The 7-Nitroindole Nucleoside as a Photochemical Precursor of 2′-Deoxyribonolactone: Access to DNA Fragments Containing This Oxidative Abasic Lesion

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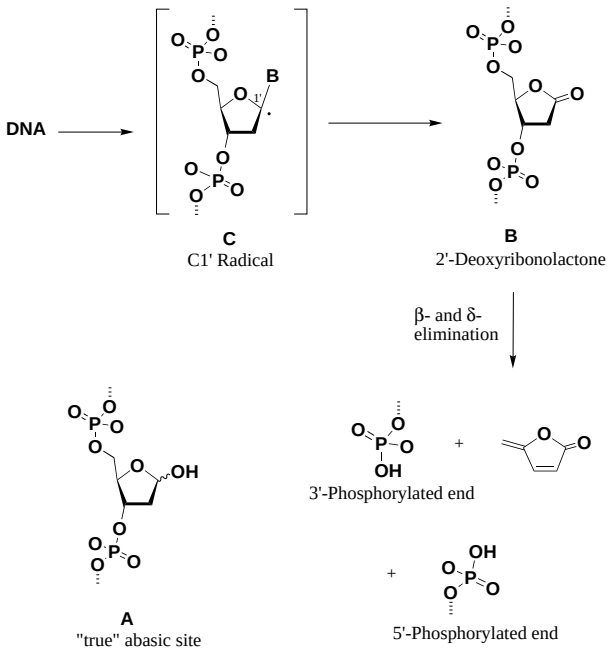
Abstract: On the basis of molecular modeling studies, the 7-nitroindole nucleoside **1** was selected as a suitable photochemical precursor for photochemical generation of the C1′ deoxyribosyl radical under irradiation, which led to 2′-deoxyribonolactone. The nitroindole nucleoside derivatives **1a** and **1b** were prepared and their conformation was determined by X-ray crystallography and NMR spectroscopy. The photoreaction of these nucleosides gave the corresponding deoxyribonolactone derivatives efficiently, with release of 7-nitrosoindole. This reaction was successfully applied to synthesis of oligonucleotides containing the deoxyribonolactone lesion.

Keywords: abasic lesions · DNA oxidation · molecular modeling · oligonucleotides · photochemistry

Introduction

A number of chemical and physical agents exist which damage DNA in the cell in a variety of ways; most of this damage is mended by enzymatic repair machinery in vivo. Loss of a nucleic base leaving a deoxyribose moiety in the DNA strand and creating a so-called abasic site (**A**) represents one of the most frequent types of DNA damage. A structurally related lesion is the 2′-deoxyribonolactone (**B**) produced in a variety of DNA oxidative processes.[1] These include UV light[2] and γ irradiation,[3] or the action of chemical agents such as the ene–diyne antibiotic neocarzinostatin.[4] The intermediacy of 2′-deoxyribonolactone has also been postulated in DNA cleavage by chemical nucleases based on metal complexes.[5] More recently, 2′-deoxyribonolactone was proposed as the alkali-labile site formed by γ irradiation of oligonucleotides[6] containing an 8-oxo deoxyguanosine moiety. In most of these cases, the mechanism of 2′-deoxyribonolactone formation involves initial formation of the C1′ radical (**C**) arising from abstraction of the anomeric hydrogen. The pathway by which ribonolactone is formed from the C1′ radical is still unclear and is the object of numerous current studies.[7–9] From the biological point of view, the 2′-deoxyribonolactone lesion has been reported to be mutagenic[4d] and to be resistant to repair enzymes.[4b] It is of poor chemical stability and is subject to successive β- and δ-elimination reactions leading to strand cleavage (Scheme 1). Because of this lability to alkali, little is known about the 2′-deoxyribonolactone site in DNA except for some special sequences.[3, 4, 9]

In the course of our continuous efforts directed toward synthesis[10] and study[11] of abasic lesions in DNA (**A**), we



Scheme 1. Deoxyribonolactone lesion formation in DNA.

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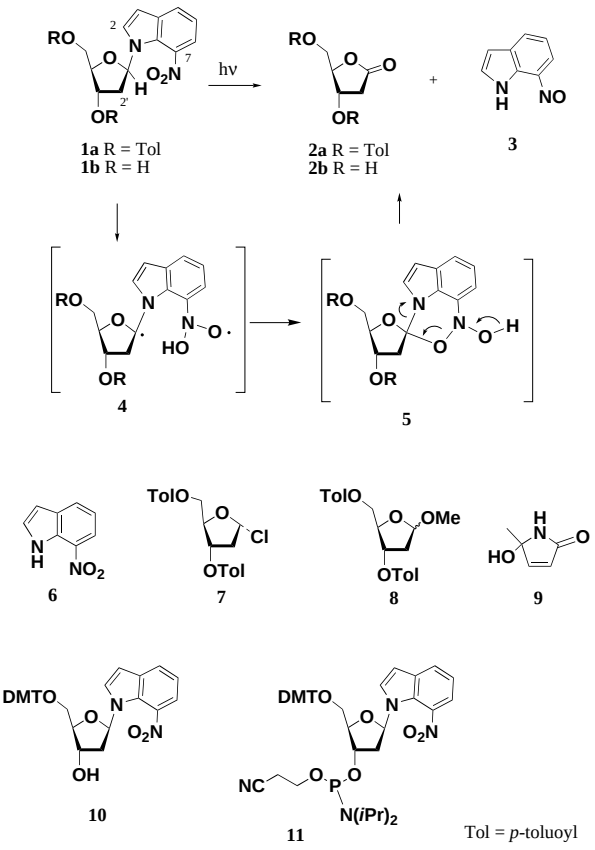
recently reported in a preliminary communication a highly efficient synthesis of short oligonucleotides containing the 2′-deoxyribonolactone at preselected positions.[12, 13] As the conventional phosphoramidite method cannot be used because of the above-mentioned alkali lability of the 2′-deoxyribonolactone site, we used a "postsynthetic approach". The approach is based on the photoreactivity of the 7-nitroindole nucleoside **1**, which gives the deoxyribonolactone moiety quantitatively under irradiation. In the present paper we report on the chemistry and concepts that underlie the synthetic approach; that is, we describe the design, synthesis, and conformation of the 7-nitroindole nucleoside models **1a** and **1b** and their photoconversion into the deoxyribonolactone derivatives **2a** and **2b** (Scheme 2). We show that this photochemical reaction occurs just as efficiently when the 7-nitroindole nucleoside is inserted inside an oligonucleotide, leading to DNA fragments containing the deoxyribonolactone lesion at any preselected position in the sequence.



