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Generation of an Abasic Site in an Oligonucleotide by Using Acid-Labile 1-Deaza-2′-deoxyguanosine and Its Application to Postsynthetic Modification

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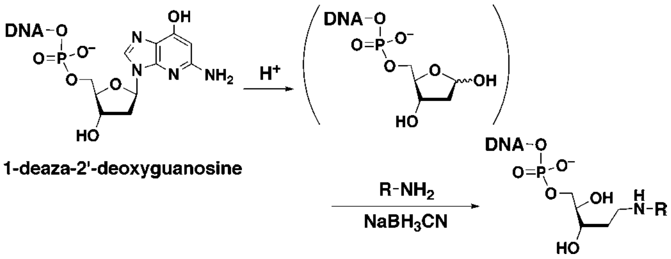
ABSTRACT



We developed a new convenient method for generation of an abasic site at the 3′-terminus of an oligonucleotide. This method uses a 1-deaza-2′-deoxyguanosine residue, which easily undergoes depurination under acidic conditions. The abasic site of the oligonucleotide can be further modified with external functional groups. We report herein the chemical stability of 1-deaza-2′-deoxyguanosine in the oligodeoxynucleotide and the application to the postsynthetic modification of an oligonucleotide by utilizing the chemical property of 1-deaza-2′-deoxyguanosine.

Oligonucleotides bearing an appropriate functional group, such as a fluorescent dye or intercalating agent, have been widely used for biological and biophysical studies.[1] Postsynthetic modifications of oligonucleotides are versatile methods for incorporating various functional groups into the oligonucleotides. Deoxynucleoside analogues, such as 4-triazolopyrimidine,[2] 4-*O*-(2,4,6-trimethylphenyl)uridine,[3] 6-(2,4-dinitrophenyl)thiopurine nucleoside,[4] 6-*O*-pentafluorophenylpurine nucleoside[5] and 5-methoxycarbonyluridine[6] were developed as convertible nucleosides for postsynthetic modification of oligodeoxynucleotides. Postsynthetic modification of RNA has also been reported by using 4-*O*-(*p*-nitrophenyl)uridine.[7] These nucleoside analogues are incorporated into oligonucleotides and then converted into various

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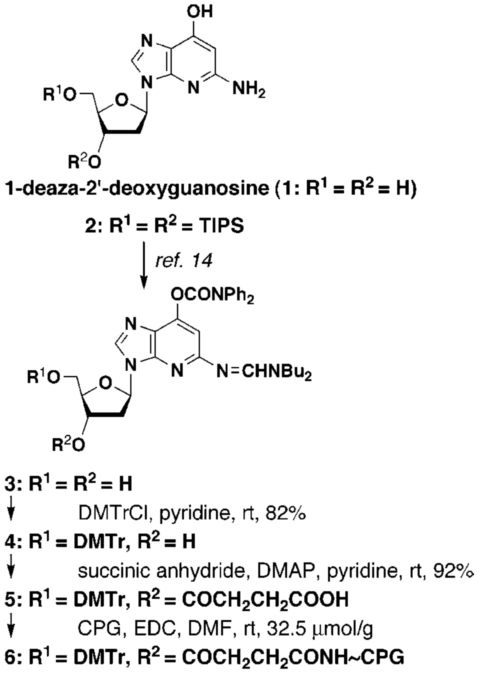
base analogues by treatment with specific reagents after oligonucleotide synthesis.

Aldehyde groups generated in the ribose moiety of oligonucleotides sometimes serve as acceptor molecules for the postsynthetic introduction of functional groups or reporter groups into oligonucleotides.[8] Selective removal of the uracil base by uracil DNA-glycosylase is a well-established technique for generation of an abasic site in an intrastrand of oligodeoxynucleotide;[9] however, this enzyme does not catalyze the deglycosidation of a deoxyuridine, which is located at the 3′-terminal position of the sequence.[10] Methods using chemical reagents have also been reported.[11] These methods involve periodate ion oxidation of the diol groups of a ribose[12] or 3′-phosphoglycerol[13] residue introduced at the 3′-terminus of oligonucelosides. All of these methods require the external addition of enzyme or oxidant molecules into a pH-controlled buffered solution containing oligonucleotides.

