest oxidation potential among the DNA bases.[8–12] In the last decade a large number of such guanine derived DNA lesions were characterized.[13–23] The four compounds depicted in Figure 1 were found to be the main degradation products.[13, 24] All four compounds are substrates for a number of repair enzymes, which remove these lesions from the genome.[2, 25–33] Most of these repair enzymes are DNA glycosylases which catalyze the hydrolysis of the N-glycosidic bond.[34–36]

Among the four main lesions the formation and repair of 8-oxo-7,8-dihydroguanine (8oxoG) has been studied most extensively.[13, 24, 27–29, 31–33, 37] 8oxoG is the main OH radical degradation product formed under aerobic conditions. The lesion is stable, which allowed the chemical synthesis of a phosphoramidite building block for the site-specific incorporation into DNA.[38–41] Crystal structures of DNA double strands containing an 8oxoG:C[42] or an 8oxoG:A[43] base pair, together with the structure of an 8oxoG-containing double strand in complex with the human repair enzyme 8-oxo-7,8-dihydroguanine DNA glycosylase (hOGG1)[44] and numerous biochemical investigations[27, 45, 46] provide today detailed information about the mutagenic potential of this lesion and its repair. The 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapydGua) is another major lesion formed predominantly when oxygen is absent[13, 24, 47] by ionizing irradiation and other agents which produce reactive oxygen species. Biological repair studies with damaged DNA, show that the FapydGua lesion is also efficiently recognized and excised by OGG1.[27] The lesion is in addition repaired by a special formamidopyrimidine glycosylase (Fpg protein)[31, 48] under-

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[a] L. T. Burgdorf, Prof. Dr. T. Carell
Fachbereich Chemie
Philipps-Universität Marburg
Hans-Meerwein Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-282-2189
E-mail: carell@mailer.uni-marburg.de

lining that the FapydGua lesion is of fundamental importance in biology.

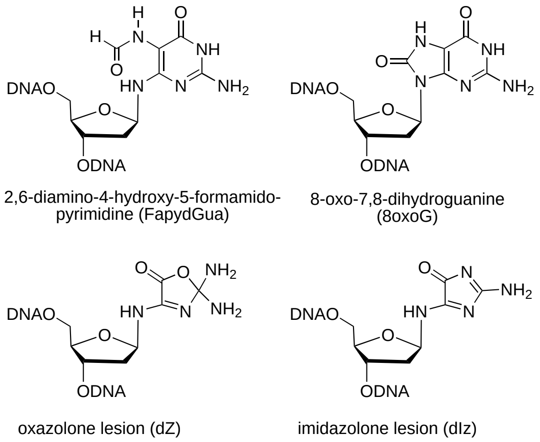
In order to investigate the biological impact of all lesions depicted in Figure 1 their chemical synthesis and incorporation into DNA is required. Towards this goal, Saito and coworkers prepared recently the 2-aminoimidazolone (dIz) lesion-containing DNA by direct irradiation of DNA single strands in the presence of riboflavin.[49–51] Greenberg and coworkers published the synthesis of a FapydGua-containing dinucleotide and the incorporation of this building block into DNA using a reverse phosphoramidite coupling protocol.[52] Today, however, neither a chemical synthesis of the pure α- and β-monomers of the FapydGua lesion nor a method that allows its direct formation in DNA are known,[14, 53, 54] which limits our current chemical and biochemical knowledge about this main DNA lesion. Even direct γ-irradiation of 2′-deoxyguanosine furnished only the rearranged α- and β-pyranosidic formamidopyrimidines in small amounts. The detection of a large amount of the “depurinated” FapydGua heterocycle established the “chemical believe” that the FapydGua lesion features a labile N-glycosidic bond, leading to rapid decomposition. As a consequence of all these studies, almost all investigations directed to elucidate repair and pairing properties of the lesion were performed with lesion analogues.

In order to gain deeper insight into the properties of the elusive FapydGua lesion, we have revisited the chemical synthesis. We were in particular interested to investigate the hypothesis that repair enzymes recognize the FapydGua- and the 8oxoG lesion by a common structural recognition motif, which allows them to distinguish both lesions simultaneously from the vast majority of guanine bases in the genome. In order to determine such a potentially common structural motif it is essential to determine the preferred conformation of the formamido group, which allows the FapydGua lesion to exist theoretically in four rotameric forms.[55, 56]

Here we report the synthesis of the FapydGua lesion as well as new NMR data, which clarify the conformation of the formamido group. Stability studies show that the FapydGua lesion is significantly more stable than originally expected.

Results and Discussion

Synthesis of the FapydGua lesion: Because of our intention to study the stability of the FapydGua lesion we targeted as the synthetic goal the cyclic silyl protected building block, which simulates the 3′,5′ substitution pattern present in DNA. The synthesis sequence is outlined in Scheme 1. Initially 2-amino-4,5-dichloropyrimidine (**1**) was i) heated with sodium hydroxide giving **2**, ii) nitrated with fuming nitric acid to **3** and iii) N-acetyl protected with acetic anhydride (→**4**), using modified procedures from the literature.[57–59] Subsequent reaction of the “vinylogous acid chloride” **4** with the 2′,3′-acetonide protected 1-amino-ribofuranoside **5**[60, 61] furnished the riboside **6** as an α/β-mixture. The desired β-isomer **6** could be isolated from this mixture by crystallization.[62, 63] Cleavage of the acetonide protection group and subsequent reaction of **7** with 1,3-dichloro-1,1,3,3-tetradiisopropyldisiloxane[64, 65] provided the key intermediate **8**. Esterification of the free 2′-hydroxyl group with 3-trifluoromethyl benzoyl chloride to allow future photodeoxygenation, furnished the nitropyrimidine ribofuranoside **9**. All these transformations were possible without causing anomerization. We believe that the reason for the stability of the anomeric centers is the presence of the strong electron-withdrawing nitro group. Reduction of the nitro group with H_2 using a Pd/C catalyst gave the labile amine, which was immediately condensed without isolation with formic acid and dicyclohexylcarbodiimide (DCC) furnishing the FapyGua-ribofuranoside **10** as a mixture of the α- and the β-anomer. It is most possible the amine intermediate which allows anomerization.



2,6-diamino-4-hydroxy-5-formamido-pyrimidine (FapydGua)

8-oxo-7,8-dihydroguanine (8oxoG)

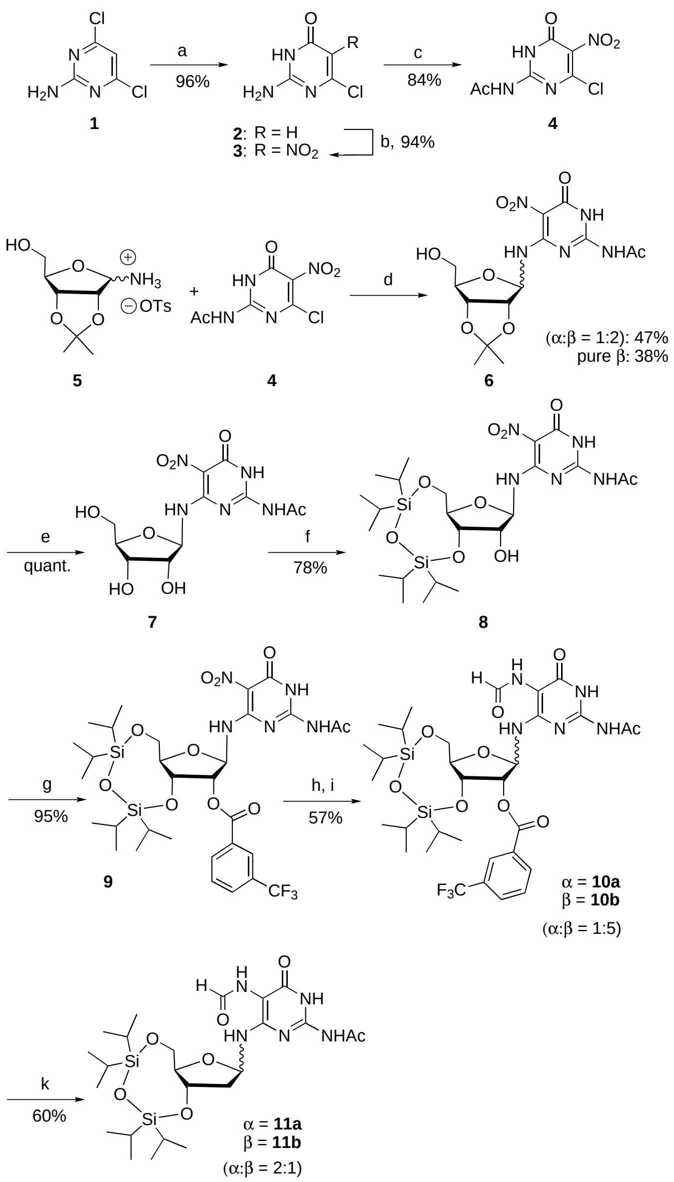
oxazolone lesion (dZ)

imidazolone lesion (dIz)