Scheme 2. Photoreaction of **1a** and **1b** leading to the deoxyribonolactones.

Abstract in French: *Par une étude de modélisation moléculaire, le nucléoside 7-nitroindolique **1** a été retenu comme précurseur photochimique pour générer le radical C1′ conduisant à la formation de la 2′-désoxyribonolactone. Les nucléosides **1a** et **1b** ont été préparés, leur conformation a été déterminée par étude radiocristallographique aux rayons X et par spectroscopie de RMN. Leur irradiation conduit exclusivement à la formation des dérivés correspondants de la désoxyribonolactone avec libération de 7-nitrosoindole. La réaction permet l'accès à des fragments d'ADN contenant la lésion abasique désoxyribonolactone.*

Results and Discussion

Design of nucleosides 1: Two particular requirements have to be fulfilled in the design of a synthetic method to generate deoxyribonolactone from an appropriate precursor inside a DNA fragment: (1) the pH must be kept at neutrality to avoid DNA cleavage by β-elimination of the 3′-phosphate in the course of the reaction; (2) the yield of ribonolactone generation must be quantitative to afford pure DNA that does not necessitate any purification for further physical and biological studies. A promising approach was to mimic the mode of generation of the lesion in DNA with a method based upon radical abstraction of the H1′ anomeric hydrogen from a nucleoside.

We anticipated that the aromatic nitro group in the 7-nitroindole nucleoside **1**, if locked into a favorable position, could be a good candidate to abstract the anomeric hydrogen intramolecularly and photochemically. We expected that abstraction of the anomeric hydrogen by the photoactivated nitro group would generate the C1′ radical **4** which, in this case, may directly evolve toward 2′-deoxyribonolactone. The photochemical reactivity of nitrobenzene derivatives has been used in synthetic applications.[15] For example, *o*-substituted nitrobenzyl groups are widely used as photoremovable protecting groups.[10b, 16] A long-range intramolecular oxidation has been demonstrated using *p*-nitrobenzene derivatives covalently attached to a steroid.[17] An example of intermolecular photochemical oxidation has been reported for an inclusion complex of *p*-nitroacetophenone in β-cyclodextrin.[18] These photochemical oxidations are mediated by the well-established hydrogen abstraction ability of the triplet state of the aromatic nitro group.[19] We first undertook a molecular modeling study to test the validity of the hypothesis and, in particular, to evaluate the distance between the oxygen atom of the nitro group and the hydrogen atom to be abstracted.

Molecular modeling study of the 7-nitroindole nucleosides 1a and 1b: The molecular modeling studies were performed on derivatives **1a** and **1b** (Figure 1), which can be viewed as models for the 7-nitroindole nucleoside inserted in a DNA fragment. The structures were built and displayed by INSIGHT II, the simulations involving molecular minimizations (MM) using the CVFF force field and the BFGS minimizer of the DISCOVER program.[20a]

Rotation around the C1′–N bond was varied and the resulting energy curves after minimization for each dihedral angle were calculated. Two minima were observed for the bistoluoyl derivative **1a** and three minima for the unprotected nucleoside **1b**.

In the two conformations of minimum energy $\mathbf{1a_1}$ and $\mathbf{1a_2}$ for the bis(toluoyl)ester **1a**, the sugar conformation is C3′-*endo* and the indole ring is in *anti*-like conformation ($\mathbf{1a_1}$: $\chi = -139°$, $\mathbf{1a_2}$: $\chi = -98°$; χ is the O4′-C1′-N1-C2 angle). The strain energy balance favors structure $\mathbf{1a_1}$ over $\mathbf{1a_2}$ by

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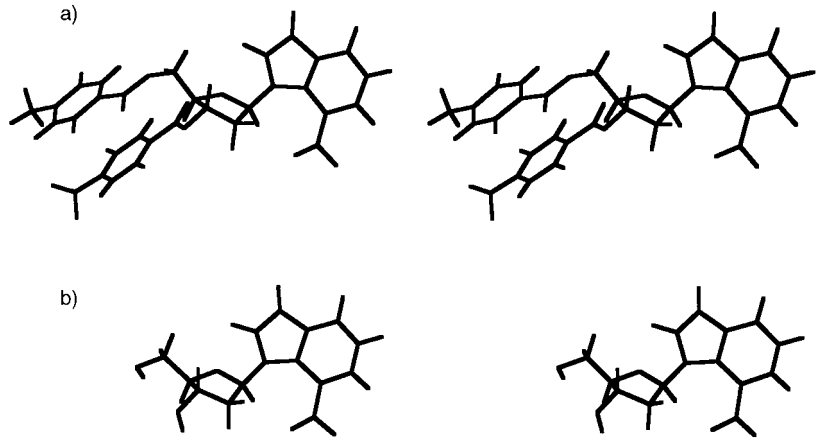

Figure 1. Stereoviews of the minimum-energy structures: a) $\mathbf{1a_1}$, b) $\mathbf{1b_1}$.

