Communications to the Editor

Design and Synthesis of a Versatile Photocleavable DNA Building Block. Application to Phototriggered Hybridization

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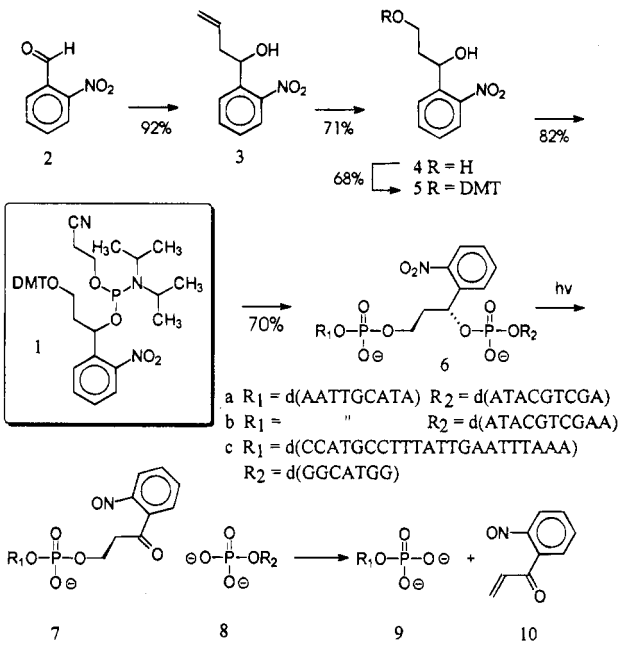
The ability to phototrigger bond cleavage has found wide application in chemistry and biology.[1,2] The ability to phototrigger single and double strand breaks in nucleic acids may be useful for the study of the dynamics of nucleic acid structural and conformational transitions, as well as complicated processes in vivo such as the repair of double strand breaks produced by ionizing radiation.[3] Phototriggered strand breaks could also be useful for the activation of nucleic acid-based probes and drugs such as antisense oligonucleotides and ribozymes. With such applications in mind, we had previously designed a building block for phototriggering strand breaks in DNA which was based on the photochemistry and photoenzymology of a cis−syn thymine dimer lacking an internucleotide linkage.[4] This building block is not generally useful because direct photocleavage requires 254 nm light, which also damages DNA, and photoenzymatic cleavage with visible light and photolyase is inefficient and may not be suitable for RNA or nucleic acid analogs. Recently it has been shown that irradiation of DNA containing the C4′-phenyl selenide derivative of adenosine with >315 nm light under anaerobic and nonreducing conditions results in phototriggered strand cleavage to give a one nucleoside gap terminated in 5′- and 3′-phosphates, a major type of strand break induced by ionizing radiation.[5a] Unfortunately, the required building block takes nine steps to synthesize from deoxyadenosine, and photocleavage in the presence of a reducing agent or oxygen leads to a number of side products.[5,6] Herein, we report the design and efficient synthesis of the building block **1** that can be used to photochemically introduce a site-specific strand break in an oxygen- and reducing agent-independent manner that ultimately leads to a gap terminated in 3′- and 5′-phosphates (Scheme 1). We also demonstrate its use in phototriggering oligonucleotide hybridization.

Scheme 1



The design of the photocleavable building block was based on the photochemistry of *o*-nitrobenzyl ethers[7,8] and the expectation that a phosphate β to the photogenerated carbonyl would be easily eliminated (Scheme 1). Building block **1** was synthesized in an overall yield of ~40% in four steps from commercially available *o*-nitrobenzaldehyde (**2**) by first adding allyltrimethylsilane in the presence of $TiCl_4$ to give **3**.[9] Diol **4** was obtained following ozonolysis of **3** and reductive workup with sodium borohydride and then converted to the building block **1** via **5** according to standard procedures.[10] The building block was incorporated into the 19-mer **6a** by standard automated DNA synthesis methodology with a coupling efficiency of ~70%. The 19-mer was 5′-end-labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase and was 3′-end-labeled by primer−extension opposite d(TTCGACGTAT) with [α-^{32}P]ATP and the Klenow fragment to give 20-mer **6b** by standard methods.[11]

Exposure of 5′-end-labeled **6a** to 355 nm pulses from a Nd:YAG laser led to light-dependent formation of a faster moving band corresponding to the expected 3′-phosphorylated 9-mer **9a** and two slower moving bands, the major of which was presumed to be the carbonyl derivative **7a** (Figure 1a). Consistent with this interpretation, hot piperidine converted the major, as well as the minor, slower moving bands to the faster moving band which comigrated with the product resulting from the Maxam−Gilbert G reaction[12] on an otherwise identical

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(1) For reviews, see: (a) Morrison, H., Ed. *Biological applications of photochemical switches*; John Wiley: New York, 1993; Vol. 2. (b) Kaplan, J. H. *Annu. Rev. Physiol.* **1990**, *52*, 897−914. (c) Lester, H. A.; Gurney, A. M. *Physiol. Rev.* **1987**, *67*, 583−617.

