A Caged Ligatable DNA Strand Break

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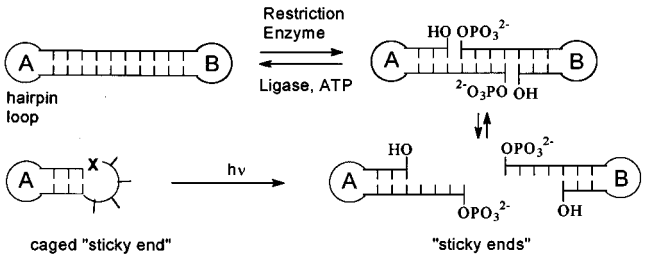
The construction of new DNA molecules for biological purposes relies heavily on the formation and ligation of sticky ends terminating in 5′-phosphates and 3′-hydroxyls (Scheme 1).[1] Self-assembly of complementary sticky ends followed by ligation is also a basic step in the construction of unnatural nanoscaled DNA structures[2−4] and in certain approaches to DNA-based computing.[5,6] In multistep DNA nanoconstruction, it is often advantageous to tie together the ends of the strands of synthetic intermediates in hairpin loops. The absence of ends helps to maintain the topology of the intermediates, and makes it possible to remove unreacted starting material and incompletely ligated molecules by degradation with exonucleases. Sticky ends are then generated at the appropriate time in a synthetic sequence with restriction enzymes. There are, however, inherent limitations to using restriction enzymes to produce sticky ends. First, most restriction enzymes produce self-complementary sticky ends not more than four nucleotides in length, and not the long asymmetric sticky ends that would be optimal for the stable self-assembly of unique structures in DNA nanoconstruction.[7,8] Seeman and coworkers have reported a method to add short extensions to sticky ends, but it is laborious and involves three enzymatic steps.[9] A second problem is that, because of their size and structure, restriction enzymes are sensitive to the local environment and may not cleave efficiently or even fail to function for certain structures.[10]

One general solution to the problem of generating sticky ends of any length or sequence would be to cage the end by incorporating it into a hairpin loop with a photocleavable precursor to a 5′-phosphate and a 3′-hydroxyl (Scheme 1, bottom). This strategy would also have the advantage of substantially reducing the length of the intermediate DNA molecules required in a synthesis. Though there have been several reports of photochemically triggered DNA strand breaks,[11,12] there have been no examples of methods for directly phototriggering the simultaneous formation of 3′-hydroxyl and 5′-phosphate termini. Herein, we report the design and synthesis of the first building block that can be used to site-specifically introduce such a caged break into oligonucleotides, and further demonstrate that the break can be enzymatically ligated. Such a caged ligatable strand break could be used to produce sticky ends of any desired length or sequence, in a structure-independent manner.

Scheme 1



The design of a building block for caging a ligatable strand break was based on two criteria: (1) that it would be sequence independent and (2) that it would be compatible with standard DNA synthesis chemistry. We had previously developed a building block based on *o*-nitrobenzyl photochemistry that released a 5′-phosphate upon 366 nm irradiation,[12] and simply needed to add functionality to trigger the release of the 3′-OH. Whereas it is very difficult to displace 3′-OH's from negatively charged phosphodiesters, neutral alkyl phosphotriesters containing a β-hydroxyl group are known to rapidly release an alkoxide ligand at pH 7 with a half-life of about 1−2 h at room temperature via intramolecular displacement by the β-hydroxyl group.[13−15] Unfortunately, with a phosphotriester, either of two alkoxide ligands could be displaced. It was expected, however, that the corresponding methyl phosphonates, which can only release a single alkoxide ligand, would react even faster based on the hydrolysis rates of simple phosphonates in comparison to phosphotriesters.[16] Based on these considerations, we designed the building block **1** (Scheme 2) that was expected to photochemically release a 3′-OH and a 5′-phosphate when incorporated into an oligonucleotide with a commercially available phosphonamidite **2**.[17]

The synthesis of building block **1** is outlined in Scheme 3. To test the efficiency of photoinduced formation of a 3′-hydroxyl and 5′-phosphate terminated break, the building block was incorporated into position 13 of the 21-mer by standard automated DNA synthesis, and the methylphosphonate of T into position 12. The oligonucleotide was deprotected under conditions compatible with methylphosphonate linkages[18] and was either 5′-end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase to give **9a** or 3′-end-labeled by primer-extension opposite d(CTTAGGCACGAGTCAATCTTATAC) with [α-^{32}P]dATP and the Klenow fragment of *E. coli* polymerase I to give 22-mer **9b**.[19] The gel-purified end-labeled oligonucleotides were then annealed to the eventual ligation scaffold **13** to form duplexes.