11 kJ mol^{-1}. On the contrary, the solvation contribution calculated according to the DelPhi procedure[20b, 20c] in two steps (60 and 90% filling) favors $\mathbf{1a_2}$. Taking account of all contributions, the final result is that $\mathbf{1a_1}$ is the more stable conformation with energy differences compared with $\mathbf{1a_2}$ of 6.4 kJ mol^{-1} in chloroform and 4.5 kJ mol^{-1} in water. Consequently, only one conformer, $\mathbf{1a_1}$, would be expected, in which the H1′ hydrogen is close to one of the oxygen atoms of the nitro group (2.32 Å) in a reactive position.

In the case of the unprotected nucleoside **1b**, a slightly different feature is apparent in the energy curves. Of the three structures of minimum energy, only the conformer $\mathbf{1b_1}$ has the *anti*-like conformation ($\chi = -138°$) and the two others possess *syn*-like conformations ($\mathbf{1b_2}$: $\chi = -41°$, $\mathbf{1b_3}$: $\chi = +28°$). The sugar conformation is C3′-*endo* for $\mathbf{1b_1}$ and $\mathbf{1b_3}$ and O4′-*endo* for $\mathbf{1b_2}$. After correction including the DelPhi solvation energy in water, $\mathbf{1b_1}$ is found to be preferred to conformer $\mathbf{1b_2}$ with a difference of 8.3 kJ mol^{-1} and to $\mathbf{1b_3}$ with a difference of 7.5 kJ mol^{-1}. In this case also, the H1′ hydrogen of conformer $\mathbf{1b_1}$ is close to an oxygen atom of the nitro group (2.37 Å).

In conclusion, calculations clearly indicate close proximity between the H1′ atom and one of the oxygen atoms of the nitro group, at distances of 2.32 and 2.37 Å in the conformations of minimum energy in the model nucleosides **1a** and **1b**.

Synthesis and structure of nucleosides 1a and 1b: On the basis of the results of the modeling study, we prepared the nitroindole nucleoside derivatives **1a** and **1b**. The sodium salt of 7-nitroindole (**6**) was generated by treatment of **6** with sodium hydride in acetonitrile and treated with a slight excess of bis(*p*-toluoyl)-α-chlorodeoxyribose (**7**) to afford the β-nucleoside **1a** in 50% yield.[21, 22] No trace of the α-anomer was detected in the reaction medium. The β-stereochemistry of the glycosyl bond was ascertained by ^{1}H NMR spectroscopy. Treatment of the ditoluoate **1a** in 1% NaOH/methanolic solution afforded the parent unprotected nucleoside **1b**.

Conformation of nucleoside **1b** in the solid, determined by X-ray crystallography, indicated an *anti* conformation for the 7-nitroindole residue, in which one of the oxygen atoms of the nitro group was located at a distance of 2.29 Å from the H1′ hydrogen.[23] This structure corresponds to the conformations predicted by the molecular modeling study except for the sugar conformation, as shown in Figure 2.

The conformation of **1a** in solution was studied by high-field NMR spectroscopy. Several NOESY spectra were recorded at 25 °C in $CDCl_3$ and interproton distances were estimated on the basis of the two-spin pair approximation assuming a single correlation time for all protons (Table 1). Neither of the two calculated structures $\mathbf{1a_1}$ and $\mathbf{1a_2}$ correctly fits the interatomic distances estimated from the NMR data. Nevertheless, a molecular dynamic simulation for 50 ps at 500 K exhibits several interconversions between the two positions and the average structure shows a better agreement, which indicates that the NMR geometrical parameters probably refer to a rapid-exchange average structure. In all cases, H1′ remains close to one oxygen of the nitro group ($d \leq$ 2.38 Å).

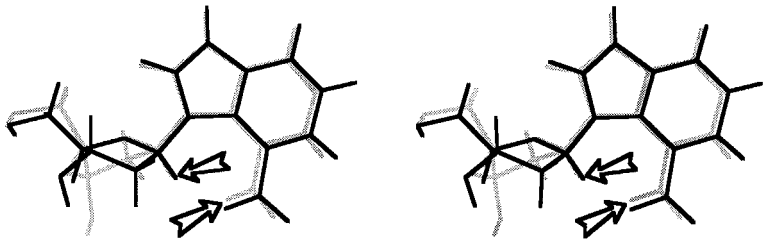

Figure 2. Stereoview of the minimum-energy structure ($\mathbf{1a_1}$: black) and X-ray structure (gray). The two arrows indicate the oxygen atom of the NO_2 group and the anomeric hydrogen atom (H1′).

Table 1. Interproton distances (Å) obtained by NMR (±10%) and molecular modeling studies.

	H2–H1′	H2–H2′	H2–H2″	H4′–H1′	H1′–O	χ	sugar pucker	ΔE[a] [kJ mol^{-1}]
1a (NMR)	3.45	2.61	>3.80	3.05	–	–	–	–
$\mathbf{1a_1}$	3.85	3.73	4.58	3.25	2.38	−139° (*anti*)	C3′-*endo*	0
$\mathbf{1a_2}$	3.90	2.55	4.00	3.28	2.35	−98° (*anti*)	C3′-*endo*	4.5
$\mathbf{1a_{dyn}}$	3.42	3.03	4.05	3.00	1.94	−148° (*anti*)	O4′-*endo*	–
1b (X-ray)	3.61	2.51	3.80	3.30	2.29	−115° (*anti*)	C2′-*endo*	–
$\mathbf{1b_1}$	3.86	3.64	4.56	3.19	2.37	−138° (*anti*)	C3′-*endo*	0
$\mathbf{1b_2}$	3.59	2.18	3.79	3.52	2.40	−41° (*syn*)	O4′-*endo*	8.3
$\mathbf{1b_3}$	2.52	4.17	3.77	2.69	4.27	+28° (*syn*)	C3′-*endo*	7.5

[a] Energy difference (vs. $\mathbf{1a_1}$or $\mathbf{1b_1}$) calculated with DelPhi process.