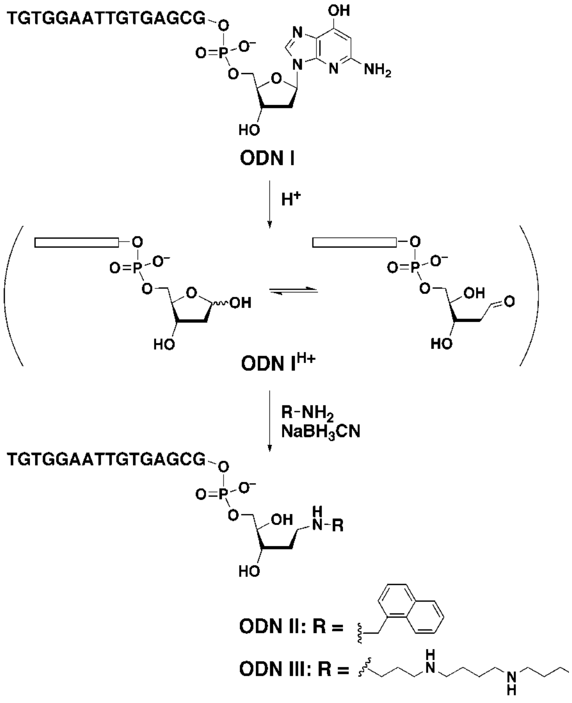
We have reported the synthesis and properties of a novel nucleoside analogue, 1-deaza-2′-deoxyguanosine (^{1C}G) (**1** of Scheme 1), and we have found that the ^{1C}G is highly sensitive to acidic conditions and easily undergoes acid-catalyzed hydrolysis of the *N*-glycosidic bond.[14] On the other hand, the base-protected form (**3** of Scheme 1) is tolerant of acidic conditions such as 3% trichloroacetic acid in dichloromethane, which is a reagent used for DNA synthesis. However, we have not studied the stability of ^{1C}G in the oligonucleotide. In this report, we describe the condition required for the depurination of ^{1C}G in the oligonucleotide. This method enables an easy preparation of a 3′-terminal aldehyde group by only changing pH to the acidic range without adding external chemical reagents or enzymes. Using the oligonucleotide containing the aldehyde group at the 3′-terminus, we were able to combine functional molecules, such as naphthalenemethylamine or spermine, with the aldehyde group. Therefore, we think that ^{1C}G is useful for the chemical modification of the oligonucleotide.

Scheme 1



We chemically synthesized an oligonucleotide containing a ^{1C}G residue to investigate whether the ^{1C}G would be easily depurinated under acidic conditions. The ^{1C}G was introduced at the 3′-end of the oligodeoxynucleotide (ODN) because it is thought that the terminal position of the oligonucleotide is subjected to the chemical reaction easily due to the direct exposure to the solution phase. The reaction should be carried out under conditions as mild as possible in order to avoid further depurination of other purine bases. To introduce the ^{1C}G (**1**) into the 3′-terminus of ODN, a CPG solid support bearing the analogue (**6**) was chemically synthesized as shown in Scheme 1. Both the exocyclic amino and the hydroxyl groups of ^{1C}G were protected with di-*tert*-butylformamidine and diphenyl carbamoyl groups as previously reported.[14] Using this CPG support (**6**), ODN **I** with the ^{1C}G at the 3′-terminus (Scheme 2) was synthesized by standard phosphoramidite chemistry. The dimethoxytrityl group of the 5′-end of the ODN was removed at the final step of the synthesis prior to its cleavage from the CPG support (trityl

Scheme 2. Postsynthetic Modification of ODN



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off mode synthesis). If the ODN is synthesized with a “trityl on” mode, the dimethoxytrityl group at the 5′-end must be removed after deprotection of the exocyclic protecting groups under acidic conditions, which may lead to depurination of the ^{1C}G.

