A Highly Efficient Synthesis of Oligodeoxyribonucleotides Containing the 2′-Deoxyribonolactone Lesion

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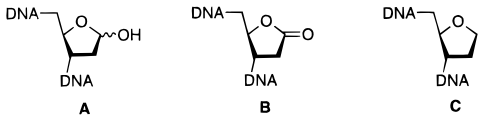
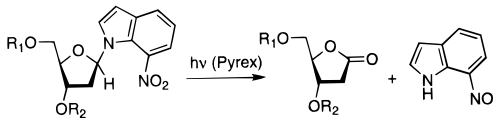
Loss of a base in DNA leaving a 2′-deoxyribonolactone residue **B** (Scheme 1) is a common abasic site damage that occurs through a variety of processes, most of which are interpreted by radical abstraction of a hydrogen atom at C-1′ of a 2′-deoxyribose unit.[1] For example, 2′-deoxyribonolactone formation has been reported to occur under the action of drugs such as enediyne antibiotics (e.g., neocarzinostatin)[2] and cationic manganese porphyrins[3] or under γ and UV irradiation.[4,5] 2′-Deoxyribonolactone has also been suspected to be formed as an intermediate in copper phenanthroline DNA cleavage.[6] The lesion has been reported to be mutagenic,[7] to be resistant to repair nucleases,[8] and to lead to DNA strand scission by β- and δ-elimination processes.[6c] Indeed this poor chemical stability of alkaline labile 2′-deoxyribonolactone in DNA has considerably limited our knowledge of the structural, chemical, and biological consequences of the lesion. Notably there is no report in the literature of a general method for preparing oligonucleotides containing the lesion.[9]

In the course of programs devoted to synthesis[11] and study[12] of "true" abasic site **A** (Scheme 1) and of its recognition by synthetic molecules,[13] we became interested in the examination of the structurally related 2′-deoxyribonolactone damage. We report here a general and efficient synthesis of oligodeoxyribonucleotides containing the 2′-deoxyribonolactone site at any preselected position in the sequence.

Scheme 1. Structure of Abasic Sites: A, 2′-Deoxyribose ("True" Abasic Site); B, 2′-Deoxyribonolactone; C, Tetrahydrofuran Analog



Scheme 2



1: $R_1 = R_2$ = Tol
2: $R_1 = R_2$ = H
3: R_1 = GC, R_2 = TA
4: R_1 = CA, R_2 = GT
5: R_1 = CGCAC, R_2 = CACGC

6: $R_1 = R_2$ = Tol
7: R_1 = GC, R_2 = TA
8: R_1 = CA, R_2 = GT
9: R_1 = CGCAC, R_2 = CACGC

The idea underlying the approach is to mimic the mode of formation of the lactone in DNA, i.e., to generate a radical at carbon C-1′ of a deoxyribose residue with total selectivity and high efficiency in nonaggressive conditions (neutral pH, room temperature). The biomimetic intramolecular H-abstraction route has been pioneered by Breslow[14] and shown to be quite successful. The nitro group, when irradiated, is an efficient radical generator that can be conveniently attached to an aromatic ring.[15] The 7-nitroindole nucleoside **2** was thus selected for intramolecular H-abstraction, on the basis of simple model examination and molecular modeling calculations, which showed that one of the oxygen atoms of the nitro group is in close proximity to the H-1′ atom to be abstracted. Nucleoside **2** was prepared by stereoselective glycosylation of 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride by 7-nitroindole followed by NaOH treatment (yield 50%). The X-ray structure of the crystalline nucleoside **2** indicated a 2.4 Å distance between H-1′ and one of the oxygens of the nitro group.[16] The 500 MHz ^{1}H NMR conformational analysis of the corresponding bis-*p*-toluoyl derivative **1** in $CDCl_3$ confirmed the close proximity of those two atoms in solution. Irradiation[17] of **1** in aqueous acetonitrile solution (1:1 by volume, $C = 2$ mM) for 1 h yielded the 2′-deoxyribonolactone derivative **6** along with 7-nitrosoindole **10**.[18] No trace of side products was detected in the HPLC chromatogram of the irradiation mixture.