Photochemical release of 2-deoxyribonolactone derivatives (2a and 2b) from 7-nitroindole nucleosides 1a and 1b: The photochemistry of the 7-nitroindole nucleosides **1a** and **1b** was studied in degassed dilute aqueous solution (0.25 mM in acetonitrile/H_2O 1:1 for **1a**, 0.17 mM in H_2O for **1b**). In both cases, the corresponding 2′-deoxyribonolactone derivatives **2a** or **2b** were formed upon irradiation by UV light with a Pyrex filter under an argon atmosphere. Very clean reactions were observed by HPLC and by UV spectroscopy when dilute solutions were irradiated. HPLC assay of the photolysis of the bistoluoyl derivative **1a** (Figure 3) indicated that **1a** (λ_{max} = 357 nm) was completely consumed within 1.5 h to yield two photoproducts, the deoxyribonolactone **2a** (λ_{max} = 241 nm) and nitrosoindole **3** (λ_{max} = 406 nm). Two isosbestic points at 310 and 365 nm were observed in the UV spectra of the irradiated solution of the free nucleoside **1b** monitored at different time intervals (Figure 4).

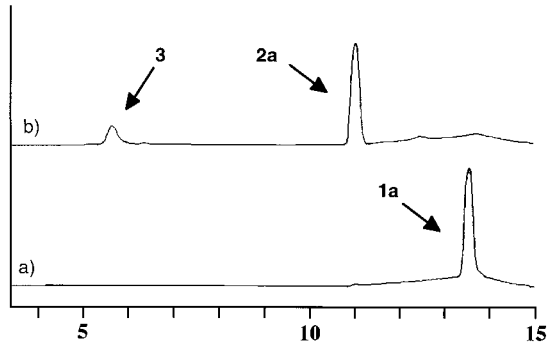

Figure 3. HPLC analysis of photoreaction of **1a**: a) before irradiation; b) after irradiation for 90 min (conditions: see Experimental Section).

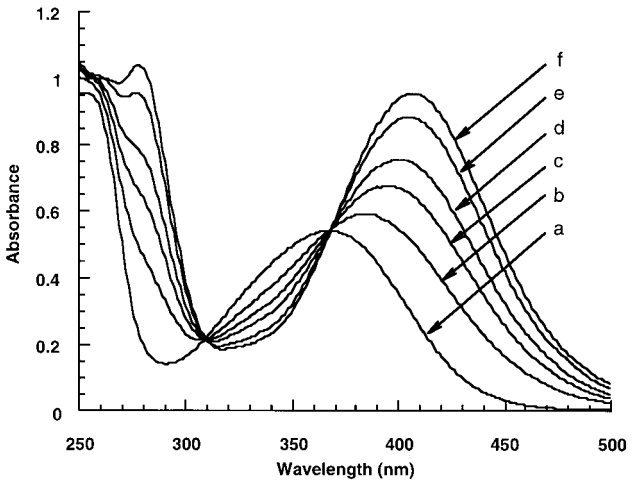

Figure 4. UV spectra of **1b** (0.17 mM) in water: a) before irradiation; b) after irradiation for 10 min; c) 20 min; d) 30 min; e) 60 min; and f) 120 min (conditions: see Experimental Section).

On the semipreparative scale, the photoreaction was less efficient, probably because of the higher concentration of the starting material (c = 1.0 – 1.4 mM). Nevertheless, the bis(toluoyl)deoxyribonolactone **2a** and 7-nitrosoindole (**3**) were isolated in 31 % and 49 % yield, respectively, after chromatographic separation. The modest yield of **2a** in this case may be explained partly by the photochemical instability of the toluoyl protecting group. In fact, 0.35 mmol-scale photolysis of the free nucleoside **1b** over a prolonged irradiation time (15 h) afforded the best yields of **2b** (crude yield 97 %) and **3** (59 %).

The deoxyribonolactone derivatives (**2a** and **2b**) were characterized by all the usual analytical data and by comparison with authentic samples. Lactone **2a** was independently prepared by means of the direct conversion method of protected lactols into lactones developed by Grieco et al.[24] The lactone **2a** was thus obtained in 88 % yield by oxidation of the methoxy sugar **8** by *m*-chloroperbenzoic acid in the presence of a catalytic amount of boron trifluoride etherate. An attempt to prepare **2b** from **2a** by treatment under mild basic hydrolysis conditions was not successful. Under basic conditions, the β-elimination reaction seems to predominate. For example, treatment of **2a** with a methanolic ammonia solution afforded the unsaturated lactam **9** in 59 % yield.[25] The deoxyribonolactone **2a** was thus prepared by the literature oxidation procedure starting from D-deoxyribose and using bromine as the oxidizing agent.[3c, 26]

The monomeric nature of 7-nitrosoindole (**3**) was confirmed by combined spectroscopic analysis. In the vibrational spectrum, the N–O stretching band was found at 1530 cm^{-1}, and in the mass spectrum (electron impact), the molecular ion peak was observed (M = 146). The UV spectral data (λ_{max} = 406 nm) and the NMR spectral data (see Experimental Section) are also in good agreement with the data described for an analogous structure (5-substituted 7-nitrosoindole: λ_{max} = 412 nm).[15a]