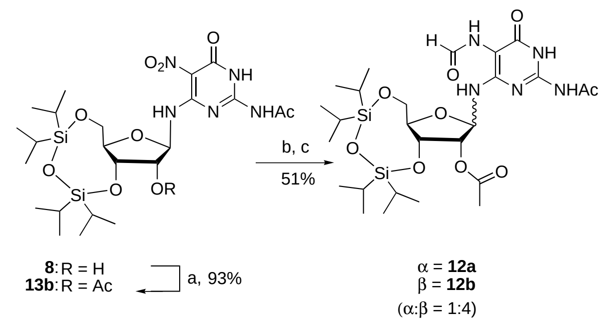
Figure 1. Chemical structures of the four main oxidative lesions (FapydGua, 8oxoG, dZ, dIz) derived from the guanosine radical cation.

The key step in the synthesis of the FapydGua lesion **11** is the deoxygenation of the building block **10**. For this step we initially prepared various 2′-thiocarbonates and explored Barton–McCombie methods.[66] All attempts, however, to deoxygenate the 2′-OH group using various hydrogen-radical donors failed in our hands leaving as the final method the photodeoxygenation procedure originally described by Saito et al.[67] and recently further developed by Rizzo and coworkers.[34, 35] Photodeoxygenation of compound **10** with *N*-ethyl-3,6-dimethyl-carbazole as the electron donor was, however, difficult as well. The FapydGua lesion possesses absorption bands in the spectral region, where the carbazole electron donor absorbs light, leading to significant photodegradation of the final compound **11** during the reaction. So far the best photodeoxygenation results were obtained with a TQ-150 medium pressure mercury lamp in a pyrex photochemical reactor. Small amounts of compound **10** were deoxygenated in a quartz cuvette with DMF used as a filter solution. Using these methods we were able to synthesize the FapydGua lesion building block in 60 % yield (estimated by TLC). NMR studies showed that the compound was synthesized as a mixture of the two anomers **11a** and **11b** with an α to β ratio of 2:1. Separation of the two anomers was difficult but finally achieved by HPLC. Both compounds **11a** and **11b** were obtained as microcrystalline powders.

For comparison reasons we also prepared the FapyGua (RNA) building blocks **12a** and **12b** as depicted in Scheme 2. Reaction of precursor **8** with acetyl chloride gave compound



Scheme 1. a) 1N NaOH, reflux, 1 h, 96%. b) conc. H_2SO_4/fuming HNO_3, 0°C, 5 min, 94%. c) Ac_2O, 90°C, 30 min, 84%. d) $NaHCO_3$, EtOH, 4 Å MS, reflux, 1.5 h. e) CF_3COOH/H_2O 9:1, 0°C, 20 min, quant. f) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, 0°C, 30 min, 78%. g) 3-(Trifluoromethyl)benzoyl chloride, DMAP, CH_2Cl_2, 0°C, 30 min, 95%. h) H_2, Pd/C, ethyl acetate, MeOH, r.t. after 7 min addition of diisopropylethylamine, 7 min. i) HCOOH, DCC, pyridine 0°C → r.t., 12 h, 47% (**10b**), 10% (**10a**). k) 9-Ethyl-3,6-dimethylcarbazole, $h\tilde{\nu}$, 15°C, 15 h, 60%).



Scheme 2. a) AcCl, DMAP, CH_2Cl_2, 0°C, 60 min, 93%. b) H_2, Pd/C, ethyl acetate, MeOH, r.t. after 5 min addition of diisopropylethylamine, 10 min. c) HCOOH, DCC, pyridine 0°C → r.t., 12 h, 41% (**12b**), 10% (**12a**).

13. Catalytic reduction of the nitro group and subsequent formylation of the amino group with formic acid and DCC provided the 2′-acetyl protected FapyGua (RNA) building blocks **12a** and **12b** as a mixture of the two anomers (α:β = 1:4). Separation of the two anomers **12a** and **12b** was possible by flash chromatography.

Stability of the glycosidic bond: The surprising fact that the FapyGua ribofuranosides **12a**, **b** and the FapydGua deoxyribofuranosides **11a**, **b** could be isolated as microcrystalline powders already demonstrated that the anomeric center of this major DNA lesion is far more stable than originally anticipated. In order to quantify the stability of compound **11** we performed temperature dependent NMR studies with compound **11a**. To our surprise, heating of **11a** in DMSO to 60°C for 6 h gave no anomerization! More significantly, we observe no cleavage of the FapydGua N-glycosidic bond as a result of the previously detected "depurination" reaction.[14, 53, 54] Contrary to all previous studies, we observe that the protected FapydGua deoxyribofuranoside **11** possesses a rather stable N-glycosidic bond. Even at 100°C over 24 h in DMSO only slow decomposition of the FapydGua lesion was observed. As expected, the FapyGua ribofuranosides **12a** and **12b** are even more stable. No anomerization and decompositon of compound **12a** in DMSO was observed even at 60°C for at least 24 h. Although these experiments provided already strong evidence for an unexpected high stability of the FapydGua lesion, we probed the anomerization reaction and the cleavage of the anomeric bond in addition in water. To this end compound **11a** was stirred in a water/acetonitrile mixture (1:1) at room temperature and at 50°C.

Figure 2a shows HPLC diagrams obtained from the reaction mixture containing the β-FapydG **11b** stirred at room temperature. After certain time intervals, small aliquots of the reaction mixture were removed and analyzed by reversed-phase HPLC. Clearly evident is the slow anomerization of **11b** to **11a** under these conditions (room temperature experiment). Although separation of both anomers was difficult it was possible to extract the amounts of both compounds from the chromatograms by peak deconvolution. The values obtained are plotted in Figure 2b, showing exponential decrease of the β-anomer **11b** and the rise of the α-anomer **11a**[68] over time. From these data we determined the relaxation time τ at 25°C in the water/acetonitrile mixture (1:1) by Equation (1).

$$\tau = \frac{1}{k_1 + k_{-1}} \tag{1}$$

for a unimolecular reaction $\mathbf{11b} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{11a}$, the value for k_1+k_{-1} was derived from Equation (2):

$$\ln\frac{[\mathbf{11b}] - [\mathbf{11b}]_{eq}}{[\mathbf{11b}]_0 - [\mathbf{11b}]_{eq}} = -(k_1 + k_{-1})t \tag{2}$$

with [**11b**] being the concentration of **11b** in solution at time t, [**11b**]$_0$ the concentration at $t = 0$, and [**11b**]$_{eq}$ the equilibrium concentration. Applying Equations (1) and (2) gives a relaxation time of $\tau = 6.5$ h, which is far higher than predicted based on the previous observations.