Irradiation of the duplex containing 5′-end labeled **9a** with 365 nm light at pH 7.0 afforded a single major band (91%) that comigrated with the expected 3′-hydroxyl terminated 12-mer **10a** (Figure 1a). Likewise irradiation of the duplex containing 3′-end labeled **9b** resulted in the formation of a single major band (91%) that comigrated with the expected 5′-phosphorylated 9-mer **11b** (Figure 1b). In another set of experiments cleavage yields of 96% and 97% were obtained. The lower mobility bands formed during irradiation presumably correspond to the intermediates in the sequential photolysis pathway (Scheme 2). As expected for a strand break terminating in a 3′-hydroxyl and a 5′-phosphate, the strand break produced from the 5′-end labeled substrate **9a** was converted by T4 DNA ligase and ATP to a band corresponding to the expected 20-mer ligation product **12a** in 64% yield (Lane

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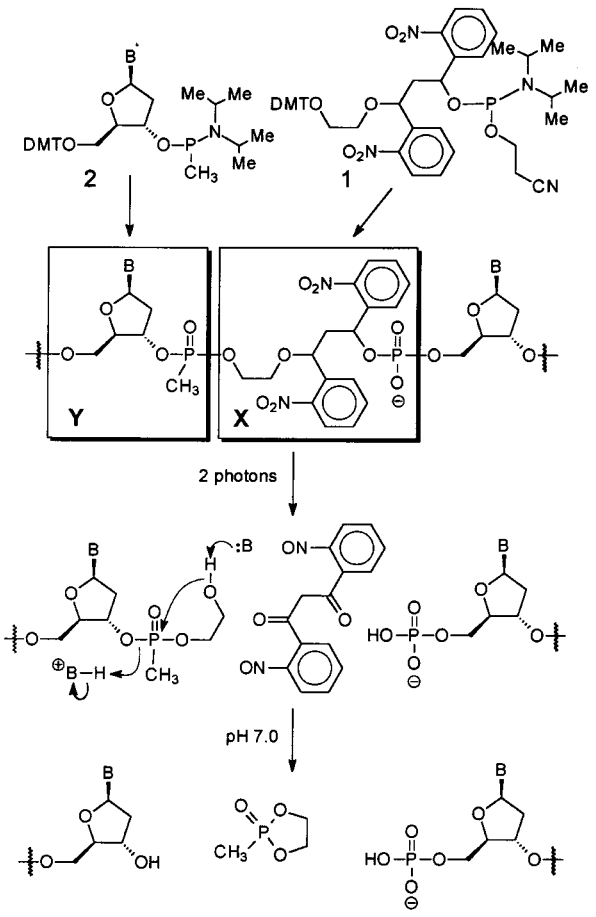
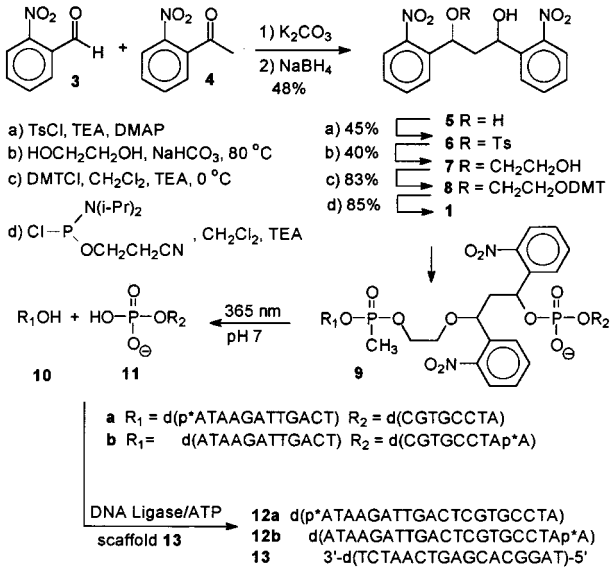
Scheme 2