The mechanism proposed for this photolysis is presented in Scheme 2. Initial abstraction of the anomeric hydrogen by the photoactivated nitro group generates the C1′ radical **4**. Because of the reduced rotational freedom of the *N*-glycosidic bond in the 7-nitroindole nucleoside, the seven-membered ring transition state[27] required for anomeric hydrogen abstraction is favored. The resulting C1′ radical **4** in this case evolves toward a cyclic intermediate (**5**) by intramolecular diradical recombination. Finally, ring-opening cleavage of **5** affords the 2′-deoxyribonolactone **2** accompanied by 7-nitrosoindole (**3**). The high efficiency of the photolysis in anaerobic conditions and formation of **3** are in agreement with this proposed mechanism based on the preceding studies.[19]

Photogeneration of deoxyribonolactone inside DNA fragments. Syntheses of oligonucleotides containing the abasic lesion: As reported in our preliminary communication,[12] the reaction was next examined at the oligonucleotide level with the aim of developing a general synthesis of DNA fragments containing the deoxyribonolactone lesion.

The 7-nitroindole nucleoside **1b** was protected and activated according to the conventional phosphoramidite method. Successive treatment with 4,4′-dimethoxytrityl chloride in pyridine and with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite gave **11** in excellent yields (87 % for tritylation and 97 % for phosphorylation). The modified building block (**Ni**) was inserted into oligonucleotides by automated solid-

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phase synthesis. The two pentamers d(GC**Ni**TA) (**12**) and d(CA**Ni**GT) (**13**) and the undecamer d(CGCAC**Ni**CACGC) (**14**) were obtained and characterized by ESMS and 500 MHz ^{1}H NMR spectroscopy.[12] Irradiation by UV light with a Pyrex filter under argon atmosphere, as carried out previously for the monomer **1**, yielded the corresponding oligomers containing the deoxyribonolactone moiety. As the 2′-deoxyribonolactone site in DNA is unstable and subject to β-elimination, the irradiations were best performed in phosphate buffer (pH 6.0). The transformations were complete within 30–45 min of irradiation. While the photoreaction is quite efficient, cleavage of the final oligonucleotides was frequently observed during our studies, in particular with prolonged reaction times in nonbuffered solution and during evaporation or lyophilization of the aqueous solutions. We also repeatedly observed 10–15% cleavage during evaporation (or lyophilization) of the HPLC-purified fractions containing ammonium acetate salt. This problem was circumvented by use of phosphate buffer for the HPLC elutions. Under these conditions, the deoxyribonolactone-containing oligonucleotides were obtained typically with 80% yield in pure material, without cleavage, as demonstrated by the ^{1}H NMR spectra (see refs. [12, 28]). The final oligomers were also characteried by their ESMS.

Conclusion

The 7-nitroindole nucleosides **1a** and **1b** were shown to be useful photochemical precursors of 2′-deoxyribonolactone derivatives **2a** and **2b**. The only secondary product of these photoreactions was 7-nitrosoindole (**3**). These nucleosides were rationally designed by molecular modeling. Conformational analysis by NMR and by X-ray studies did indeed validate the initial prediction of molecular modeling. The photochemistry of 7-nitroindole nucleoside was further used for postsynthetic incorporation of the deoxyribonolactone lesion in DNA.[12] This approach constitutes a very efficient and general process to prepare oligonucleotides containing the labile deoxyribonolactone modification at a preselected position. The method was applied to prepare an undecamer containing the lesion in the middle of the sequence that was hybridized with the complementary strand. The structural properties of the resulting duplex have been studied using high-field NMR spectroscopy and molecular modeling.[28] The biological consequence of the presence of the lesion in DNA fragments is currently under investigation.

Experimental Section

General: All commercially available chemical reagents were used without purification. The α-chloro sugar **7** was prepared as described.[22] 7-Nitroindole (**6**) is commercially available from Lancaster. Alternatively we prepared **6** from *o*-nitroaniline or from ethyl 7-nitroindole 2-carboxylate (ACROS) by described procedures.[29, 30] Analytical TLC was performed on 0.25 mm silica 60 coated aluminum foils with F254 indicator (Merck). Preparative column chromatography was executed using silica gel (Merck 60, 0.063–0.200 mm). Analytical and semipreparative HPLC was performed with Millipore–Water equipment (two M-510 pumps, solvent gradient M 680) with a UV detector (M 490 and diode array 990). Melting points were measured on a Reichert Thermovar apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC200 spectrometer. Spectra were referenced to the residual proton solvent peaks. Fourier transform infrared spectra were measured with an Impact 400 spectrophotometer and UV spectra with a Perkin–Elmer spectrophotometer. Mass spectra were recorded on a Delsi–Nermag R10–10. Elemental analyses were performed by the Service Central de Microanalyse du CNRS.