After the synthesis of ODN **I**, we investigated the chemical stability of the ^{1C}G residue in an acidic condition. ODN **I**, which was dissolved in sodium phosphate buffer of pH 2.0, was incubated at 40 °C. After aliquots of the solution had been taken from the reaction solution at various time points, the reaction products were analyzed by reverse-phase HPLC (Figure 1). A decrease in the intact ODN **I** peak was accompanied by the appearance of a new single peak $\mathbf{I}^{\mathbf{H}+}$ with earlier retention time. The peak $\mathbf{I}^{\mathbf{H}+}$ was identified to be depurinated ODN (Scheme 2) by matrix-assisted laser desorption/ionization time-of-flight mass (MALDI-TOF/MS) spectrometry analysis.[15] The reaction proceeded efficiently, and more than 90% of ODN **I** had been converted into depurinated ODN $\mathbf{I}^{\mathbf{H}+}$ at 20 min after the start of the reaction. Efficient depurination of ^{1C}G was also observed in 10% aqueous acetic acid solution at 40 °C. In these conditions, the oligonucleotide containing a deoxyguanosine at the 3′-end instead of ^{1C}G did not show a depurinated product (data not shown), and these results indicate that ^{1C}G in ODN **I** was specifically depurinated in these conditions. When the reaction was carried out at 60 °C for 20 min, a small amount of a side product was detected. It is thought that this side product was derived from depurination of the guanine base, neighboring the terminal ^{1C}G, after ^{1C}G had been first depurinated.

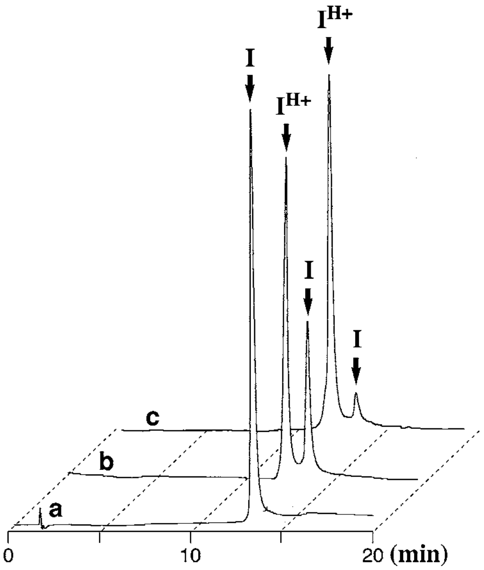

Figure 1. HPLC profiles of depurination reaction: (a) ODN **I**; (b) ODN **I** in phosphate buffer (pH 2.0) at 40 °C after 5 min; (c) ODN **I** in phosphate buffer (pH 2.0) at 40 °C after 20 min. Conditions: Waters μ-Bondasphere C18, 7% to 15% acetonitrile in 0.1 M TEAA buffer, pH 7.0, 20 min.

We measured the observed rate constant (k_{obs}) of the depurination reaction of ^{1C}G in ODN **I** at pH 2.0.[16] The value of k_{obs} for the depurination of ^{1C}G in ODN **I** was calculated to be 1.9×10^{-3} s^{-1}. This rate constant is almost comparable to k_{obs} of a ^{1C}G monomer ($k_{obs} = 1.2 \times 10^{-3}$ s^{-1} at pH 2.2, 37 °C).[14] Since the ^{1C}G of ODN **I** is located at the 3′-end of the oligonucleotide, the ^{1C}G residue exhibited almost the same reactivity as that of the nucleoside monomer.

We also synthesized ODN containing ^{1C}G in an intrastrand position and the ODN was subjected to the acidic treatment. We found that the depurination of the ^{1C}G residue proceeded slowly when it located in an intrastrand position. The depurination of the ^{1C}G needed more stringent condition or extended reaction time. From these results, we think that terminals are suitable positions for effective depurination of the ^{1C}G residue.

