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Site-Specific Generation of Deoxyribonolactone Lesions in DNA Oligonucleotides

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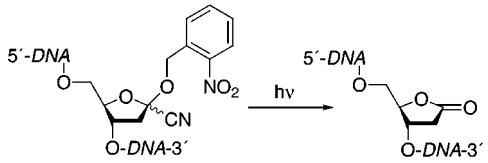
ABSTRACT



An efficient method for the site-specific generation of 2-deoxyribonolactone oxidative DNA damage lesions from a "photocaged" nucleoside analogue was developed. A nucleoside phosphoramidite bearing a C-1′ nitrobenzyl cyanohydrin was prepared and incorporated into DNA oligonucleotides using automated DNA synthesis. The caged analogue, which was stable in aqueous solution, was converted to the 2-deoxyribonolactone lesion by UV irradiation. DNA containing the caged analogue and the deoxyribonolactone site were characterized by electrospray mass spectrometry (ES-MS).

DNA damage by toxic agents compromises the coding potential and strand integrity of the genetic material.[1] Oxidative DNA damage, mediated by free radicals and reactive oxygen species (ROS), represents a major contributor to cellular DNA damage.[2] Oxidation of DNA may occur at the nucleobase[3] or sugar[4] moieties of DNA nucleotides. The resulting lesions may labilize the otherwise stable DNA backbone and/or induce mutations during cellular nucleic acid synthesis. Thus, evolution has developed complex enzymatic pathways that recognize and repair oxidative DNA damage.[5] An understanding of the chemical mechanisms of DNA damage and repair is central to uncovering the biological effects of genomic lesions.

Oxidation of the C-1′ position of DNA nucleotides (**1**) by exogenous toxic agents[6] and endogenous lipid peroxidation pathways[7] produces 2-deoxyribonolactone, or C-1′ oxidized abasic site, lesions (**2**) within DNA. Recent studies from the laboratories of Sigman,[8] Greenberg,[9] and Chatgilialoglu[10] have enumerated diverse chemical mechanisms that produce

(1) Lindahl, T. *Nature* **1993**, *362*, 709−715.
(2) Aust, A. E.; Eveleigh, J. F. *Proc. Soc. Exp. Biol. Med.* **1999**, *222*, 246−252.
(3) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1151.
(4) (a) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089−1107. (b) Povirk, L. F.; Steighner, R. J. *Mutat. Res.* **1989**, *214*, 13−22. (c) Burger, R. M. *Chem. Rev.* **1998**, *98*, 1153−1169.
(5) (a) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis*; American Society for Microbiology: Washington, DC, 1995. (b) Crouteau, D. L.; Bohr, V. A. *J. Biol. Chem.* **1997**, *272*, 25409−25412. (c) Demple, B.; Harrison, L. *Annu. Rev. Biochem.* **1994**, *63*, 915−948.
(6) For a review, see: Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1235−1248.
(7) (a) Harkin, L. A.; Burcham, P. C. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 1−5. (b) Harkin, L. A.; Butler, L. M.; Burcham, P. C. *Chem. Res. Toxicol.* **1997**, *10*, 575−581.
(8) (a) Sigman, D. S.; Graham, D. G.; D'Aurora, V.; Sterm, A. M. *J. Biol. Chem.* **1979**, *254*, 12269−12272. (b) Goyne, T. E.; Sigman, D. S. *J. Am. Chem. Soc.* **1987**, *109*, 2846−2848. (c) Meijler, M. M.; Zelenko, O.; Sigman, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 1135−1136. (d) Zelenko, O.; Gallagher, J.; Sigman, D. S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2776−2778.
(9) (a) Tronche, C.; Goodman, B. K.; Greenberg, M. M. *Chem. Biol.* **1998**, *5*, 263−271. (b) Tallman, K. A.; Tronche, C.; Yoo, D. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 4903−4909. (c) Chen, T.; Greenberg, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3815−3816. (d) Greenberg, M. M.; Yoo, D. J.; Goodman, B. K. *Nucleosides Nucleotides* **1997**, *16*, 33−40. (e) Goodman, B. K.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 2−3. (f) Hwang, J.-T.; Tallman, K. A.; Greenberg, M. M. *Nucleic Acids Res.* **1999**, *27*, 3805−3810. (g) Hashimoto, M.; Greenberg, M. M.; Kow, Y. W.; Hwang, J. T.; Cunningham, R. P. *J. Am. Chem. Soc.* **2001**, *123*, 3161−3162.
(10) (a) Chatgilialoglu, C.; Gimisis, T. *Chem. Commun.* **1998**, 1249−1250. (b) Emanuel, C. J.; Newcomb, M.; Ferreri, C.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **1999**, *121*, 2927−2928. (c) Chatgilialoglu, C.; Ferreri, C.; Bazzanini, R.; Guerra, M. Choi, S.-Y.; Emanuel, C. J.; Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **2000**, *122*, 9525−9533.