1-(2′-Deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-pentafuranosyl)-7-nitroindole (1a): Sodium hydride (60% dispersion in oil, 0.82 g) was added to a solution of 7-nitroindole (**6**, 3.00 g, 18.5 mmol) in acetonitrile (40 mL) and the solution was stirred at room temperature for 30 min. The deoxyribosyl chloride **7** (7.90 g, 20.3 mmol) was then added and the solution stirred overnight. The solution was filtered through Celite and the solvent removed in vacuo. The oily residue (10.4 g) was directly purified by column chromatography (CH_2Cl_2) affording compound **1a** (4.82 g, 50% yield); m.p. 105–110 °C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.39$ (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.73 and 2.90 (2m, 2H, H2′ and H2″), 4.50 (m, 3H, H4′, H5′ and H5″), 5.59 (m, 1H, H3′), 6.55 (t, $J = 6$ Hz, 1H, H1′), 6.64 (d, $J = 3$ Hz, 1H, H3), 7.08–7.28 (m, 5H, 4H-Tol and H5), 7.57 (d, $J = 3$ Hz, 1H, H2), 7.77–7.97 (m, 6H, 4H-Tol and H4, H6); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 22.0$, 39.0, 64.0, 74.0, 82.0, 87.5, 104.0, 119.0, 120.0, 126.0, 127.0, 128.0, 129.0, 131.0, 133.5, 137.0, 144.0, 145.5, 166.0; MS (DCI, NH_3/isobutane): *m*/*z*: 532 $[M+NH_4]^+$; UV (EtOH): λ_{max} (ε) = 240 (38050), 357 (3500) nm ($mol^{-1}\,dm^3\,cm^{-1}$); IR (KBr): $\tilde{\nu} = 2955$, 2360, 1725, 1634, 1516, 1260, 1105, 755 cm^{-1}; elemental analysis calcd (%) for $C_{29}H_{26}N_2O_7$ (514.5): C 67.69, H 5.09, N 5.44; found C 67.71, H 5.06, N 5.52.

1-(2′-Deoxy-β-D-*erythro*-pentafuranosyl)-7-nitroindole (1b): Ditoluoate **1a** (1.5 g, 2.9 mmol) was dissolved in 1% NaOH/methanolic solution and stirred for 50 min at room temperature. The solvent was then evaporated and free nucleoside **1b** was purified by column chromatography (CH_2Cl_2/MeOH: 90/10, v/v). Nucleoside **1b** was obtained as yellow needles in 93% yield (0.75 g). M.p. 92–97 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 2.40$ (m, 2H, H2′ and H2″), 3.37 (m, 2H, H5′ and H5″), 3.76 (m, 1H, H4′), 4.28 (m, 1H, H3′), 4.87 (t, $J = 4$ Hz, 1H, O5′H), 5.29 (d, $J = 4$ Hz, 1H, O3′H), 6.19 (t, $J = 6$ Hz, 1H, H1′), 6.77 (d, $J = 3$ Hz, 1H, H3), 7.20 (t, $J = 8$ Hz, 1H, H5), 7.76 (d, $J = 8$ Hz, 1H, H4), 7.88 (d, $J = 3$ Hz, 1H, H2), 7.94 (d, $J = 8$ Hz, 1H, H6); ^{13}C NMR (50 MHz, [D$_6$]DMSO): $\delta = 40.5$, 61.2, 70.4, 86.3, 86.9, 103.4, 118.9, 119.1, 125.3, 126.7, 128.5, 132.9, 136.6; MS (DCI, NH_3/isobutane): *m*/*z*: 296 $[M+NH_4]^+$, 278 $[M]^+$; UV (MeOH): λ_{max} (ε) = 256 (5700), 360 (3300) nm ($mol^{-1}\,dm^3\,cm^{-1}$); IR (KBr): $\tilde{\nu} = 3313$, 3241, 2957, 2866, 1628, 1510, 1269, 1017, 701 cm^{-1}; elemental analysis calcd (%) for $C_{13}H_{14}N_2O_5$ (278.3): C 56.11, H 5.07, N 10.07; found C 56.30, H 5.30, N 9.86.

Photolysis of the 7-nitroindole compounds 1a and 1b: The photolysis lamp, suspended in a jacketed water-cooled immersion well, was a 100 W high-pressure mercury-arc Hanovia lamp with pyrex filter.

Irradiation of **1a** (analytical run): A solution of **1a** (0.25 mM) in H_2O/CH_3CN (1:1) was irradiated and the reaction was followed by HPLC analysis. Reverse phase μ-Bondapak C-18 column (Millipore–Waters: 3.9 × 300 mm) was used with methanol/water pH 2.5 gradient, flow 2 mL min^{-1} for 10 min.

Irradiation of **1a** (preparative run): A solution of **1a** (129 mg, 0.251 mmol) in acetonitrile/*i*PrOH (250 mL, 95:5, *v*/*v*) was irradiated under argon for 2 h. After removal of solvent, crude residue was purified by chromatography (CH_2Cl_2) affording the nitrosoindole **3** (18 mg, 0.123 mmol, 49%) and the lactone **2a** (29 mg, 0.079 mmol, 31%).

Data for **3**: m.p. 76–78 °C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.59$ (dd, 1H, $J = 3$ and 2 Hz, H3), 7.26 (d, 1H, $J = 2$ Hz, H2), 7.53 (t, 1H, $J = 8$ Hz, H5), 8.03 (d, 1H, $J = 8$ Hz, H4), 9.16 (d, 1H, $J = 8$ Hz, H6), 10.4 (br s, 1H, H1); MS (EI): *m*/*z*: 146 $[M]^+$, 116 $[M-NO]^+$, 89, 63; UV [$c = 50$ μM L^{-1} in H_2O/CH_3CN (99:1)]: λ_{max} (ε) = 228 (7500), 278 (5900), 406 (7000) nm ($mol^{-1}\,dm^3\,cm^{-1}$); IR (KBr): $\tilde{\nu} = 3420$, 3380, 3290, 3180, 1640, 1570, 1534, 1443, 1397, 1346, 1283, 1161, 1118, 995, 870, 827, 804, 733, 690, 636, 587, 464 cm^{-1}.