Heating of the α/β mixture **11a/11b** in the water/acetonitrile mixture to 50 °C for 24 h resulted in a small amount of depurination as shown in Figure 2c. We observed the acetyl protected sugar free FapyG heterocycle with a retention time of 1.8 min. Both anomers **11a** and **11b** hydrolyze only slowly even under these rather harsh conditions. Figure 2d quantifies the decomposition reaction of **11a** and **11b** under these conditions. Under the assumption that the decay follows first-order kinetics we can determine the half-life to 37.8 h for the β-anomer **11b** and 65.2 h for the α-anomer **11a**.[36]

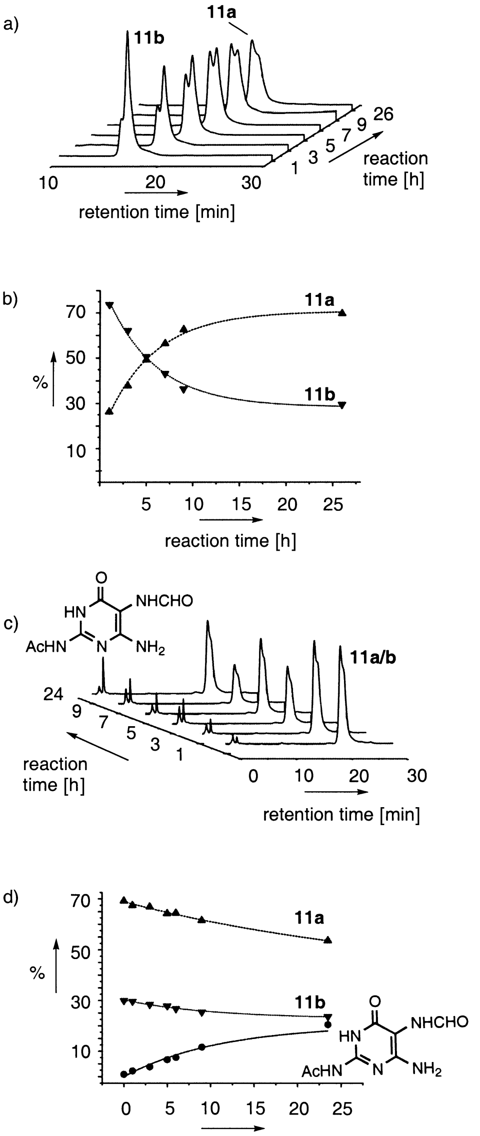


Figure 2. Anomerization and stability studies of the FapydGua lesion investigated with **11a** and **11b**. a) HPLC diagrams of **11b** stirred in water/acetonitrile at r.t. over 26 h. b) Time dependent formation of **11a** (▲) and decay of **11b** (▼). c) HPLC diagrams of a solution of **11a** and **11b** in water/acetonitrile at 50 °C over 24 h. d) Decomposition of **11a** (▲) and **11b** (▼) and formation of the acetyl protected FapyGua heterocycle (●). HPLC conditions: mobile phase A: water, mobile phase B: acetonitrile. Gradient: 50 to 75 % B over 45 min.

Structural properties of the FapydGua lesion relevant for the recognition by repair enzymes: The two main guanine derived lesions in DNA are 8oxoG and FapydGua.[13, 24] Both are recognized and repaired by the glycosylase hOGG1 and the Fpg protein.[2, 25–33, 69] It was argued that both enzymes recognize the C(8) carbonyl group as the common structural feature.[32] Although lesion recognition by the Fpg protein is so far not clear, the recent X-ray structure of the human OGG1 protein in complex with 8oxoG-lesioned DNA provided a detailed molecular picture of the binding motif.[44] Here it was found that hOGG1 distinguishes the 8oxoG amidst the vast excess of guanine not by a contact to the most characteristic C(8) carbonyl group. Instead, hOGG1 recognises exclusively the N(7)-H donor group, which is a hydrogen-bond acceptor group in guanine itself.

The FapydGua lesion can exist as four conformers (**11-1**–**11-4**) due to rotation around the C(5)–N(7) and N(7)–C(8) bonds.[55, 56] All conformers are depicted in Figure 3. It is currently believed that repair enzymes bind the rotamer **11-2**, which most closely mimics the structure of the 8oxoG lesion.

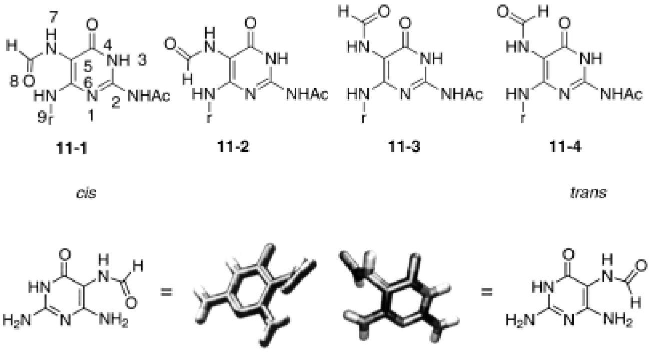


Figure 3. Chemical structure of all four possible FapyGua lesion conformers **11-1**–**11-4** and molecular modelling calculations (macromodel, MMFF94s force field, gas phase)[38] of the two only observed conformers **11-1** and **11-2**.

In order to clarify the conformational preference of the FapydGua lesion, temperature dependent NMR studies were performed (Figure 4b). Figure 4 shows the results of a study obtained with **11** (as an α,β-mixture) in [D$_6$]DMSO (Figure 4a).The NMR spectra contains four signals for the protons N(9)-H (N*H*C1′). The anomers of **11a** and **11b** give accordingly two signals each for the single N(9)-H proton, which couples with C(1′)-H of the sugar. It is therefore clear that each anomer exists in two different conformations. Two-dimensional NMR analysis of the compounds clearly showed that the two sets of signals belong to the α- and β-, *cis*- and *trans*-formamides **11-1** and **11-2**. The *trans*-conformation should be thermodynamically more stable for steric reasons. The fact that both, the *cis* and the *trans* isomer, are observed leads us to conclude that the *cis*-amide **11-1** is stabilized by an intramolecular hydrogen bond giving even in the highly competitive solvent DMSO a 66:34 mixture ratio of the *cis* to the *trans* rotamers.

In contrast to earlier assumptions we do not observe further signals belonging to the second set of rotamers **11-3** and **11-4**. Force field calculations (macromodel, MMFF94s force

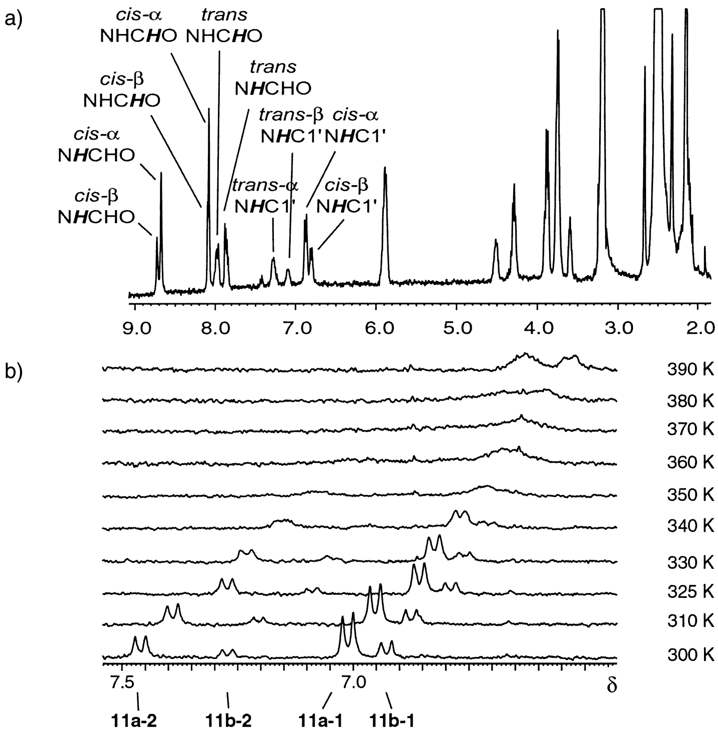


Figure 4. NMR investigation of the rotation barrier around N(7)–C(8). a) 400 MHz spectrum of a mixture of **11a** and **11b** in [D_6]DMSO. b) Temperature dependent NMR study (300 to 390 K) in [D_6]DMSO. Depicted is the N(9)-H region. **11a-2**:α-anomer, trans; **11b**-2: β-anomer, *trans*; **11a-1**: α-anomer, *cis*; **11b-1**: β-anomer, *cis*.

