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Photolabile thymidine cleavable with a 532 nanometer laser

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ABSTRACT

The synthesis and characterization of a novel 3′ *p*-hydroxyphenacyl-caged thymidine bearing a 5′ *N*,*N*-diisopropylcyanoethylphosphoramidite is presented representing a new methodology for the photoregulation of PCR and gene expression. Solid phase oligonucleotide synthesis affords a primer blocked at the 3′ position, which could function as a phototrigger for polymerase activity. The caging group exhibits quantitative photolysis in 15 s using a 532 nm green hand laser.

Spatiotemporal regulation through light irradiation presents an attractive method to trigger biochemical events, as it enables high-resolution control for the release of active molecular species, often for the elucidation of complex biological processes. Enabling the site-specific control of a cellular process in its native environment is intriguing, and many recent developments have been made.[1–3] A multitude of caged compounds and macromolecules have been reported with a variety of applications, including proteins,[4–6] peptides,[7] neurotransmitters,[8,9] and nucleotides.[10,11] Photoactivatable oligonucleotides have been shown to regulate genetic events, such as transcription,[12,13] translation,[14] DNAzyme reactions,[15,16] RNA interference,[17] aptamer binding,[18] and PCR,[19,20] but the need for an improved caging methodology is apparent, especially for nucleic acids.

Two main approaches to caging DNA have been utilized. The first involves alteration of the DNA backbone, either a statistical but nonspecific caging of the phosphodiester bonds[21,22] or the insertion of a photocleavable linker in the backbone.[23] While this approach has proven effective, it is difficult to achieve a completely binary (on/off) system capable of fully restoring function. The second approach is to cage the nucleobase, disrupting the Watson–Crick base pairing. This method is effective in disrupting DNA hybridization and is more capable of achieving binary behavior. Here we report a novel alternative by caging the 3′ position to inhibit primer extension while still allowing hybridization (Fig. 1). This provides facile access to 3′ caged primers via reverse phase oligonucleotide synthesis.

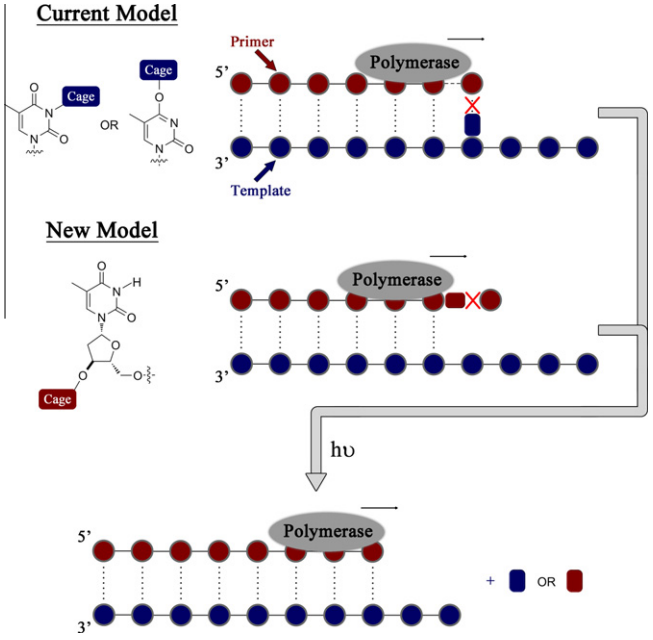

Figure 1. Caging methodologies to regulate oligo synthesis.

Of the common *ortho*-nitrobenzyl, coumarin, and benzoin caging groups, the *para*-hydroxyphenacyl (*p*HP) group is a promising alternative given its rapid photolysis, aqueous solubility, and biocompatibility.[24,25] A variety of *p*HP caged bioactive compounds have been reported (including ATP, GABA, and bradykinin), with

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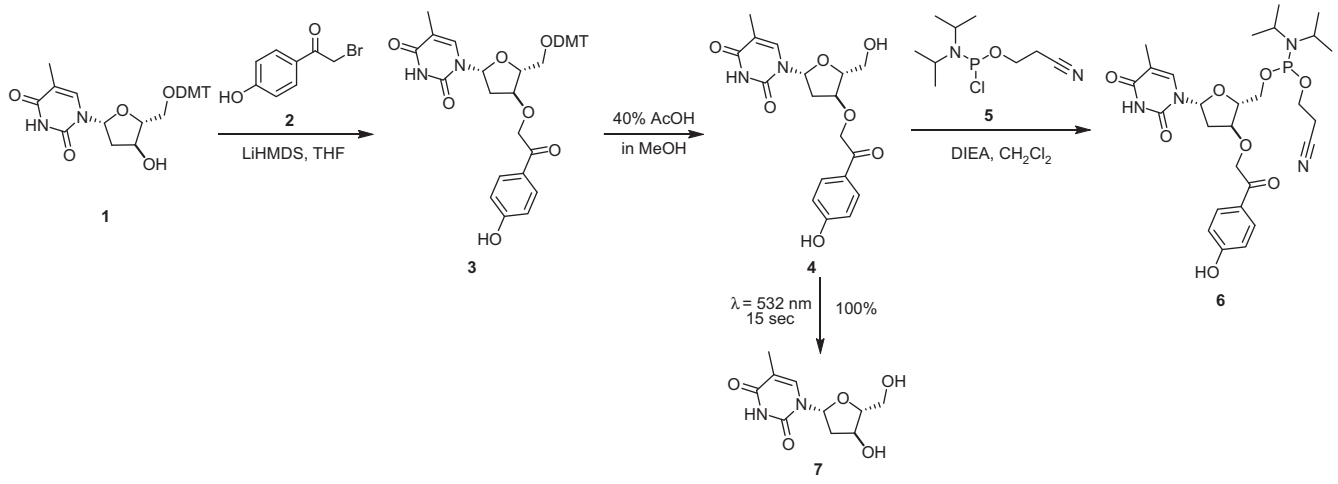