Data for **2a**: m.p. 115–117 °C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.35$ (2s, 6H, $2CH_3$), 2.78 (dd, $J = 12$ Hz, 2 Hz, 1H, H2′), 3.15 (dd, $J = 12$ Hz, 7 Hz, 1H, H2″), 4.60 (ABX, 2H, H5′ and H5″), 4.90 (m, 1H, H4′), 5.56 (m, 1H, H3′), 7.19 (m, 4H, ArH), 7.84 (m, 4H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 21.6$, 34.9, 63.7, 71.6, 82.5, 125.8, 126.2, 129.3, 129.5, 129.7, 144.3, 144.7, 165.7, 165.8, 174.1; MS (DCI, NH_3/isobutane): *m*/*z*: 386 $[M+Na]^+$,

369 $[M+H]^+$, 250, 233; UV (EtOH): λ_{max} (ε) = 241 (30000) nm ($mol^{-1}dm^3cm^{-1}$); IR (KBr): $\tilde{\nu}$ = 2968, 2357, 1795 (C=0), 1733 (C=0), 1622, 1283, 1097, 764 cm^{-1}.

Irradiation of **1b** (analytical run): An aqueous solution of **1b** (0.17 mM, 2.5 mL) in a quartz cube was irradiated at 5 cm distance from the photolysis lamp and the UV spectra were recorded at different intervals.

Irradiation of **1b** (preparative run): A solution of **1b** (100 mg, 0.35 mmol) in water/methanol (250 mL, 92:8, *v*/*v*) was irradiated under argon for 15 hours. The reaction mixture was extracted with dichloromethane (2 × 50 mL), the organic phase was dried ($MgSO_4$), and the dichloromethane was removed under vacuum, affording the nitrosoindole **3** (30 mg, 0.21 mmol, 59% yield). The aqueous phase was filtered on Celite and lyophilized to afford a colorless oil (45 mg, 0.34 mmol, 97% yield). As described previously, the deoxyribonolactone **2b** is in equilibrium between the open carboxylate form and the five-membered lactone form.[26b] The ^{1}H NMR spectrum of the crude **2b** in D_2O showed the presence of about 20% of the open form which decreased to about 10% upon addition of trifluoroacetic acid. We also found that the lactone form is predominant (about 95%) at pD 7.4 in phosphate buffer.

Most of the data for **2b** are found in ref. [26]. ^{1}H NMR (200 MHz, D_2O, pD 7.4): δ = 2.55 (dd, J = 18 and 3 Hz, 1H, H2′), 3.02 (dd, J = 18 and 6 Hz, 1H, H2″), 3.73 (dd, J = 13 and 4 Hz, 1H, H2′), 3.85 (dd, J = 13 and 3 Hz, 1H, H2″), 4.53 (m, 2H, H3′ and H4′).

3,5-Di-*O*-*p*-toluoyl-2-deoxy-D-ribono-1,4-lactone (2a): $BF_3 \cdot Et_2O$ (5 mg) and *m*CPBA (0.274 g, 1.4 mmol) were added to a solution of **8** (0.5 g, 1.3 mmol) in CH_2Cl_2. The mixture was stirred overnight under argon at room temperature. Et_2O was then added and the organic layer was washed with aqueous $NaHCO_3$ solution and saturated NaCl aqueous solution, and then dried over $MgSO_4$. After evaporation, the lactone **2a** was obtained as a white powder in 88% yield.

5-Hydroxy-3-pyrrolin-2-one (9): A solution of **2a** (2.0 g, 5.4 mmol) in saturated NH_3/MeOH (30 mL) was stirred at room temperature overnight. After removal of the solvent, the residue was passed through a silica gel column (AcOEt) to afford the hydroxylactam **9** (360 mg, 59%).^{1}H NMR (200 MHz, [D_6]DMSO): δ = 1.38 (s, 3H, CH_3), 5.77 (s, 1H, OH), 5.82 (d, J = 5 Hz, 1H, H3), 6.95 (d, J = 5 Hz, 1H, H4), 8.31 (s, 1H, NH); ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 25.5, 86.9, 125.1, 153.5, 171.3; MS (DCI, NH_3/isobutane): m/z: 114 $[M+H]^+$.

Molecular modeling study: The structures were built and displayed by INSIGHT II, version 2.3.5, the simulations involving molecular minimizations (MM) using the CVFF force field and the BFGS minimizer of the DISCOVER program, version 2.95, from Biosym[20a] implemented on IRIS or INDIGO workstations (SGI) and on RISC RS6000–540 (IBM). Molecular dynamics simulations were performed in 1 fs steps over 50 ps at T = 500 K with dielectric constant ε_r = 1. Solvent contributions were calculated with the DelPhi process,[20b, 20c] in two focusing steps, with ε_r = 80 for the solvent, ε_r = 1 for the solute.

NMR study: NMR experiments with **1a** were conducted at 25 °C in $CDCl_3$ on a Unity+500 Varian spectrometer equipped with a 5 mm indirect probe. NOESY spectra were recorded with mixing times of 50, 75, 100 and 150 ms, and were acquired using 2048 complex points in t_2, and 300 in t_1 increments with 24 scans for each t_1 value. The interproton distances were estimated on the basis of the two-spin pair approximation assuming a single correlation time for all protons. The volume integrals of NOE cross-peaks as a function of the four mixing times were measured to generate the build-up curves. The distances (r_{ij}) were calculated using the relationship $r_{ij} = r_{ref}(\sigma_{ref}/\sigma_{ij})^{1/6}$, where r_{ref} is a known calibration distance and σ_{ref} and σ_{ij} are the initial cross-relation rates for the calibration and unknown distances. The nitroindole H2–H3 distance of 2.67 Å was used as a reference (r_{ref}). The upper and lower bound ranges on the calculated distances were determined from the resolution of NOE cross-peaks and the quality of the NOE build-up plots.