Scheme 3



L, Figure 1a). In another experiment an 80% yield of ligated product was obtained. In the presence of added unlabeled **11a** the ligation could be driven to 91% of **12a** along with 9% of what appeared to be a −1 deletion product (Lane E). This minor side product also appeared in the control ligation reaction between authentic **10a** and **11a** (Lane C), but in other ligation reactions this band was less than 3%. Ligation of the photolysis products of 3′-end labeled **9b** resulted in the expected 21-mer **12b** in 36% yield, along with a second band in 64% yield which migrates such as an 11-mer (Lane L, Figure 1b). In another experiment, these two products were produced in 63% and 33% yields,

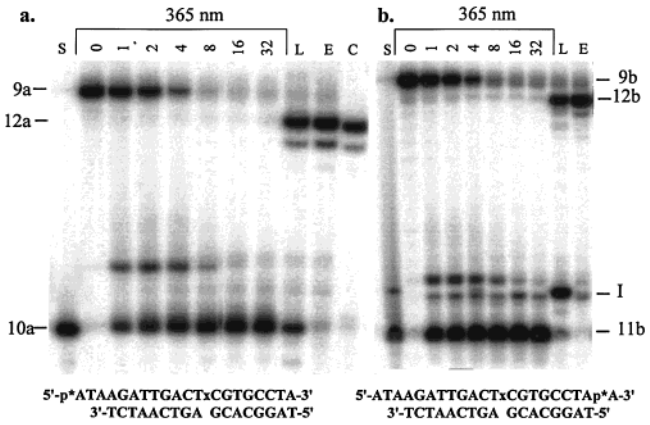


Figure 1. Photoactivated cleavage (pH 7.0) and ligation of 83 nM (a) 5′-end labeled 21-mer **9a** or (b) 3′-end labeled 22-mer **9b** in the presence of an equimolar amount of the 17-mer template **13** (shown below the figure). Irradiation with 365 nm light was conducted at 0 °C with a hand held UV lamp (720 μW/cm^2 at 3 in.) and aliquots were taken at the indicated times in minutes. Lane L: Ligation of photocleaved products using T4 DNA ligase and ATP for 16 h at 16 °C after 32 min of irradiation and 2 h at room temperature. Lane S: Authentic 5′-[^{32}P]-labeled **10a** in panel a, or **11b** in panel b. Lane E: Ligation in the presence of 5 equiv of nonradioactive **11a** in panel a or **10b** in panel b. Lane C: Ligation of authentic **10a** and 5 equiv of **11a** in the presence of the template. Band I in lane L of panel b is presumably the 5′-AMP derivative of d(pCGTGCCTAA) that forms as an intermediate in the ligation reaction.

respectively. The second band is presumably the 5′-AMP derivative of d(pCGTGCCTAA) that forms as an intermediate in ligation reactions and can sometimes be detected.[20,21] When additional authentic unlabeled **10b** was added, the ligation could be driven almost completely to the fully ligated product (Lane E). These results verify that the two oligonucleotides produced in the photolysis reaction are indeed terminated in the expected 3′-OH or 5′-phosphate and can be fully ligated under driving conditions.

In summary, we have reported the first caged ligatable DNA strand break, which could be used to cage DNA sticky ends of arbitrary length, sequence, and symmetry, as well as other structures, that would be useful for DNA nanoconstruction, and possibly DNA computation. Because the breaks are phototriggered they could also be used to spatially address the assembly and ligation of DNA. The spontaneous release of an alcohol from a β-hydroxyphosphonate at neutral pH and room temperature may also be useful for caging other substrates, or for prodrug design. DNA strand breaks terminating in a 3′-hydroxyl and a 5′-phosphate are also important intermediates in the replication, repair, and recombination of nucleic acids.[22,23] The ability to cage ligatable breaks may also be expected to aid in the study or application of these processes by affording a method for photo-triggering the formation of such breaks in vitro and in vivo.

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Supporting Information Available: Experimental procedures as well as chromatographic and spectroscopic data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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