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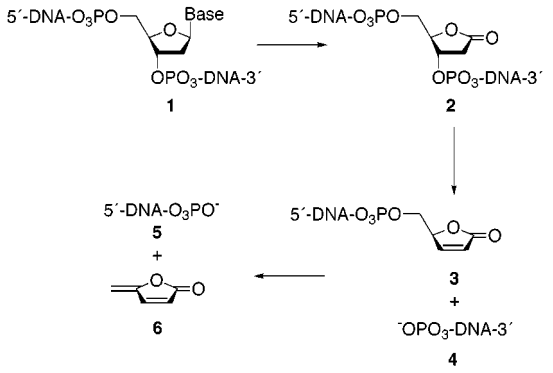
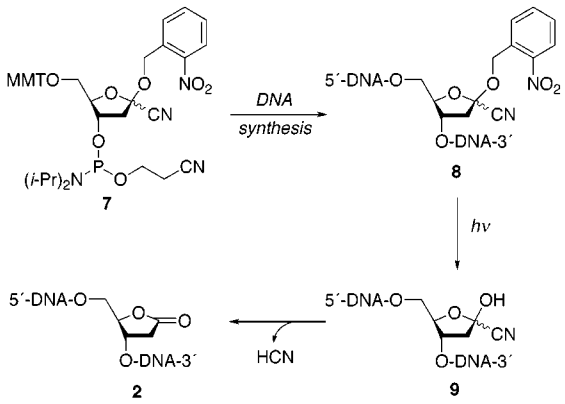
Scheme 1. Deoxyribonolactone Lesion Formation and Cleavage



Scheme 2. Site-Specific Generation of DNA Deoxyribonolactone Sites



deoxyribonolactone lesions in DNA. Scheme 1 shows a generalized pathway for the formation of and subsequent DNA strand scission at deoxyribonolactone sites. The C-1′ oxidative damage reaction is initiated by hydrogen abstraction at a deoxyribonucleotide in DNA (**1**, Scheme 1) to produce a C-1′ sugar radical. Once formed, the radical species may undergo one of several possible oxygenation pathways, which ultimately results in the extrusion of the nucleobase and the production of the oxidized abasic site, **2**. By virtue of its lactone character, **2** may undergo β-elimination to produce the α,β-unsaturated lactone DNA strand, **3**, and a 5′-phosphorylated DNA product (**4**). The ene−lactone **3** may decompose further to produce a 3′-phosphorylated cleavage product (**5**) and methylene furanone (**6**).

Because of the lability of the deoxyribonolactone lesion, methods for the controlled introduction of oxidized abasic site lesions into DNA are required. Furthermore, such methods would provide direct experimental insight into the chemical properties and biological effects of this oxidative damage lesion. Existing approaches to deoxyribonolactone lesions within DNA have relied on pathways analogous to the natural C-1′ oxidative damage reaction. In particular, photolysis of C-1′ *tert*-butyl ketone[9,10] or nitroindole[11] nucleoside analogues produces C-1′ deoxyribose radicals, which are efficiently oxygenated to provide oxidized abasic sites within DNA. These approaches have provided the first methods for specific deoxyribonolactone generation and have offered significant insights into the properties of these DNA damage lesions.[9g,11c]

We now report an efficient method for the site-specific introduction of 2-deoxyribonolactone lesions in DNA oligonucleotides that is independent of the C-1′ damage reaction. Our approach, which is outlined in Scheme 2, involves the generation of lactone sites within DNA from a stable, photochemically "caged" form of the lesion. The C-1′ cyanohydrin 2-nitrobenzyl ether (**7**) was selected as a photolabile lactone precursor based upon (1) its predicted compatibility with DNA synthesis and deprotection conditions,[12] (2) the expected stability of the caged analogue **8** under aqueous conditions,[13] and (3) the known photochemistry of *o*-nitrobenzyl ethers.[14] Thus, incorporation of analogue **7** during solid-phase DNA synthesis would produce the caged-lactone DNA (**8**). The *o*-nitrobenzyl ether of **8** then would be cleaved by UV irradiation to produce the lactone cyanohydrin, **9**, which would be expected to decompose to the lactone lesion within DNA (**2**). Herein we describe the synthesis of caged phosphoramidite **7**, its incorporation into DNA (**8**), and the efficient photochemical generation and characterization of 2-deoxyribonolactone lesions (**2**, Scheme 2) within DNA.