field, gas phase)[70, 71] confirm that these two rotamers are higher in energy, which supports our experimental result (data not shown). The two rotamers **11-1** and **11-2** depicted in Figure 3 are clearly the dominating species in solution. Upon heating of the NMR solution (Figure 4b) we observe coalescence at approximately 370 K. At this temperature we calculated the rotation barrier around the N(7)–C(8) bond for both anomers **11a** and **11b**. A value of approximately 74 kJ mol^{-1} was obtained using line shape analysis and the Eyring equation.[53, 72]

In order to investigate the conformational preference of the FapyGua lesion in a more apolar solvent, mimicking the typical environment of a protein and DNA, we re-measured the NMR spectrum in $CDCl_3$. In contrast to the assumption in the literature that the FapydGua lesions exists in a conformational equilibrium containing four rotameric species, we observed that **11** strictly prefers the *cis*-amide conformation **11-1**. The formation of the seven-membered hydrogen-bonded ring system clearly shifts the equilibrium fully towards this rotamer. Even heating of the $CDCl_3$ solution to 60 °C does not change the equilibrium as observed by NMR spectroscopy. In $CDCl_3$, the second *trans*-conformer is not observed at all!

If we compare the structure of 8oxoG with the structure of the FapydGua lesion in order to extract a possible common recognition motif adressable by repair enzymes we can conclude that in the major FapydGua rotamer the C(8)=O carbonyl group and the N(9)-H are involved in a rather strong hydrogen bond. This carbonyl group is only accessible when the enzyme provides an interaction strong enough to compete successfully with this intramolecular hydrogen bond. This would be energetically costly. Furthermore, computer modeling studies depicted in Figure 3 (macromodel, MMFF94s force field)[70, 71] show that the formamide group in the *trans*-isomer is for steric reasons almost orthogonal to the pyrimidine ring. Hence, even if the *trans*-isomer would dominate, the 8oxo-carbonyl group would be oriented completely different compared with the 8oxoG lesion. In conclusion the C(8)=O carbonyl group is not easily acceptable as a common structural element recognizable by repair enzymes.

The only structural motif which is common to both the FapydGua and the 8oxoG lesions is the N(7)-H hydrogen bond donor group. This N(7) is a hydrogen-bond acceptor in guanine. This group alone allows the combined recognition of both lesions by repair protein in the presence of intact guanine bases.

This result draws our attention to repair results obtained with N(7)-methyl FapydGua derivatives, frequently employed as a FapydGua analogues in biological studies.[28, 29, 33, 48] Our results suggest that the biologically relevant lesion prefers a conformation, which allows formation of a seven-membered ring system containing a hydrogen bond between the formamide and the N(7)-H. This might limit the ability of the N(7)-methyl FapydGua derivative and of other FapydGua derivatives, devoid of this critical hydrogen bond, to mimic the properties of the biologically relevant FapydGua lesion.

Conclusions

The FapydGua lesion is one of the major lesions formed by the reaction of OH-radicals with DNA.[13, 24] Due to the lack of methods allowing either synthesis of this lesion or direct generation of the lesion in DNA, our chemical knowledge about the FapydGua is very limited. It was so far assumed that the N-glycosidic bond of the lesion is highly unstable leading to rapid decomposition, accompanied by the cleavage of the FapydGua heterocycle.[14, 53, 54] Because enzymes, which repair oxidative lesions accepts both, the FapydGua lesion and the 8oxoG lesion as substrates, it was suggested that they may target for recognition the C(8)=O carbonyl group as the common structural motif.[32]

In order to clarify the stability of the FapydGua lesion and to determine the conformation of the lesion a new chemical synthesis of the FapydGua lesion was developed. It was found that the N-glycosidic bond is much more stable than previously anticipated. Anomerization was observed in a water/acetonitrile mixture at room temperature with a relaxation time of $\tau = 6.5$ h. In the same solvent decomposition only takes place at 50 °C with half-life of 37.8 h for the β-anomer and of 65.2 h for the α-anomer.

NMR and modelling investigation of the structure of the FapydGua lesion showed that the lesion prefers in solvents such as $CHCl_3$, which mimic the polarity of the interior of proteins and DNA, the *cis*-conformation. Driving force is the formation of a hydrogen bond within a seven-membered ring system. The *cis*-rotamer was even present up to about 66 % in the highly competitive solvent DMSO. The *trans*-rotamer was found to have a formamide group almost perpendicular to the pyrimidine system (modelling). The rotational barrier was determined as 74 kJ mol^{-1}. The C(8)=O carbonyl group is hence not acceptable as the structural motif common to both oxidative lesions. In agreement with the recent X-ray structure of an 8oxoG-containing double strand in complex with the hOGG1 repair enzyme,[44] we conclude that the structural motif recognizable by repair enzymes is the N(7)-H donor group, which is a hydrogen-bond acceptor in guanine.

Experimental Section

General procedures: Melting points are uncorrected. ^{1}H NMR spectra were recorded on Varian Gemini 200, 300, Bruker AMX 300, 400, and 500 spectrometers. The chemical shifts were referenced to $CHCl_3$ (δ 7.27) in $CDCl_3$, and DMSO (δ 2.50) in [D$_6$]DMSO. ^{13}C NMR spectra were recorded on a Bruker DRX 200 (50 MHz) and AMX 500 (125 MHz) spectrometer, and the chemical shifts were referenced to $CHCl_3$ in $CDCl_3$ and DMSO in [D$_6$]DMSO. Standard pulse sequences were employed for ^{1}H two-dimensional NOESY and ^{1}H – ^{1}H correlation studies. IR spectra were recorded in KBr and measured in cm^{-1} on a Bruker IFS 25 Fourier transform infrared spectrophotometer. Mass spectra and high-resolution mass spectra were recorded using VG ZAB2-SEQ (nitrobenzylalcohol matrix), Finnigan TSQ 700, Finnigan MAT 95S and HP5989B mass spectrometers, respectively. HPLC was performed with a Merck – Hitachi system equipped with a L-7400 UV detector. Analytical separations were performed with a Nucleosil 100-5, C18 (250 × 4 mm) column from Macherey – Nagel. For preparative separations a Nucleoprep 100-12, C18 column (250 × 40 mm) was used.

All solvents were of the quality puriss. p.a., or purum. Purum solvents were distilled prior to use. The commercially available reagents were used as received without further purification.

General procedures

2-Amino-6-chloro-3*H*-pyrimidine-4-one (2):[28] 2-Amino-4,6-dichloropyrimidine (7.0 g, 42.6 mmol) was suspended in 1N NaOH (100 mL) and heated to reflux until everything was dissolved (ca. ½ – 1 h). The product was precipitated through addition of acetic acid and was filtered off. Compound **2** was obtained as a yellowish powder (5.96 g, 40.96 mmol, 96 %). M.p. > 220 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 5.57$ (s, 1 H, CH), 6.96 (s, 2 H, NH_2), 11.09 (s, 1 H, NH); ^{13}C NMR (50 MHz, [D$_6$]DMSO): $\delta = 97.67$, 153.79, 157.84, 160.52.

2-Amino-6-chloro-5-nitro-3*H*-pyrimidine-4-one (3):[28] Chloropyrimidine **2** (5 g, 34.4 mmol) was dissolved at 0 °C in conc. H_2SO_4 (10 mL) using an ultrasonic bath. Fuming HNO_3 (10 mL) was added at 0 °C. The solution was stirred for 5 min. The reaction mixture was slowly added onto ice. The reaction product was filtered off and dried in high vacuum. Compound **3** was obtained in form of a yellowish powder (6.1 g, 32.2 mmol, 94 %). M.p. > 220 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 7.20$ (s, 1 H, NH), 8.41 (s, 1 H, NH), 12.12 (s, 1 H, NH); ^{13}C NMR (50 MHz, [D$_6$]DMSO): $\delta = 126.57$, 153.12, 154.09, 154.96.