(2) For specific examples, see: (a) Walker, J. W.; Somlyo, A. V.; Goldman, Y. E.; Somlyo, A. P.; Trentham, D. R. *Nature* **1987**, *327*, 249−252. (b) Hess, G. P.; McCray, J. A.; Denk, W.; Marque, J.; Webb, W. W.; Carpenter, B. K.; Walker, J. W.; Udgaonkar, J. B.; Billington, A. P.; Matsubara, N.; Milburn, T. *Biochemistry* **1989**, *28*, 49−55. (c) Horn, T.; Downing, K.; Gee, Y.; Urdea, M. S. *Nucleosides Nucleotides* **1991**, *10*, 299−302. (d) Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D.; Read, J. L.; Fodor, S. P. A. *Science* **1991**, *251*, 767−773. (e) Nunn, D. S.; Pirrung, M. C. *Biomed. Chem. Lett.* **1992**, *2* (12), 1489−1492. (f) Hong, J.-I.; Feng, Q.; Rotello, V.; Rebek, J. J. *Science* **1992**, *255*, 848−850. (g) Givens, R. S.; Athey, P. S.; Kueper, L. W., III; Matuszewski, B.; Xue, J. *J. Am. Chem. Soc.* **1992**, *114*, 8708−8710. (h) Givens, R. S.; Kueper, W., III. *Chem. Rev.* **1993**, *93*, 55−66. (i) Wender, P. A.; Zercher, C. K.; Beckham, S.; Haubold, E. M. *J. Org. Chem.* **1993**, *58*, 5867−5869. (j) Greenberg, M. M. *Tetrahedron Lett.* **1993**, *34* (2), 251−254. (k) Greenberg, M. M.; Gilmore, J. L. *J. Org. Chem.* **1994**, *59*, 746−753. (l) Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 373−374. (m) Gee, K. R.; Wieboldt, R.; Hess, G. P. *J. Am. Chem. Soc.* **1994**, *116*, 8366−8367.

(3) Price, A. *Cancer Biol.* **1993**, *4*, 61−71.

(4) Nadji, S.; Wang, C.-I.; Taylor, J.-S. *J. Am. Chem. Soc.* **1992**, *114*, 9266−9269.

(5) (a) Giese, B.; Dussy, A.; Elie, C.; Erdmann, P.; Schwitter, U. *Angew. Chem., Int. Ed. Engl.* **1994**, *33* (18), 1861−1863. (b) Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Giraud, L.; Imwinkelried, P.; Muller, S. N.; Schwitter, U. *J. Am. Chem. Soc.* **1995**, *117*, 6146−6147.

(6) Takaku, H.; Morita, K.; Sumiuchi, T. *Chem. Lett.* **1983**, 1661−1664.

(7) Walker, J. W.; Reid, G. P.; McCray, J. A.; Trentham, D. R. *J. Am. Chem. Soc.* **1988**, *110*, 7170−7177.

(8) Patchornik, A.; Amit, A.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333−6335.

(9) Hosomi, A.; Sakurai, H. *Tetrahedron Lett.* **1976**, 1295−1298.

(10) Gait, M. J., Ed. *Oligonucleotide synthesis. A practical approach*; IRL Press: Washington, DC, 1984.

(11) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular cloning: A laboratory manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989; Vol. 1.

(12) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499−560.