A versatile synthetic route to the caged nucleoside analogue **7** was developed (Scheme 3). The approach centered on the construction of a ribosyl donor, cyanobromide **12**, which was prepared from the known[15] 1-chloro-3,5-di-*p*-chlorobenzoyl-D-ribofuranose (**10**). Conversion of **10** into C-1 cyanide (**11**) was accomplished by reaction with diethylaluminum cyanide.[16] Free-radical bromination of **11** with NBS produced the anomeric mixture of C-1 bromocyanides **12** in 80% yield. The nitrobenzyl group was installed using silver triflate-promoted glycosylation,[17] to produce **13** in 70% yield as a mixture of C-1′ isomers.

To convert **13** into a form suitable for automated DNA synthesis, the 3′- and 5′-*O*-*p*-chlorobenzoyl protecting groups were removed with methanolic ammonia[18] to produce an inseparable mixture of the anomeric nucleosides **14**. Transformation of **14** to the corresponding mono-methoxytrityl

(11) (a) Kotera, M.; Bourdat, A.; Defrancq, E.; Lhomme, J. *J. Am. Chem. Soc.* **1998**, *120*, 11810−11811. (b) Kotera, M.; Bourdat, A.-G.; Defrancq, E.; Jourdan, M.; Garcia, J.; Coulombeau, C.; Lhomme, J. *Nucleosides Nucleotides* **1999**, *18*, 1323−1324. (c) Jourdan, M.; Garcia, J.; Defrancq, E.; Kotera, M.; Lhomme, J. *Biochemistry* **1999**, *38*, 3985−3995. (d) Kotera, M.; Roupioz, Y.; Defranq, E.; Bourdat, A.-G.; Garcia, J.; Coulombeau, C.; Lhomme, J. *Chem. Eur. J.* **2000**, *6*, 4163−4169.

(12) Ordoukhanian, P.; Taylor, J.-S. *J. Am. Chem. Soc.* **1995**, *117*, 9570−9571.

(13) Olah, G. A.; Arvanaghi, M.; Surya Prakash, G. K. *J. Am. Chem. Soc.* **1982**, *104*, 1628−1631.

(14) Pillai, V. N. Rajasekharan. *Synthesis* **1980**, 1−26.

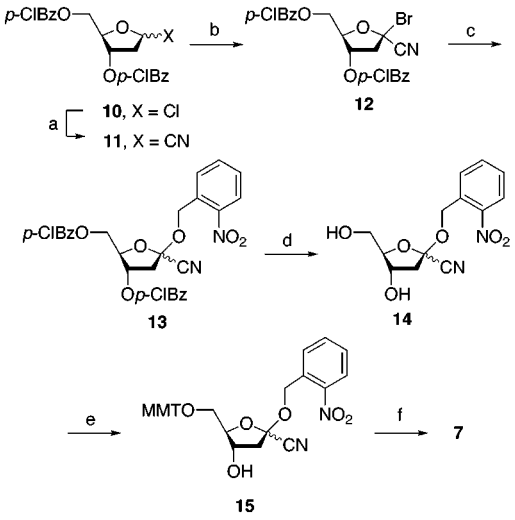
(15) Eger, K.; Jalalian, M.; Schmidt, M. *Tetrahedron* **1994**, *50*, 8371−8380.

(16) Iyer, R.; Phillips, L.; Egan, W. *Synth. Commun.* **1991**, *20*, 2053−2063.

(17) Buchanan, J. G.; Clelland, A. P. W.; Wightman, R. H.; Johnson, T.; Rennie, R. A. C. *Carbohydr. Res.* **1992**, *237*, 295−301.

(18) Uteza, V.; Chen, G.; Tuoi, J.; Fenet, B.; Grouiller, A. *Tetrahedron* **1993**, *49*, 8579−8588.

Scheme 3. Synthesis of Phosphoramidite **7**[a]



[a] (a) Et_2AlCN, THF, 4 °C to rt, 4 h, 75%; (b) NBS, benzoyl peroxide, CCl_4, 85 °C, 2 h, 80%; (c) 2-nitrobenzyl alcohol, 2,6-lutidine, AgOTf, CH_2Cl_2, 3 h, 70%; (d) 4.5 M NH_3/MeOH, THF, 40 °C, 3 h, 76%; (e) MMTCl, DMAP, pyridine, 18 h, 80%; (f) $[(i\text{-}Pr)_2N]_2POCH_2CH_2CN$, $(i\text{-}Pr)_2NH_2{\bullet}CHN_4$, CH_2Cl_2, 2.5 h, 83%.