2-Acetylamino-6-chloro-5-nitro-3*H*-pyrimidine-4-one (4):[28] Nitropyrimidine **3** (3 g, 15.7 mmol) was suspended in Ac_2O (30 mL). Three drops of H_2SO_4 were added and the mixture was heated to 90 °C for 30 min. The reaction mixture was cooled to r.t. and allowed to stand over night. The precipitate was filtered off and the filtrate was concentrated to half of the volume. Additional precipitate formed over night, which was filtered off. The combined products were dried in high vacuum. Compound **4** was obtained in form of yellowish crystals (3.1 g, 13.2 mmol, 84 %). M.p. > 220 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 2.23$ (s, 3 H, CH_3CO), 12.44 (s, 2 H, NH, AcNH); ^{13}C NMR (50 MHz, [D$_6$]DMSO): $\delta = 23.90$, 131.89, 150.70, 151.62, 153.00, 174.42; MS (ESI$^-$): *m*/*z* (%): 231.0 (100) [$M - H$]$^-$.

6-(2′-*O*,3′-*O*-Isopropylidene-*β*-D-ribofuranose-1′-yl)amino-2-acetylamino-5-nitro-3*H*-pyrimidine-4-one (6): Acetylchloronitropyrimidine **4** (1 g, 4.3 mmol) and ribofuranonsylamine[29] **5** (3.7 g, 10.2 mmol, 2.4 equiv) was suspended in dry EtOH (20 mL) and stirred for 1 h over 4 Å molecular sieves. After addition of $NaHCO_3$ (860 mg, 10.2 mmol, 2.4 equiv), the reaction mixture was stirred for additional 2 h. The mixture was subsequently heated to reflux for 1.5 h. After cooling to r.t., the mixture was filtered. The filtrate was evaporated to dryness in vacuo. The residual material was dissolved in water (25 mL). Product **6** crystallized over night. The crystalline material was filtered off and dried in high vacuum. Compound **6** was obtained in form of a light yellowish powder (630 mg, 1.7 mmol, 38 %). $R_f = 0.19$ (ethyl acetate/MeOH 20:1); m.p. 146 – 148 °C; IR (KBr): $\tilde{\nu} = 645$, 668, 790, 1105, 1246, 1264, 1319, 1384, 1431, 1466, 1512, 1551, 1595, 1637, 1672, 2928, 3463 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.27$ (s, 3 H, CH_3C), 1.44 (s, 3 H, CH_3C), 2.18 (s, 3 H, CH_3CO), 3.49 (d, ^2J(C5′H$_a$,C5′H$_b$) = 11.2 Hz, 1 H, C5′H$_a$), 3.60 (d, ^2J(C5′H$_a$,C5′H$_b$) = 11.2 Hz, 1 H, C5′H$_b$), 4.24 (s, 1 H, C4′H), 4.69 (d, ^3J(C2′H,C3′H) = 6.0 Hz, 1 H, C2′H), 4.83 (d, ^3J(C3′H,C2′H) = 6.0 Hz, 1 H, C3′H), 5.66 (d, 1 H, OH), 6.22 (d, ^3J(C1′H,NH) = 9.2 Hz, 1 H, C1′H), 10.27 (d, ^3J(NH,C1′H) = 9.2 Hz, 1 H, NH), 11.49 (s, 1 H, NH), 11.75 (s, 1 H, NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 24.13$, 24.51, 26.47, 62.24, 82.25, 86.20, 86.68, 88.10, 111.47, 112.62, 150.53, 154.15, 157.29, 174.49; MS (ESI$^-$): *m*/*z* (%): 769.4 (11) [$M_2 - H$]$^-$, 556.3 (45), 474.2 (100) [M]$^-$; HR-MS (ESI$^-$): calcd for [$C_{14}H_{19}N_5O_8 - H$]$^-$: 384.1155, found 384.1169.

6-(*β*-D-Ribofuranose-1′-yl)amino-2-acetylamino-5-nitro-3*H*-pyrimidine-4-one (7): Isopropylidene protected sugar **6** (1.68 g, 4.89 mmol) was dissolved at 0 °C in CF_3COOH/H_2O (9:1, 15 mL) and stirred for 20 min. The solvent was removed in vacuo. Then ethanol (3 × 5 mL) was added and the solvent was removed in vacuo (3 ×). Product **7** was obtained after drying in high vacuum as a yellowish powder (1.6 g, quant). $R_f = 0.01$ ($CHCl_3$/MeOH 20:1); m.p. 197 °C (decomp); IR (KBr): $\tilde{\nu} = 528$, 751, 787, 844, 1027, 1085, 1152, 1230, 1324, 1384, 1427, 1476, 1535, 1592, 1620, 1678, 3418 cm^{-1}; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 2.19$ (s, 3 H, CH_3), 3.43 (dd, ^2J(C5′H$_a$,C5′H$_b$) = 11.6 Hz, ^3J(C5′H$_a$,C4′H) = 3.0 Hz, 1 H, C5′H$_a$), 3.51 (dd, ^2J(C5′H$_b$,C5′H$_a$) = 11.6, ^3J(C5′H$_b$,C4′H) = 3.7 Hz, 1 H, C5′H$_{2b}$), 3.79 (m, 1 H, C4′H), 3.98 – 4.05 (m, 2 H, C2′H, C3′H), 4.91 (br s, 3 H, 3OH), 5.81 (dd, ^3J(C1′H,NH) = 8.6, ^3J(C1′H,C2′H) = 4.3 Hz, 1 H, C1′H), 9.62 (d, ^3J(NH,C1′H) = 8.7 Hz, 1 H, NH), 11.53 (s, 1 H, NH), 11.90 (s, 1 H, NH); ^{13}C NMR (50 MHz, [D$_6$]DMSO): $\delta = 25.48$, 62.19, 71.70, 76.32, 85.81, 86.94, 114.22, 152.04, 155.39, 159.71, 175.92; MS (ESI$^-$): *m*/*z* (%): 345.2 (15) [M]$^-$, 344.1 (100) [$M - H$]$^-$; HR-MS (ESI$^-$): calcd for [$C_{11}H_{15}N_5O_8 - H$]$^-$: 344.0842, found 344.0833.

6-(3′-*O*,5′-*O*-[1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl]-*β*-D-ribofuranose-1′-yl)amino-2-acetylamino-5-nitro-3*H*-pyrimidine-4-one (8): Sugar **7** (2.2 g, 6.5 mmol) was dissolved in pyridine (15 mL) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.4 mL, 7.8 mmol, 1.2 equiv) was added dropwise at 0 °C. After 30 min stirring, the solution was diluted with ethyl acetate (150 mL) and extracted three times with 1N HCl (40 mL), sat. $NaHCO_3$ (40 mL) and sat. NaCl (40 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. Compound **8** was obtained after flash chromatography ($CHCl_3$, 2 – 10 % MeOH) as a yellowish crystalline powder (3.0 g, 5.1 mmol, 78 %). $R_f = 0.34$ (ethyl acetate/MeOH 20:1); m.p. 111 – 114 °C; IR (KBr): $\tilde{\nu} = 3454$, 2946, 2868, 1678, 1625, 1586, 1536, 1466, 1433, 1384, 1326, 1229, 1150, 1126, 1039, 885, 859, 789, 697 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.02 - 1.07$ (m, 28 H, *i*Pr), 2.28 (s, 3 H, CH_3CO), 3.06 (s, 1 H, HO), 3.88 – 3.93 (m, 1 H, C4′H), 3.97 (d, ^3J(C5′H,C4′H) = 3.3 Hz, 2 H, C5′H), 4.09 (d, ^3J(C2′H,C3′H) = 5.3 Hz, 1 H, C2′H), 4.33 (dd, ^3J(C3′H,C4′H) = 8.0, ^3J(C3′H,C2′H) = 5.3 Hz, 1 H, C3′H), 5.62 (d, ^3J(C1′H,NH) = 6.3 Hz, 1 H, C1′H), 9.38 (d, ^3J(NH,C1′H) = 6.3 Hz, 1 H, NHC1′), 10.88 (s, 1 H, NH), 11.49 (s, 1 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.75$, 12.80, 13.21, 13.47, 17.03, 17.11, 17.14, 17.18, 17.30, 17.37 (2 × CH_3CSi), 17.48, 24.47, 61.89, 71.41, 76.33, 81.34, 87.86, 113.64, 150.63, 155.25, 157.85, 173.62; MS (ESI$^+$): *m*/*z* (%): 587 (100) [M]$^+$; HR-MS: calcd for [$C_{23}H_{41}N_5O_9Si_2 + Na$]$^+$: 610.2340, found 610.2336.