5′-Dimethoxytrityl-7-nitroindolenucleoside (10): 4,4′-Dimethoxytrityl (DMT) chloride (0.85 g, 2.5 mmol), Et_3N (0.25 g, 3.5 mmol), and 4-dimethylaminopyridine (0.011 g, 0.1 mmol) were added to a solution of **1b** (0.50 g, 1.8 mmol) in pyridine (20 mL), and the solution was stirred at room temperature for 1.5 h, then poured into $NaHCO_3$ (5%) aqueous solution (100 mL). The resulting mixture was extracted twice with Et_2O (100 mL). The organic phase was dried with $MgSO_4$ and concentrated to an oily residue. This material was coevaporated twice with a small volume of toluene in order to remove the residual pyridine before purification by passage through a silica gel column (5% AcOEt/CH_2Cl_2) to afford **10** (0.91 g, 87%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.57 (t, J = 6 Hz, 2H, H2′ and H2″), 3.26 (d, J = 4 Hz, 2H, H5′ and H5″), 3.76 (s, 6H, OMe), 3.97 (m, 1H, H4′), 4.48 (m, 1H, H3′), 6.41 (t, J = 6 Hz, 1H, H1′), 6.58 (d, J = 3 Hz, 1H, H3), 6.73–6.79 (m, 4H, ArH), 7.13 (t, J = 7 Hz, 1H, H5), 7.16–7.37 (m, 9H, ArH), 7.78 and 7.81 (d × 2, J = 7 Hz, 1H × 2, H4 and H6); MS (DCI, NH_3/isobutane): m/z: 582 $[M+2H]^+$.

Phosphoramidite 11: 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.45 mL, 2.0 mmol) was added dropwise to a solution of **10** (580 mg, 1.0 mmol) and EtN(*i*Pr)$_2$ (0.70 mL, 4.0 mmol) in CH_2Cl_2 (5 mL). The solution was stirred at room temperature for 30 min. The reaction mixture was then passed immediately through a column of silica gel (eluent: 1:2 AcOEt/cyclohexane) to afford **11** as a yellow powder (0.76 g, 97%). ^{31}P NMR (81 MHz, $CDCl_3$): δ = 147.3 and 146.9; MS (DCI, NH_3/isobutane): m/z: 780 $[M]^+$.

Oligonucleotide synthesis: Oligodeoxyribonucleotides were synthesized by means of standard solid-phase cyanoethyl phosphoramidite chemistry on a Milligen/Biosearch 8700 DNA synthesizer. The standard 15 μmol cycle was used except for incorporation of the nitroindole nucleoside **11**, which was done with prolonged coupling time (15 min). The coupling yields for **11** were estimated from the trityl effluent to be higher than 90%. The oligomers were purified twice (trityl on mode and trityl off mode) by HPLC on a reverse-phase nucleosil C-18 column (Macherey–Nagel 10 × 250 mm) with a linear gradient of acetonitrile (trityl on: 10–35% in 15 min; trityl off: 5–35% in 20 min) in 20 mM ammonium acetate solution (pH 7). The following ε values (260 nm, $mol^{-1}dm^3cm^{-1}$) were used for the determination of the concentration of single-stranded oligonucleotides in aqueous solution: $\varepsilon = 44 \times 10^3$ for **12** and **13** and $\varepsilon = 94 \times 10^3$ for **14** [$\varepsilon = (15.4N_A + 11.5N_G + 7.4N_C + 8.7N_T + 5.7N_{Ni}) \times 0.9 \times 10^3$]. Final yields of purified oligomers were 134 unit OD_{260} (3.0 μmol) for **12**, 112 unit OD_{260} (2.5 μmol) for **13**, and 430 unit OD_{260} (4.6 μmol) for **14**; ESMS (TEA/water/acetonitrile) **12** and **13**: $[M-H]^-_{obs}$ = 1514 ($[M-H]^-_{calcd}$ = 1514); **14**: $[M-H]^-_{obs}$ = 3298 ($[M-H]^-_{calcd}$ = 3298).

Photolysis of oligonucleotides: For the photolysis of oligonucleotides, the same lamp as above was used. Typical experimental procedure is as follows: A solution of **14** (15 μM × 100 mL) in 5 mM sodium phosphate buffer (pH 6) was irradiated for 30 min under Ar at room temperature while being stirred. The total conversion was verified by HPLC (see ref. [12]). The solution was extracted twice with Et_2O and concentrated by rotary evaporator at 45 °C. The residue was further purified by HPLC in three injections (reverse-phase nucleosil C-18 column; Macherey–Nagel 10 × 250 mm) with a linear gradient of methanol (5–50% in 20 min) in 5 mM sodium phosphate solution (pH 6). The flow rate was 4 mL min^{-1}. Recovered fractions (22 mL) were evaporated under vacuum at 45 °C and then transferred to an eppendorf with a small volume of water and lyophilized. The deoxyribonolactone-containing oligonucleotide thus obtained (with phosphate salts) was used without desalting, except for ESMS analysis, for which a small amount was desalted by HPLC (MeOH/water). The masses corresponding to the cleaved products were observed (see ref. [12]). Yield of purified oligonucleotide was typically 80%. ESMS (TEA/water/acetonitrile) from **12** and from **13**: $[M-H]^-_{obs}$ = 1368 ($[M-H]^-_{calcd}$ = 1368); from **14**: $[M-H]^-_{obs}$ = 3151 ($[M-H]^-_{calcd}$ = 3152).

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