(MMT) ethers (**15a**, **15b**) was accomplished in 80% yield by reaction of **14** with MMTCl in pyridine. Addition of the hydrophobic MMT group facilitated the chromatographic separation of the two C-1′ isomers **15a** and **15b**.[19] The MMT derivatives (**15a** and **15b**) were converted independently into the corresponding phosphoramidite derivatives (**7a** or **7b**) using the method of Caruthers.[20]

Caged phosphoramidite analogues (**7**) were incorporated with high efficiency into DNA oligonucleotides with minor modifications of standard automated DNA synthesis methods.[21] Caged oxidized abasic sites were inserted independently into several oligonucleotide sequences, **16**, **18**, and **19**, shown in Figure 1. After DNA synthesis, DNA strands

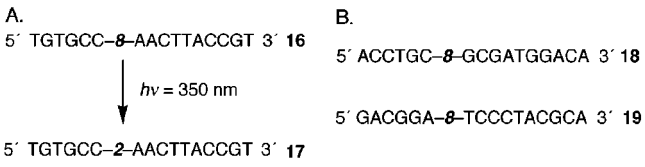


Figure 1. Caged and lactone-containing DNA sequences.

containing **8** were deprotected at room temperature for 14−16 h with 1.5 M NH_3 in methanol. These mild conditions completely deprotected the oligonucleotides with minimal modification of the caged analogue. Reverse-phase HPLC (RP-HPLC) purification was used to provide oligonucleotides containing **8** in high synthetic yield and purity.

Oligonucleotides containing the caged analogue were characterized by electrospray mass spectrometry (ES-MS) and base composition analysis. Oligonucleotide **16** was subjected to ES-MS analysis. As illustrated in Figure 2A

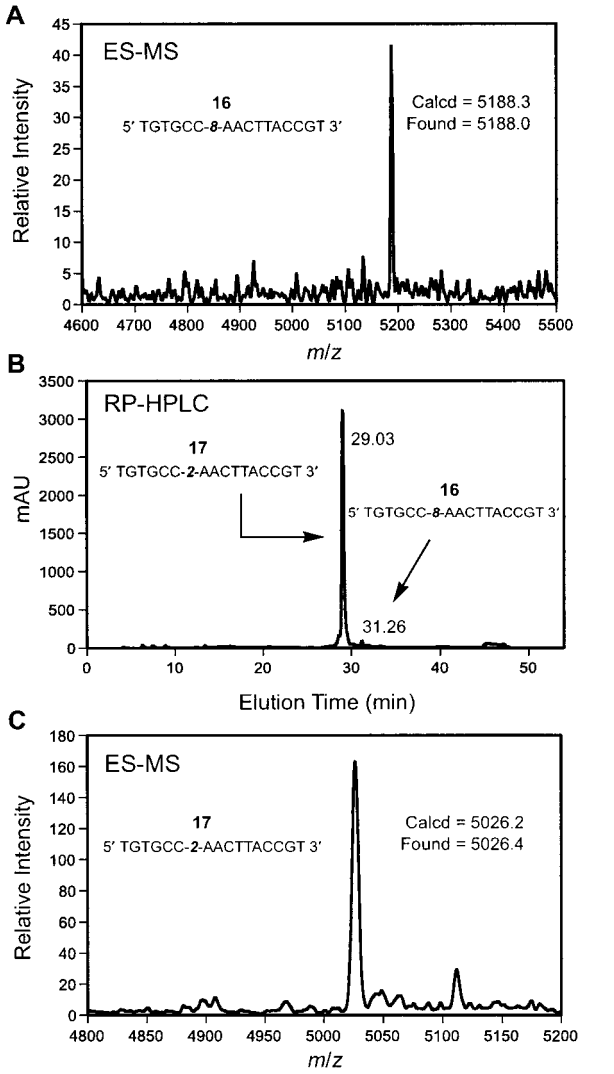


Figure 2. ES-MS and RP-HPLC characterization of DNA oligonucleotides containing the caged analogue (**16**) and the oxidized abasic site (**17**). (A) ES-MS analysis of RP-HPLC purified **16**, containing the caged lactone analogue (**8**). (B) RP-HPLC trace of a crude photolysis reaction of caged-DNA **16** (t_R 31.26 min) to form lactone-containing DNA **17** (t_R 29.03 min). (C) ES-MS analysis of oligonucleotide **17** containing the 2-deoxyribonolactone lesion (**2**).

the observed *m*/*z* for **16** was 5188.0, which corresponded well with the calculated value of 5188.33. In addition, enzymatic digestion of **16** to its component nucleosides and quantitative RP-HPLC revealed that **16** contained deoxynucleosides and analogue **14** in the expected ratios.[22]

(19) The stereochemistry of the two cyanohydrin anomers, **15a** and **15b**, was assigned by conversion to the corresponding **13a** and **13b** analogues. X-ray structural analysis revealed that **13b** possessed β-stereochemistry at the C-1′ position. See Supporting Information.