Scheme 1. Synthesis of *p*HP caged thymidine phosphoramidite **6**. DMT = dimethoxytrityl.

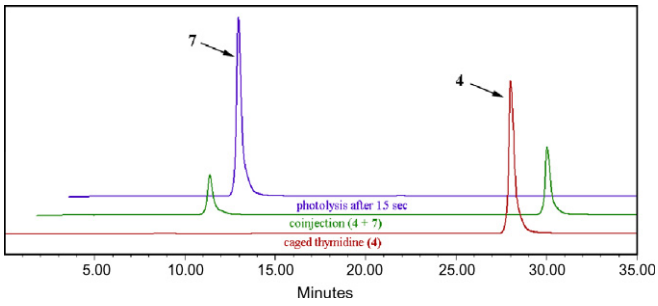

Figure 2. RP-HPLC photolysis studies of compound **4** at 532 nm (5–30% MeCN in H_2O, observed at 280 nm).

fast photolysis rates and favorable photolysis quantum yields (Φ).[26,27] The byproduct, *p*-hydroxyphenylacetic acid, is non-toxic and its absorbance is blue-shifted so as to not interfere with the photolysis reaction. Two-photon removable groups are promising for biological applications by replacing UV excitation with simultaneous absorption of two IR photons equal in total energy.[28] This approach eliminates potential thymine dimerization, which is known to occur through a reversible [2+2] photocycloaddition under UVA exposure.[29,30] Application of a two-photon group allows the selected irradiation source to be well outside the UVA region, and better tissue penetration with less phototoxicity is observed with longer wavelength sources. Two-photon excitation also enables three-dimensional control over substrate release. However, it remains difficult to predict two-photon uncaging efficiency from caging group structure;[31] *ortho*-nitrobenzyl groups have proven particularly resistant to efficient two-photon uncaging.[32]

Alkylation of the 3′ position of 5′-DMT-thymidine with *p*-hydroxyphenacyl bromide was initially attempted with NaH (in varying stoichiometric ratios). Unsuccessful efforts were originally attributed to competing substitution between the *p*-OH and the 3′-OH. Yet, attempts with *p*-OMe substituted phenacyl bromide (data not shown) also did not yield the desired product. Subsequent experiments revealed that any significant excess of NaH is sufficient to deprotonate the α-position of **2** and form phenacyl dimers prior to nucleophilic attack by the alkoxide. But, without a large excess of hydride, primarily N-alkylation of the nucleobase is observed (as with K_2CO_3 and other bases). 1,8-Diazabicyclo[5.4.0]undec-7-ene forms a mixture of N- and O-alkylated products on the thymine ring, but only lithium hexamethyldisilazide gives the desired 3′ alkylation product **3** (70% yield); lithium diisopropylamide fails to produce **3** under the same conditions (Scheme 1).

DMT deprotection with acetic acid followed by phosphitylation of the free 5′-OH gives the desired phosphoramidite **6**. A minor reaction product (10%) with substitution at the *p*-OH was isolated, and the structure was confirmed by gCOSY and NOESY experiments (see Supplementary data). Given flash chromatography following each step (three overall), the total isolated yield is 19.1%. Importantly, *p*HP caging of the substrate does not introduce a chiral center (as opposed to some other caging groups), alleviating the need to separate diastereomers.

Absorbance spectra of **4** indicate a maximum at 273 nm (see Supplementary data). An optimal two-photon irradiation wavelength will be twice this value, or 546 nm. HPLC photolysis studies confirm quantitative conversion to thymidine **7** (10 μM solution in 1:1 MeOH:H_2O) in 15 s using a 532 nm green hand laser (Fig. 2). This photolysis wavelength and source are substantially cheaper to use than the conventional one- and two-photon uncaging sources.[28] Also, the photolysis efficiency appears substantially higher than that typically achieved with *ortho*-nitrobenzyl caging groups.[32] Compound **4** is stable, neat or in solution for at least one week when exposed to ambient light, while the phosphoramidite **6** undergoes partial photolysis (~50%) after four days in DMSO-d_6 at room temperature exposed to ambient light. Compound **6** is not stable under aqueous conditions following the initial flash chromatography over deactivated silica.