The 7-nitroindole nucleoside **2** was then incorporated into oligonucleotides according to the classical phosphoramidite technology. Two pentamers were first prepared, containing the four natural bases with the nitroindole nucleoside (**Ni**) in the middle of the sequence flanked either by pyrimidines or by purines, respectively d(GC**Ni**TA) **3** and d(CA**Ni**GT) **4**. Coupling of the **Ni** nucleoside occurred in satisfactory yield. The oligomers

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(9) Deoxyoligonucleotides containing a 5′-A^{Br}U site in the middle of a duplex structure undergo photoreaction to produce a 2′-deoxyribonolactone residue with release of free adenine.[10]

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(17) The photolysis lamp, suspended in a jacketed, water-cooled immersion well, is a 100-W high-pressure mercury arc Hanovia lamp with Pyrex filter. Owing to the known character of the ribonolactone moiety, the irradiation time and concentration as indicated must be strictly controlled to avoid strand cleavage during irradiation and workup.

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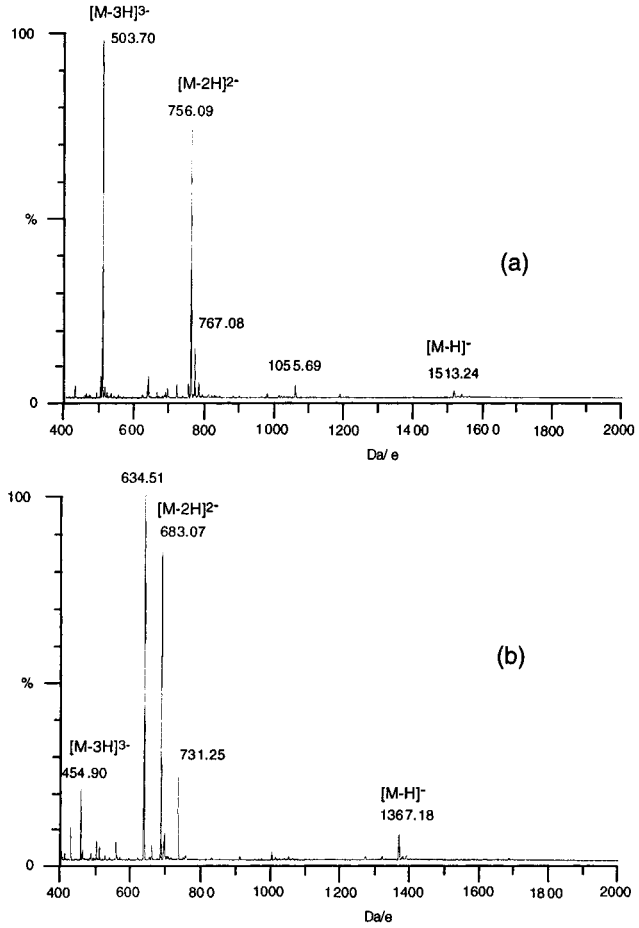

Figure 1. ESMS spectra of the (a) 7-nitroindole-containing oligonucleotide **3** and (b) 2′-deoxyribonolactone-containing oligonucleotide **7**.

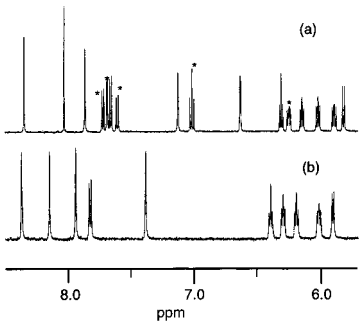

Figure 2. 500 MHz ^{1}H NMR spectra of (a) oligonucleotide **3** containing the nitroindole nucleoside (the asterisks indicate the signals of aromatic and anomeric protons of the nitroindole nucleoside) and (b) oligonucleotide **7** resulting from irradiation of **3** containing the 2′-deoxyribonolactone residue.