6-(3′-*O*,5′-*O*-[1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl]-2′-*O*-acetyl-*β*-D-ribofuranose-1-yl)amino-2-acetylamino-5-nitro-3*H*-pyrimidine-4-one (13): Silyl protected sugar **8** (0.5 g, 0.9 mmol) and DMAP (260 mg, 2.1 mmol, 2.5 equiv) were dissolved in CH_2Cl_2 (1 mL) and acetyl chloride (0.70 mL, 0.9 mmol, 1.1 equiv) was added dropwise at 0 °C. After stirring for 60 min, the reaction mixture was diluted with ethyl acetate (10 mL) and extracted three times with 1N HCl (2 mL), sat. $NaHCO_3$ (2 mL) and sat. NaCl (2 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. Compound **13** was obtained as a yellowish powder (0.50 g, 0.8 mmol, 93 %). $R_f = 0.63$ (ethyl acetate/MeOH 20:1); m.p. 112 – 115 °C; IR (KBr): $\tilde{\nu} = 3470$, 2946, 2868, 1754, 1678, 1620, 1585, 1536, 1466, 1433, 1384, 1229, 1158, 1039, 886, 860, 789, 696, 665, 599 cm^{-1}; ^{1}H NMR

(300 MHz, $CDCl_3$): $\delta = 0.94-1.00$ (m, 28 H, *i*Pr), 2.12 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 3.88–3.99 (m, 3 H, C4′H, C5′H), 4.38 (dd, ^3J(C3′H,C4′H) = 8.0, ^3J(C3′H,C2′H) = 5.0 Hz, 1 H, C3′H), 5.35 (d, ^3J(C2′H,C3′H) = 5.0 Hz, 1 H, C2′H), 5.52 (d, ^3J(C1′H,NH) = 5.8 Hz, 1 H, C1′H), 8.68 (s, 1 H, NH), 9.35 (d, ^3J(NH,C1′H) = 5.8 Hz, 1 H, NH), 11.30 (s, 1 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.60$, 12.67, 12.99, 13.34, 16.85, 16.90, 16.98 (2 × CH_3CSi), 17.16 (3 × CH_3CSi), 17.29, 20.93, 24.41, 60.92, 69.29, 73.47, 77.13, 81.64, 85.94, 149.82, 154.22, 157.55 (2 C), 170.96, 171.96; MS (ESI$^+$): *m*/*z* (%): 630.2 (100) $[MH]^+$; HR-MS (ESI$^-$): calcd for $[C_{25}H_{43}N_5O_{10}Si_2-H]^-$: 628.2470, found: 628.2083.

6-(3′-*O*,5′-*O*-[1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl]-2′-*O*-[4-trifluoromethyl-benzoyl]-*β*-D-ribofuranose-1-yl)amino-2-acetylamino-5-nitro-3*H*-pyrimidine-4-one (9): Silyl protected sugar **8** (1.5 g, 2.6 mmol) and DMAP (780 mg, 6.4 mmol, 2.5 equiv) were dissolved in CH_2Cl_2 (15 mL). 3-(Trifluoromethyl)benzoyl chloride (0.4 mL, 2.6 mmol, 1.0 equiv) was added dropwise at 0 °C. After stirring for 30 min, ethyl acetate was added (150 mL) and the reaction mixture was three times extracted with 1N HCl (20 mL), sat. $NaHCO_3$ (20 mL) and sat. NaCl (20 mL). After drying of the organic phase over $MgSO_4$ and removal of the solvent in vacuo, compound **9** was obtained as a yellowish powder (1.8 g, 2.4 mmol, 95 %). $R_f = 0.44$ (ethyl acetate/MeOH 20:1); m.p. 195–197 °C; IR (KBr): $\tilde{\nu} = 3460$, 2947, 2869, 1733, 1683, 1620, 1584, 1539, 1465, 1436, 1384, 1336, 1250, 1169, 1134, 1073, 1039, 994, 885, 695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.01-1.09$ (m, 28 H, *i*Pr), 2.25 (s, 3 H, CH_3CO), 4.05–4.10 (m, 3 H, C4′H, C5′H), 4.56 (dd, ^3J(C3′H,C4′H) = 8.0, ^3J(C3′H,C2′H) = 5.0 Hz, 1 H, C3′H), 5.65 (d, ^3J(C2′H,C3′H) = 5.0 Hz, 1 H, C2′H), 5.83 (d, ^3J(C1′H,NH) = 6.3 Hz, 1 H, C1′H), 7.60–7.65 (m, 1 H, $C_{ar}H$), 7.84–7.86 (m, 1 H, $C_{ar}H$), 8.23–8.34 (m, 2 H, $C_{ar}H$), 9.35 (s, 1 H, NH), 9.47 (d, ^3J(NH,C1′H) = 6.3 Hz, 1 H, NH), 11.46 (s, 1 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.78$, 12.81, 13.19, 13.53, 17.14 (8 C), 24.54, 61.17, 70.04, 77.93, 82.03, 85.89, 113.78, 123.79 (q, $^1J = 272$ Hz), 126.74 (q, $^3J = 4$ Hz), 129.58, 130.30 (q, $^3J = 4$ Hz), 131.10, 131.93 (q, $^2J = 33$ Hz), 133.20, 150.15, 154.61, 157.71, 164.49, 172.66; ^{19}F NMR (188 MHz, $CDCl_3$): $\delta = -63.01$; MS (ESI$^+$): *m*/*z* (%): 782 (6) $[M+Na]^+$, 760 (18) $[MH]^+$, 261 (63), 186 (100); HR-MS (ESI$^+$): calcd for $[C_{31}H_{44}N_5O_{10}Si_2+H]^+$: 760.2680, found 760.2679.