Future endeavors will include feasibility studies of the oligonucleotide primers synthesized using **6**, as photo-initiators of the PCR reaction.

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Supplementary data

Supplementary data (experimental procedures and associated characterization data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.07.052.

References and notes

1. Lee, H.; Larson, D. R.; Lawrence, D. S. *ACS Chem. Biol.* **2009**, *4*, 409–427.
2. Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.

3. Young, D. D.; Deiters, A. *Org. Biomol. Chem.* **2007**, *5*, 999–1005.
4. Edwards, W. F.; Young, D. D.; Dieters, A. *ACS Chem. Biol.* **2009**, *4*, 441–445.
5. Georgianna, W. E.; Lusic, H.; McIver, A. L.; Dieters, A. *Bioconjugate Chem.* **2010**, *21*, 1404–1407.
6. Gautier, A.; Nguyen, D. P.; Lusic, H.; An, W.; Deiters, A.; Chin, J. W. *J. Am. Chem. Soc.* **2010**, *132*, 4086–4088.
7. Mizukami, S.; Hosoda, M.; Satake, T.; Okada, S.; Hori, Y.; Furuta, T.; Kikuchi, K. *J. Am. Chem. Soc.* **2010**, *132*, 9524–9525.
8. Sjulson, L.; Miesenböck, G. *Chem. Rev.* **2008**, *108*, 1588–1602.
9. Sabbatini, P.; Wellendorph, P.; Høg, S.; Pedersen, M. H. F.; Bräuner-Osborne, H.; Martiny, L.; Frølund, B.; Clausen, R. P. *J. Med. Chem.* **2010**, *53*, 6506–6510.
10. Lusic, H.; Uprety, R.; Deiters, A. *Org. Lett.* **2010**, *12*, 916–919.
11. Lusic, H.; Lively, M. O.; Deiters, A. *Mol. BioSyst.* **2008**, *4*, 508–511.
12. Kröck, L.; Heckel, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 471–473.
13. Deiters, A.; Garner, R. A.; Lusic, H.; Govan, J. M.; Dush, M.; Nascone-Yoder, N. M.; Yoder, J. A. *J. Am. Chem. Soc.* **2010**, *132*, 15644–15650.
14. Ando, H.; Furuta, T.; Tsien, R. Y.; Okamoto, H. *Nat. Genet.* **2001**, *28*, 317–325.
15. Ting, R.; Lermer, L.; Perrin, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 12720–12721.
16. Young, D. D.; Lively, M. O.; Dieters, A. *J. Am. Chem. Soc.* **2010**, *132*, 6183–6193.
17. Casey, J. P.; Blinder, R. A.; Monroe, W. T. *Mol. Pharmacol.* **2009**, *6*, 669–685; Shah, S.; Rangarajan, S.; Friedman, S. H. *Angew. Chem. Int. Edit* **2005**, *44*, 1328–1332.
18. Heckel, A.; Mayer, G. *J. Am. Chem. Soc.* **2005**, *127*, 822–823.
19. Tanaka, K.; Katada, H.; Shigi, N.; Kuzuya, A.; Komiyama, M. *ChemBioChem* **2008**, *9*, 2120–2126.
20. Yamaguchi, S.; Chen, Y.; Nakajima, S.; Furuta, T.; Nagamune, T. *Chem. Commun.* **2010**, *46*, 2244–2246.
21. Monroe, W. T.; McQuain, M. M.; Chang, M. S.; Alexander, J. S.; Haselton, F. R. *J. Biol. Chem.* **1999**, *274*, 20895–20900.
22. Ghosn, B.; Haselton, F. R.; Gee, K. R.; Monroe, W. T. *Photochem. Photobiol.* **2005**, *81*, 953–959.
23. Ordoukhanian, P.; Taylor, J. *Bioconjugate Chem.* **2000**, *11*, 94–103.
24. Pelliccioli, A. P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441–458.
25. Givens, R. S.; Lee, J. *J. Photosci.* **2003**, *10*, 37–48.
26. Givens, R. S.; Weber, J. F. W.; Conrad, P. G., II; Orosz, G.; Donahue, S. L.; Thayer, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 2687–2697.
27. Park, C.; Givens, R. S. *J. Am. Chem. Soc.* **1997**, *119*, 2453–2463.
28. Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619–628.
29. Taylor, J.-S. *Acc. Chem. Res.* **1994**, *27*, 76–82.
30. Carell, T.; Burgdorf, L. T.; Kundu, L. M.; Cichon, M. *Curr. Opin. Chem. Biol.* **2001**, *5*, 491–498.
31. Gagey, N.; Neveu, P.; Benbrahim, C.; Goetz, B.; Aujard, I.; Baudin, J.-B.; Jullien, L. *J. Am. Chem. Soc.* **2007**, *129*, 9986–9998.
32. Aujard, I.; Benbrahim, C.; Gouget, M.; Ruel, O.; Baudin, J.-B.; Neveu, P.; Jullien, L. *Chem. Eur. J.* **2006**, *12*, 6865–6879.