When the ^{1C}G residue is depurinated, an aldehyde group is generated at the 3′-end of ODN (ODN $\mathbf{I}^{\mathbf{H}+}$), and the primary amino group should react with the aldehyde group. Therefore, we carried out a coupling reaction of ODN $\mathbf{I}^{\mathbf{H}+}$ with 1-naphthalenemethylamine or spermine to investigate whether these molecules, having primary amino groups, would connect with ODN **I**. After ODN **I** had been depurinated by acidic treatment as described above, followed by desalting, the crude mixture was combined with 1-naphthalenemethylamine in the presence of sodium cyanoborohydride as a reductant.[17] As a result of the reaction, smooth conversion of depurinated ODN $\mathbf{I}^{\mathbf{H}+}$ into ODN with a naphthalenemethylamino group (ODN **II** of Scheme 2) was observed (Figure 2). In a similar manner, spermine was connected with ODN $\mathbf{I}^{\mathbf{H}+}$, giving ODN **III**.[18] The coupling reaction of ODN $\mathbf{I}^{\mathbf{H}+}$ with spermine gave a single-peak product by HPLC analysis using either reverse-phase or ion-exchange column. The molecular weights of ODNs **II** and **III** were confirmed by MALDI-TOF/MS spectrometer analysis.[15]

Thermal denaturation studies were carried out with these modified ODNs (Figure 3). The complementary strand (ODN **VI**) has five extra bases at the 5′-end. It was thought that

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(15) MALDI-TOF/MS spectra were obtained on a Voyager-DE pro (PerSeptive Biosystems, Inc., Framingham, MA). ODN **I**: observed *m/z* 5317.46, calcd for $C_{170}H_{210}N_{67}O_{102}P_{16}$ (M − H) 5319.45. ODN $\mathbf{I}^{\mathbf{H}+}$: observed *m/z* 5185.93, calcd for $C_{164}H_{206}N_{63}O_{102}P_{16}$ (M − H) 5187.33. ODN **II**: observed *m/z* 5328.73, calcd for $C_{175}H_{217}N_{64}O_{101}P_{16}$ (M − H) 5328.54. ODN **III**: observed *m/z* 5369.39, calcd for $C_{174}H_{232}N_{67}O_{101}P_{16}$ (M − H) 5373.67.

(16) Conditions: 100 μM ODN **I** was dissolved in 0.25 M sodium phosphate buffer (pH 2.0), and the reaction solution was incubated at 40 °C. After the reaction mixture had been desalted by a NAP 10 cartridge (Amersham Biosciences, Piscataway, NJ), reverse-phase HPLC analysis was carried out. Percentages of the products were calculated from the peak areas, and the observed rate constants were calculated by the first-order reaction equation.

(17) Conditions: 40 μM ODN $\mathbf{I}^{\mathbf{H}+}$, 50 mM 1-naphthalenemethylamine, 100 mM $NaBH_3CN$, 5.0 mM NaCl, 100 mM HEPES (pH 6.5), DMF, 37 °C. The reaction mixture was desalted by a NAP 10 cartridge.

(18) Conditions: 40 μM ODN $\mathbf{I}^{\mathbf{H}+}$, 50 mM spermine, 100 mM $NaBH_3CN$, 5.0 mM NaCl, 100 mM HEPES (pH 6.5), DMF, 37 °C. The reaction mixture was desalted by a NAP 10 cartridge.