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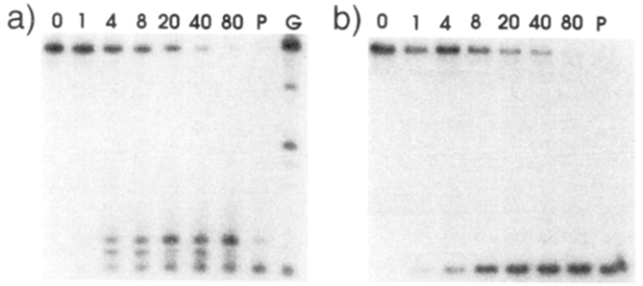

Figure 1. Autoradiograph of a polyacrylamide electrophoresis gel of the products resulting from exposure of 7.5 pmol of (a) 5′-^{32}P-end-labeled 19-mer **6a** or (b) 3′-end-labeled 20-mer **6b** in 200 μL of 100 mM Tris-HCl (pH 7.0) buffer in a 4 mm × 1 cm quartz cuvette to the indicated number of 10 ns, 36 mJ, 355 nm pulses from a Quantel Q-switched Nd:YAG laser. Lane P: a second 80 pulse aliquot that was heated in 100 μL of a 1 M piperidine solution at 90 °C for 30 min. Lane G: a Maxam−Gilbert G reaction that was carried out on 5′-end-labeled d(AATTGCATAGATACGTCGA), a sequence in which the photochemical building block of **6a** was replaced by the indicated G.

sequence containing a G in place of the building block (Figure 1a, lanes P and G). Also consistent with the proposed cleavage chemistry, irradiation of 3′-end-labeled **6b** resulted in light-dependent formation of a single piperidine-insensitive band corresponding to the 5′-phosphorylated 10-mer **8b** (Figure 1b). The initial appearance of the faster moving band in the 5′-labeled substrate is puzzling, however, as the half-life for the conversion of the slower moving band to the faster moving band is about 8 days at room temperature and pH 7. To determine whether the high pH of the acrylamide gel (pH 8.3) was inducing the β-elimination, electrophoresis was carried out on a pH 7.0 acrylamide gel with similar results. Photocleavage could also be induced by irradiation with 254, 302, or 366 nm light and was independent of the presence or absence of dithiothreitol.

To demonstrate the potential usefulness of this building block, we designed a system for phototriggered oligonucleotide hybridization. An oligonucleotide complementary to an 18-nt section of the (+) strand of the single-stranded M13 clone **CW1** was joined to a shorter complementary sequence via a 4-nt sequence and the photocleavable building block to give **6c** (Figure 2). As a result of the hairpin structure, **6c** failed to hybridize well to **CW1** on nitrocellulose, but nearly quantitative photocleavage of **6c** yielded **7/9c**, which hybridized with about 9 times the binding affinity of **6c**.

The photocleavable building block is easily synthesized and could also be used to trigger site-specific double strand breaks

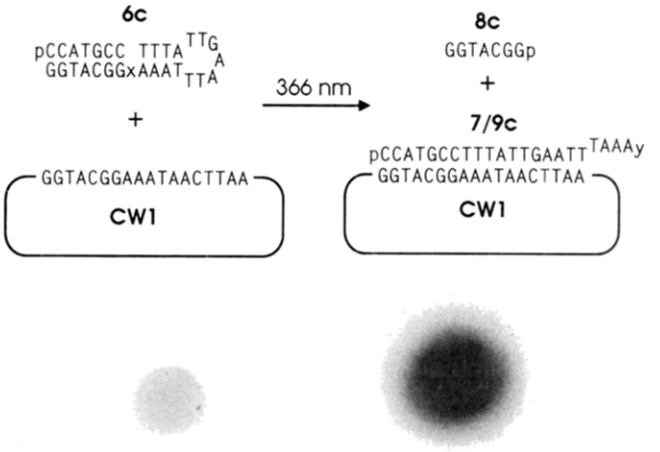

Figure 2. Autoradiograph of two 6 mm diameter circular nitrocellulose filter papers impregnated with 112.5 ng of **CW1** each that were incubated overnight at room temperature with 12 nM of annealed 5′-^{32}P-end-labeled oligonucleotide **6c** before (left) and after (right) irradiation with 90 min of 0.5 mW, 366 nm light from a hand-held light source and then washed for 20 min at room temperature. In comparision, photocleavage was complete in 3 min when exposed to 0.2 mW of 302 nm light from a transluminator.

of variable spacing under wavelengths that cause little or no damage to DNA or protein, and may thus be useful for the study of certain aspects of double strand break repair. The building block may also be of use for the photoactivation (or deactivation) of biologically active forms of nucleic acids and analogs, such as primer−templates, antisense or triplex-targeting oligonucleotides, and ribozymes.

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Supporting Information Available: Experimental procedures and spectroscopic data (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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