***α*- and *β*-6-(3′-*O*,5′-*O*-[1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl]-2′-*O*-[4-trifluoromethyl-benzoyl]-D-ribofuranose-1-yl)amino-2-acetylamino-5-formylamino-3*H*-pyrimidine-4-one (10a and 10b)**: Sugar **9** (500 mg, 0.66 mmol) was dissolved in ethyl acetate (0.5 mL). Under argon, Pd/C (10 %, 500 mg) was added and the reaction mixture was diluted with MeOH (5 mL). The solution was then stirred under an H_2 atmosphere. After 7 min, diisopropylethylamine (0.5 mL) was added and the hydrogenation was continued for another 7 min. The reaction mixture was filtered through Celite and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in pyridine (5 mL) under argon and DCC (270 mg, 1.32 mmol, 2 equiv) was added. After cooling to 0 °C, HCOOH (50 µL, 1.32 mmol, 2 equiv) was added. The reaction mixture was allowed to warm to r.t. over 12 h. The mixture was diluted with ethyl acetate (60 mL) and extracted two times with 1N HCl (15 mL), sat. $NaHCO_3$ (15 mL) and sat. NaCl (15 mL). Drying of the organic phase over $MgSO_4$ and removal of the solvent in vacuo afforded crude **10**. Both anomers were separated by flash chromatography ($CHCl_3$, 1–4 % MeOH). **10b** (240 mg, 0.3 mmol, 47 %) and **10a** (50 mg, 0.07 mmol, 10 %) were obtained as yellowish powders. Compound **10b**: $R_f = 0.48$ (ethyl acetate/MeOH 20:1); m.p. 94–96 °C; IR (KBr): $\tilde{\nu} = 605$, 695, 1038, 1073, 1135, 1251, 1335, 1422, 1467, 1530, 1552, 1643, 1727, 2869, 2947, 3454 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.83-1.11$ (m, 28 H, *i*Pr), 2.22 (s, 3 H, CH_3CO), 3.96–4.04 (m, 3 H, C4′H, C5′H), 4.57–4.62 (m, 1 H, C3′H), 5.55 (dd, ^3J(C2′H,C3′H) = 5.0, ^3J(C2′H,C1′H) = 1.3 Hz, 1 H, C2′H), 5.78 (dd, ^3J(C1′H,NHC1′) = 8.0, ^3J(C1′H,C2′H) = 1.3 Hz, 1 H, C1′H), 7.61 (t, ^3J($C_{ar}H$,$C_{ar}H$) = 8.0 Hz, 1 H, C_{ar}5H), 7.73 (d, ^3J(NHC1′,C1′H) = 8.0 Hz, 1 H, NHC1′), 7.81–7.86 (m, 2 H, *H*NCHO, C_{ar}6H), 8.16 (d, ^3J(C*H*O,*H*NCHO) = 2 Hz, 1 H, C*H*O), 8.25 (d, ^3J(C4H,C5H) = 8.0 Hz, 1 H, C_{ar}4H), 8.32 (s, 1 H, C_{ar}2H), 8.45 (s, 1 H, NH), 11.54 (s, 1 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.54$, 12.58, 12.99, 13.16, 16.63, 16.87, 16.93 (2C), 17.17, 17.23 (2C), 17.31, 24.11 (*C*H3CON), 62.51 (C5′), 71.30 (C3′), 77.91 (C1′), 81.36 (C2′), 84.53 (C4′), 99.11, 123.84 (q, $^1J = 273$ Hz), 126.84 (q, $^3J = 4$ Hz), 129.43, 130.00, 130.02 (q, $^3J = 4$ Hz), 131.40 (q, $^2J = 33$ Hz), 133.24, 146.16, 151.60, 157.48, 159.59, 164.44, 171.82; ^{19}F NMR (188 MHz, $CDCl_3$): $\delta = -63.21$; MS (ESI$^+$): *m*/*z* (%): 788 (36) $[M+Na]^+$, 758 (100) $[MH]^+$; HR-MS (ESI$^-$): calcd for $[C_{32}H_{46}F_3N_5O_8Si_2-H]^-$: 756.2708, found 756.2709.

Compound **10a**: $R_f = 0.50$ (ethyl acetate/MeOH 20:1); m.p. 97–99 °C; IR (KBr): $\tilde{\nu} = 1039$, 1073, 1134, 1247, 1334, 1507, 1521, 1540, 1559, 1577, 1653, 1683, 1734, 2869, 2947, 3447 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.96-1.01$ (m, 28 H, *i*Pr), 2.14 (s, 3 H, CH_3CO), 3.82–3.92 (m, 2 H, C5′H), 3.97–4.00 (m, 1 H, C4′H), 4.44 (dd, ^3J(C3′H,C4′H) = 8.3, ^3J(C3′H,C2′H) = 4.9 Hz, 1 H, C3′H), 5.71 (t, ^3J(C1′HC2′*H*C3′H,C1′*H*C2′HC3′*H*) = 4.9 Hz, 1 H, C2′H), 6.04 (dd, ^3J(C1′H,NHC1′) = 9.0, ^3J(C1′H,C2′H) = 4.9 Hz, 1 H, C1′H), 7.33 (d, ^3J(NHC1′,C1′H) = 9.0 Hz, 1 H, NHC1′), 7.55 (t, ^3J($C_{ar}H$,$C_{ar}H$) = 8.0 Hz, 1 H, $C_{ar}H$), 7.78 (d, ^3J($C_{ar}H$,$C_{ar}H$) = 8.0 Hz, 1 H, $C_{ar}H$), 7.86 (d, ^3J(CHON*H*,C*H*O) = 1.7 Hz, 1 H, CHON*H*), 8.02 (d, ^3J(C*H*O,*H*NCHO) = 1.7 Hz, 1 H, C*H*O), 8.30 (d, ^3J($C_{ar}H$,$C_{ar}H$) = 8.0 Hz, 1 H, $C_{ar}H$), 8.35 (s, 1 H, $C_{ar}H$), 9.14 (s, 1 H, NH), 11.52 (s, 1 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.53$, 12.75, 12.95, 13.32, 16.57, 16.61, 16.79, 16.81, 16.98 (2 C), 17.16, 17.26, 24.12, 60.66, 70.56, 77.13, 80.01, 80.46, 98.92, 123.81 (q, $^1J = 273$ Hz), 126.76 (q, $^3J = 4$ Hz), 129.42, 130.03 (q, $^3J = 4$ Hz), 130.05, 131.38 (q, $^2J = 33$ Hz), 133.21, 146.51, 152.57, 157.75, 159.99, 164.03, 171.85; ^{19}F NMR (188 MHz, $CDCl_3$): $\delta = -63.21$; MS (ESI$^+$): *m*/*z* (%): 788 (36) $[M+Na]^+$, 758 (100) $[MH]^+$; HR-MS (ESI$^-$): calcd for $[C_{32}H_{46}F_3N_5O_8Si_2-H]^-$: 756.2708, found 756.2709.

***α* and *β*-6-(3′-*O*,5′-*O*-[1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl]-2′-deoxy-D-ribofuranose-1′-yl)amino-2-acetylamino-5-formylamino-3*H*-pyrimidine-4-one (11)**: The sugar **10** (100 mg, 0.13 mmol), $MgClO_4 \times 6H_2O$ (9 mg, 0.04 mmol, 0.3 equiv) and 9-ethyl-3,6-dimethylcarbazole (5 mg, 0.02 mmol, 0.15 equiv) were dissolved in a mixture of isopropanol/water (9:1, 10 mL) After purging of the solution with N_2 for 45 min, the solution was irradiated with a medium pressure mercury lamp in a pyrex reaction vial for 15 h at 15 °C. The solvent was removed in vacuo and the residual material purified by flash chromatography. Compound **11** was obtained as a yellowish powder as a mixture of the two anomers (yield 60 %). Separation of the anomers was possible, with a small amount (12 mg) of the obtained material, by HPLC. **11a** (minor anomer): $R_f = 0.38$ (ethyl acetate/MeOH 10:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.02-1.05$ (m, 28 H, *i*Pr), 2.00–2.05 (m, 1 H, C2′H_a), 2.22 (s, 3 H, CH_3CO), 2.48–2.53 (m, 1 H, C2′H_b), 3.71–3.76 (m, 1 H, C5′H_a), 3.92–3.94 (m, 1 H, C4′H), 3.99–4.02 (m, 1 H, C5′H_b), 4.42–4.46 (m, 1 H, C3′H), 5.87–5.91 (m, 1 H, C1′H), 7.06 (d, ^3J(NH,C1′H) = 8.1 Hz, 1 H, NHC1′), 7.38 (s, 1 H, N*H*CHO), 8.07 (s, 1 H, NH), 8.23 (s, 1 H, C*H*O), 11.41 (s, 1 H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.21$, 12.62, 13.00, 13.13, 16.60, 16.71 (3C), 16.90, 17.01, 17.04, 17.13, 23.97, 39.49, 62.16, 71.65, 80.38, 83.26, 146.00, 152.74, 157.33, 159.27, 170.84, 170.89. HRMS (ESI$^-$): calcd for $[C_{24}H_{43}N_5O_7Si_2-H]^-$: 568.2623, found 568.2613.