(20) Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J. Y. *Methods Enzymol.* **1987**, *154*, 287−313.

Oligonucleotides **18** and **19** also gave the expected *m*/*z* values by ES-MS. Thus, solid-phase DNA synthesis using phosphoramidite **7** provides an effective route to oligonucleotides containing the masked lactone analogue **8**.

Oligonucleotides containing the caged oxidized abasic site precursor **8** were converted efficiently by photolysis to deoxyribonolactone-modified DNA. Irradiation of sequence **16** in buffered aqueous solution provided the oxidized abasic site **2** within sequence **17** (Figure 1). The time course of the decaging reaction was analyzed by gel electrophoresis: photolysis of **16** demonstrated the time-dependent formation of an alkali labile site over 40 min of irradiation (Supporting Information). The efficiency of the decaging process is demonstrated in Figure 2B, which shows a RP-HPLC trace for the crude reaction mixture resulting from photolysis of **16** for 40 min at 350 nm. Oligonucleotide **16** (retention time 31.26 min) was converted in ~90% yield to a single new product that displayed a shorter retention time (29.03 min). This faster moving material was isolated and subjected to ES-MS analysis (Figure 2C), which confirmed the identity of the product as sequence **17** containing the deoxribonolactone lesion, **2** (calcd $m/z = 5026.2$; found 5026.4). The high photolytic efficiency for conversion of the masked analogue **8** to an oxidized abasic site (**2**) was independent of the anomeric identity of the starting phosphoramidite (**7a** or **7b**).

Our approach to deoxyribonolactone-containing DNA has been generalized to other DNA sequences and to duplex DNA. Analysis of sequences **18** and **19** demonstrated that the photolytic decaging of **8** to oxidized abasic sites (**2**) was independent of the sequence flanking the caged analogue. In addition, the decaging process occurs with similar efficiency in double-stranded DNA. For example, photolysis of **16** in the presence of a complementary strand with dA or dC paired opposite **8** produced lesion **2** in ~90% yield in 40 min. Thus, photolytic decaging of lactone analogue **8** offers a clean route to DNA containing the deoxyribonolactone lesion **2**.

The synthetic route outlined in Scheme 3 provides a versatile approach for the construction of other DNA deoxyribonolactone lesion synthons. For example, a series of caged lactones with tunable photochemical properties such as faster deprotection rates or altered absorption maxima could be synthesized from **12**. Other nonphotochemical caging strategies also could be employed. In addition, photolabile alkoxycyanohydrins may offer a new protection strategy for lactones and other carbonyl functional groups. Several *O*-protected ester cyanohydrin derivatives have been reported.[23] However, to our knowledge, this report represents the first demonstration of photolabile 2,2-dialkoxyalkanenitriles as masked lactones.

We have outlined an efficient independent synthesis of the 2-deoxyribonolactone lesion in DNA oligonucleotides, which is independent of the C-1′ radical DNA damage pathway. This general strategy will facilitate the further investigation of the chemical biology of DNA oxidized abasic site lesions.

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Supporting Information Available: Detailed experimental protocols and spectral data for compounds **7** and **10**−**15**, X-ray crystal data for **13b**. An X-ray crystallographic file in cif format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Phosphoramidite **7a** coupled at ~64% and was extended with an efficiency of >95%. Analogue **7b** coupled at ~70% and extended at >90% efficiency. Because DNA contains β-nucleosides, our studies employed primarily the β-isomer, **7b**.

(22) Eadie, J. S.; McBride, L. J.; Efcavitch, J. W.; Hoff, J. B.; Cathcart, R. *Anal. Biochem.* **1987**, *165*, 442−447.

(23) (a) Utimoto, K.; Wakabayashi, Y.; Shishiyama, Y.; Inoue, M.; Nozaki, H. *Tetrahedron Lett.* **1981**, *22*, 4279−4280. (b) Kirchmeyer, S.; Mertens, A.; Arvanaghi, M.; Olah, G. A. *Synthesis* **1983**, 498−499. (c) Tomoda, S.; Takeuchi, Y.; Nomura, Y. *Chem. Lett.* **1982**, 1733−1734.