3 and **4** were characterized by their ESMS and 500 MHz ^{1}H NMR spectra (see spectra of **3** given as an example in Figures 1a and 2a). **3** and **4** were both irradiated in dilute aqueous solution (5 mM sodium phosphate buffer, pH 6.0, C = 15 μM) at room temperature.[17] After 30 min the nitroindole-containing pentamers were totally transformed into new oligonucleotides, respectively, **7** and **8**, that appeared as single peaks in the HPLC spectrum, accompanied by the 7-nitrosoindole **10** peak. 7-Nitrosoindole **10** was eliminated by ether extraction. The two pentamers **7** and **8** were isolated and identified by ESMS and ^{1}H NMR spectroscopy. The 500 MHz ^{1}H NMR spectrum of **7** (given for illustration in Figure 2b) shows absence of signals between 7.00 and 7.70 ppm corresponding to indole protons and disappearance of the signal at 6.25 ppm corresponding to the anomeric proton of the nitroindole nucleoside in **3**. Furthermore, new signals are

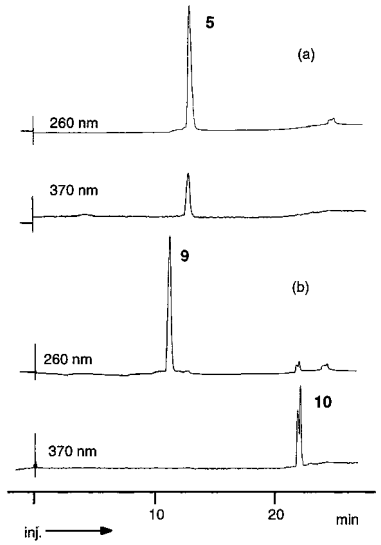

Figure 3. Irradiation of **5** with Pyrex filter (5 mM sodium phosphate buffer, pH 6.0, C = 15 μM). HPLC profiles of **5** (a) before irradiation and (b) after 30 min of irradiation. **9** is formed along with 7-nitrosoindole **10**. HPLC analysis was performed using a Nucleosil C_{18} column (Macherey-Nagel) with a detection at 260 and 370 nm; elution was carried out with a linear gradient of MeOH (5−50%) for 20 min in 5 mM sodium phosphate buffer (pH 6). The flow rate was 4 mL/min.

observed at 3.14 and 2.71 ppm corresponding to the methylene protons α to the carbonyl of the deoxyribonolactone moiety. In the ESMS spectra, the presence of the peaks at *m*/*z* 1367.18 and *m*/*z* 683.07, corresponding, respectively, to the calculated molecular ions $[M - H]^-$ and $[M - 2H]^{2-}$, confirmed the structure (Figure 1b).

The method was extended to synthesis of longer DNA fragments. The undecamer **9** was obtained in the same conditions with identical yields (no secondary peaks visible in the HPLC spectrum, Figure 3). It was characterized by the ESMS and the 500 MHz ^{1}H NMR spectra. The undecamer **9** was hybridized with the complementary strand, and the resulting duplex was examined for stability. The melting temperature T_m = 35.6 °C was determined. This value can be compared to that measured for the reference unmodified duplex (T_m = 56.9 °C). This melting temperature decrease (ΔT_m = 21.3 °C) is indicative of strong destabilization of the double helix induced by the presence of the 2′-deoxyribonolactone site. This stability decrease is indeed quite close to that previously determined for the duplex undecamer containing the tetrahydrofuran abasic site analog[19] **C** (Scheme 1) in the same position in the sequence (T_m = 36.9 °C).[13b]

As a conclusion the described approach constitutes a highly efficient route to obtain oligonucleotides containing 2′-deoxyribonolactone. Studies are in progress to determine the influence of this damage on the structure of the DNA helix and on the behavior of polymerases and repair enzymes.

Supporting Information Available: Experimental procedures for the preparation of **1** and **2**, electrospray mass spectra of oligodeoxynucleotides **4**, **5**, and **9**, and ^{1}H NMR spectra of oligonucleotides **3**, **4**, and **7** (8 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(18) Compounds **6** and **10** were characterized by their MS and ^{1}H NMR spectra. Furthermore, for comparison of the retention time in HPLC, an authentic sample of **6** was prepared by mCPBA oxidation of 2-deoxy-1-*O*-methyl-3,5-bis-*p*-toluoylribofuranose.

(19) As the 2′-deoxyribose site in DNA is unstable and subject to β-elimination, the chemically stable analogue **C** is most frequently used in structural and biological studies. It is indeed a substrate for repair endonucleases.[20]

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