Compound **11b** (major anomer): $R_f = 0.38$ (ethyl acetate/MeOH 10:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.02-1.10$ (m, 28 H, *i*Pr), 2.16–2.19 (m, 1 H, C2′H_a), 2.23 (s, 3 H, CH_3CO), 2.30–2.37 (m, 1 H, C2′H_b), 3.78–3.80 (m, 2 H, C5′H_a, C4′H), 3.98–4.04 (m, 1 H, C5′H_b), 4.54–4.58 (m, 1 H, C3′H), 5.83–5.90 (m, 1 H, C1′H), 7.44 (d, ^3J(NH,C1′H) = 8.7 Hz, 1 H, NHC1′), 7.59 (s, 1 H, N*H*CHO), 8.00 (s, 1 H, NH), 8.16 (s, 1 H, C*H*O), 11.45 (s, 1 H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.21$, 12.50, 13.00, 13.13, 16.62, 16.70 (2C), 16.77, 16.93, 17.01, 17.08, 17.17, 24.01, 40.43, 64.19, 72.59, 80.07, 84.35, 145.57, 152.74, 157.06, 158.99, 170.86, 170.89; MS (ESI$^-$): *m*/*z* (%): 604 (101), 658 (100) $[M-H]^-$; HR-MS (ESI$^-$): calcd for $[C_{24}H_{43}N_5O_7Si_2-H]^-$: 568.2623, found 568.2613.

***α*- and *β*-6-(3′-*O*,5′-*O*-[1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl]-2′-*O*-acetyl-*β*-D-ribofuranose-1′-yl)amino-2-acetylamino-5-formylamino-3*H*-pyrimidine-4-one (12)**: Sugar **13b** (253 mg, 0.40 mmol) was dissolved in degassed ethyl acetate (0.2 mL) under argon. Pd/C (250 mg) was added and the mixture was diluted with MeOH (2.5 mL). The solution was stirred under an H_2 atmosphere for 5 min and diisopropylethylamine (0.2 mL) was added. The hydrogenation was continued for another 10 min. The reaction mixture was filtered through Celite and the filtrate was concentrated to dryness in vacuo. The residual material was dissolved in degassed pyridine (2.5 mL). DCC (165 mg, 0.8 mmol, 2 equiv) was added and at 0 °C, HCOOH (30 µL, 0.8 mmol, 2 equiv) was added. The reaction was allowed to warm to r.t. over 12 h and was diluted with ethyl acetate (50 mL). The organic phase was extracted two times with 1N HCl (15 mL), sat. $NaHCO_3$ (15 mL) and sat. NaCl (15 mL) and dried over $MgSO_4$. After removal of the solvent in vacuo and separation of **12a** and **12b** by flash chromatography ($CHCl_3$, 1–4 % MeOH) were both compounds obtained in form of yellowish powders. **12b**: (104 mg, 0.16 mmol, 41 %). $R_f = 0.38$ ($CHCl_3$/MeOH 10:1); m.p. 122–124 °C; IR (KBr): $\tilde{\nu} = 702$, 1039, 1075, 1154, 1237, 1384, 1467, 1555, 1591, 1649, 1656, 1741, 1753, 2868, 2946, 3417 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.00-1.09$ (m, 28 H, *i*Pr), 2.14 (s, 3 H, CH_3), 2.22 (s, 3 H, CH_3), 3.87–3.92 (m, 1 H, C4′H), 3.94–4.01 (m, 2 H, C5′H), 4.46 (dd, ^3J(C3′H,C4′H) = 7.6, ^3J(C3′H,C2′H) = 5.3 Hz, 1 H, C3′H), 5.28 (dd, ^3J(C2′H,C3′H) = 5.3, ^3J(C2′H,C1′H) = 2.0 Hz, 1 H, C2′H), 5.63 (dd, ^3J(C1′H,NH) = 8.2, ^3J(C1′H,C2′H) = 2.0 Hz, 1 H, C1′H), 7.64 (d, ^3J(NH,C1′H) = 8.2 Hz, 1 H, N*H*C1′), 7.68 (s, 1 H, NH), 8.14 (d, ^3J(C*H*O,CHON*H*) = 2.0 Hz, 1 H, C*H*O), 8.40 (d, ^3J(N*H*CHO, C*H*O) = 2.0 Hz, 1 H, CHON*H*), 11.50 (s, 1 H, NH); ^{1}H NMR (400 MHz, $[D_6]$DMSO): $\delta = 0.97-1.06$ (m, 28 H, *i*Pr), 2.05 (s, 3 H, CH_3), 2.17 (s, 3 H, CH_3), 3.65–3.70 (m, 1 H, C4′H), 3.85–4.01 (m, 2 H, C5′H), 4.41–4.56 (m, 1 H, C3′H), 5.34–5.46 (m, 1 H, C2′H), 5.79–5.84 (m, 1 H, C1′H), 7.43 (d, ^3J(NHC1′,C1′H) = 9.6 Hz, 0.62 H, NHC1′(*cis*-amide)), 7.84 (d, ^3J(NHC1′,C1′H) = 9.0 Hz, 0.38 H, NHC1′(*trans*-amide)), 7.87 (d, ^3J(N*H*CHO,C*H*O) = 11.6 Hz, 0.38 H, N*H*CHO(*trans*-amide)), 8.18 (d, ^3J(N*H*CHO,C*H*O) = 0.8 Hz, 0.62 H, N*H*CHO (*cis*-amide)), 8.23 (d, ^3J (C*H*O, N*H*CHO) = 11.6 Hz, 0.38 H, C*H*ONH(*trans*-amide)), 8.87 (d, ^3J(N*H*CHO,C1′H) = 0.8 Hz, 0.62 H, C*H*O(*cis*-amide)), 11.55 (s, 2 H, 2 × NH); ^{13}C NMR (50 MHz, $[D_6]$DMSO): $\delta = 12.63$, 12.71, 13.06, 13.24, 16.88, 16.93 (2 × $\mathit{C}H_3CSi$), 16.96, 17.24, 17.27 (2 × $\mathit{C}H_3CSi$), 17.37, 20.76, 24.19, 62.51, 70.94, 76.58, 81.28, 84.56, 99.34, 145.88, 151.17, 157.39, 159.21, 169.91, 171.52; MS (ESI$^-$): *m*/*z* (%): 626 (100) $[M-H]^-$; HR-MS (ESI$^-$): calcd for $[C_{26}H_{45}N_5O_9Si_2 - H]^-$: 626.2678, found 626.2694. **12a**: (10 mg, 0.016 mmol, 10 %). $R_f = 0.42$ ($CHCl_3$/MeOH 9:1). M.p. 119–121 °C; IR (KBr): $\tilde{\nu} = 696$, 777, 860, 1038, 1156, 1232, 1386, 1419, 1465, 1507, 1559, 1586, 1632, 1761, 2869, 2946, 3446 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.93-1.00$ (m, 28 H, *i*Pr), 2.15 (s, 3 H, CH_3), 2.16 (s, 3 H, CH_3), 3.87–3.93 (m, 3 H, C4′H, C5′H), 4.33 (dd, ^3J(C3′H,C4′H) = 8.5, ^3J(C3′H,C2′H) = 4.6 Hz, 1 H, C3′H), 5.45 (t, ^3J = 4.6 Hz, 1 H, C2′H), 5.94 (dd, ^3J(C1′H,NH) = 8.7, ^3J(C1′H,C2′H) = 4.6 Hz, 1 H, C1′H), 7.48 (d, ^3J(NH,C1′H) = 8.7 Hz, 1 H, NH), 7.83 (d, ^3J(N*H*CHO,C*H*O) = 1.7 Hz, 1 H, N*H*CHO), 8.18 (d, ^3J(C*H*O,CHON*H*) = 1.7 Hz, 1 H, C*H*O), 8.98 (s, 1 H, NH), 11.45 (s, 1 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.67$ (2 CHSi), 12.97, 13.38, 16.84 (3 $\mathit{C}H_3CHSi$), 17.19 (4 $\mathit{C}H_3CHSi$), 17.29, 20.55, 24.16, 60.56, 70.23, 71.60, 79.98, 80.20, 98.96, 146.28, 152.29, 157.70, 159.70, 169.53, 171.73; MS (FD$^+$): *m*/*z* (%): 627 (100) $[MH]^+$; MS(ESI$^-$): *m*/*z* (%): 628 (100) $[M-H]^-$; HR-MS (ESI$^-$): calcd for $[C_{26}H_{45}N_5O_9Si_2 - H]^-$ 626.2678, found 626.2694.

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