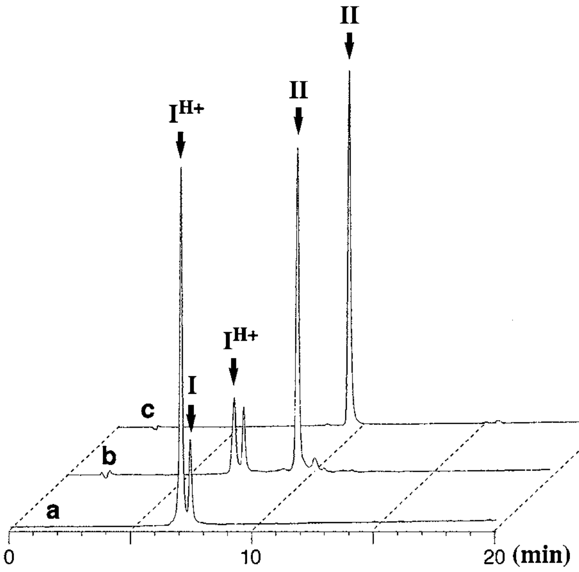

Figure 2. HPLC profiles of postsynthetic modification of ODN **I** with 1-naphthalenemethylamine. (a) Desalted mixture of depurinated ODN **I**. Depurination reaction was carried out in phosphate buffer (pH 2.0) at 40 °C for 20 min. (b) Reaction mixture of ODN $\mathbf{I}^{\mathbf{H+}}$ and 1-naphthalenemethylamine after 16 h. (c) HPLC-purified ODN **II**. Conditions: Waters μ-Bondasphere C18, 8% to 25% acetonitrile in 0.1 M TEAA buffer, pH 7.0, 20 min.

there would be some interactions between the functional groups and the extra sequence on ODN **VI**. Double-stranded complex **II**−**VI** showed a melting temperature (T_m) of 61.8 °C in the presence of 100 mM NaCl. This was 2.3 °C more stable than the unmodified ODN complex **IV**−**VI**. Complex **III**−**VI** showed a T_m 1.9 °C higher than that of **IV**−**VI**. It is thought that the higher stability of ODN **II** is derived from the stacking effect of the naphthalene residue. On the other hand, ODN **III** stabilized the duplex by electrostatic interactions between the sperimine residue and the phosphordiester groups of the duplex **III**−**VI** or the extra single stranded residue of ODN **VI**. These electrostatic interactions were more significant at a low salt concentration (10 mM NaCl), and the complex **III**−**VI** showed a T_m 3.0 °C higher than that of the unmodified complex **IV**−**VI**. ODN **I** containing ^{1C}G at the 3′-terminus destabilized the duplex compared with ODN **V** with a normal guanine base. This is consistent with our previous finding that ^{1C}G has little ability to form base pairs with any nucleoside at the complementary strand.[14]

a

I : 5'-TGTGGAATTGTGAGCG-^{1C}G
II : 5'-TGTGGAATTGTGAGCG-naphthalene
III : 5'-TGTGGAATTGTGAGCG-spermine
IV : 5'-TGTGGAATTGTGAGCG
V : 5'-TGTGGAATTGTGAGCGG
VI : 3'-ACACCTTAACACTCGCCTATTG-5'

b

	Tm (°C)	
complex	100 mM NaCl	10 mM NaCl
I-VI	59.5	45.9
II-VI	61.8	49.2
III-VI	61.4	49.3
IV-VI	59.5	46.3
V-VI	62.5	49.3

Figure 3. (a) Sequences of ODNs used in the thermal denaturation study. (b) Results of the thermal denaturation study. Conditions: 3.0 μM each strand in 100 mM Na cacodylate buffer (pH 7.0) and 100 or 10 mM NaCl.

In summary, we have developed a method for postsynthetic modification of ODN that involves selective generation of an abasic site at the 3′-termininus of ODN. Generation of the abasic site can be easily achieved by the incorporation of acid-labile ^{1C}G into the oligonucleotide. This reaction does not require any additive reagents such as enzymes or chemical oxidants and can be achieved only by changing the pH range. We focused on 3′-end modification and prepared ^{1C}G-bonded CPG. The CPG is versatile and the modified analogue can be incorporated into many synthetic ODNs by using this CPG. The method using ^{1C}G provides an alternative approach to incorporate functional groups into the 3′-terminus of ODNs.

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Supporting Information Available: Experimental procedure and spectral data (^{1}H and ^{13}C NMR) for the compounds synthesized in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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