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Mechanistic Studies on the Repair of a Novel DNA Photolesion: The Spore Photoproduct

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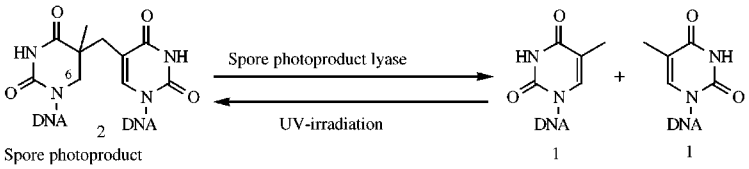
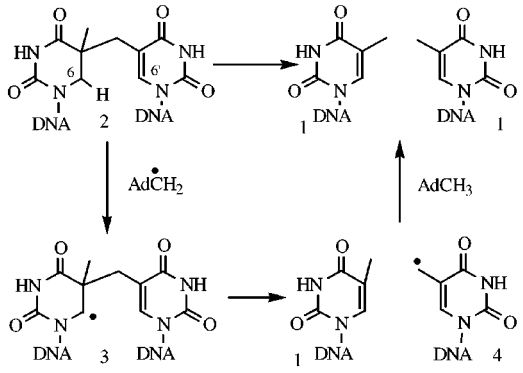
ABSTRACT



UV irradiation of spores results in the formation of the spore photoproduct. This novel DNA photolesion is repaired in the germinating spore in a reaction catalyzed by the spore photoproduct lyase. Model studies, using a simple bispyrimidine, suggest that this repair reaction proceeds by hydrogen abstraction from C6 of the spore photoproduct followed by β-scission of the bond linking the two pyrimidines and back hydrogen atom transfer.

In contrast to the photochemistry of hydrated DNA, where the cyclobutane pyrimidine photodimer is the major photoproduct, irradiation of DNA in spores results in the formation of the spore photoproduct **2**.[1] One of the pathways for the repair of this photolesion involves its conversion back to two thymines in a reaction catalyzed by the spore photoproduct lyase.[2] This enzyme has recently been overexpressed from *Bacillus subtilis* and found to contain a 2Fe-2S cluster. The overexpressed enzyme does not catalyze the spore photoproduct repair reaction, possibly due to a requirement for another protein or due to the instability of the cluster during enzyme purification. However, the repair activity can be detected in spore extract and was found to require *S*-adenosyl methionine (SAM).[3] This suggests that the adenosyl radical, which can be generated by reduction of SAM by a reduced iron sulfur cluster,[4] is involved in the repair reaction and that the cleavage of the spore photoproduct is likely to proceed via a radical intermediate (Scheme 1). Thus,

Scheme 1. Proposed Repair Mechanism for the Spore Photoproduct



hydrogen atom abstraction from C6 of the spore photoproduct **2** by the adenosyl radical, followed by β-scission of the bond linking the pyrimidines and back transfer of the hydrogen

(1) (a) For a review, see: Begley, T. P. *Comprehensive Natural Products Chemistry*; Poulter, C. D., Ed.; Elsevier Science Ltd.: New York, 1999; Vol. 5, pp 371−399. (b) Cadet, J.; Vigny, P. *Bioorganic Photochemistry*; John Wiley & Sons: New York, 1990; Vol. 1, pp 96−99. (c) Setlow, P. *J. Bacteriol.* **1992**, *174*, 2737.

(2) (a) Fajardo-Cavazos, P.; Salazar, C.; Nicholson, W. L. *J. Bacteriol.* **1993**, *175*, 1735. (b) Van Wang, T.-C.; Rupert, C. S. *Photochem. Photobiol.* **1977**, *25*, 123−127.

(3) Rebeil, R.; Yubo, S.; Chooback, L.; Pedraza-Reyes, M.; Kinsland, C.; Begley, T. P.; Nicholson, W. L. *J. Bacteriol.* **1998**, *180*, 4879.

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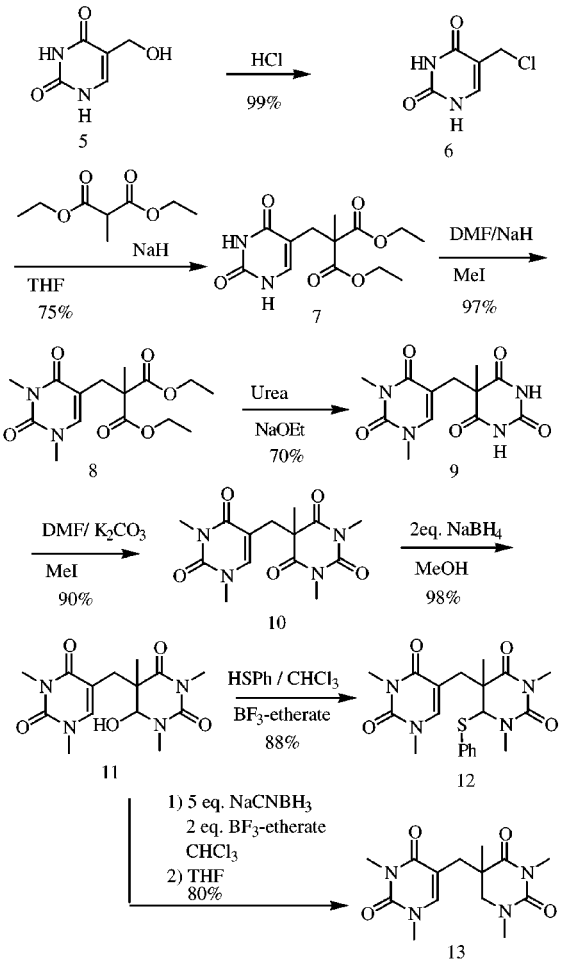
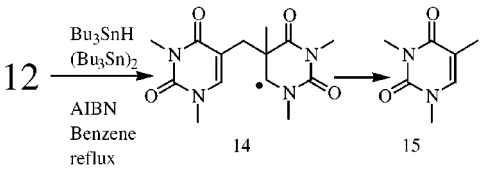
Scheme 2. Synthesis of the Model System



atom would complete the cleavage reaction. The energetically unfavorable back transfer of the hydrogen atom may be facilitated by the stabilization of the adenosyl radical by the iron−sulfur cluster. Alternatively, the spore photoproduct hydrogen atom transfer chemistry may be mediated by a glycyl radical formed by hydrogen atom abstraction from the protein by the adenosyl radical.[4] In this Letter, we describe our efforts to test this hypothesis using a simple bispyrimidine model system (**12**).

We have synthesized **12**, a precursor to the C6 spore photoproduct radical, and the spore photoproduct **13** as outlined in Scheme 2. This route involved the development of new barbiturate reduction chemistry for the conversion of **10** to **11**, **12**, and **13**. Treatment of **12** under radical-generating conditions resulted in the clean formation of dimethylthymine **15** in 85% yield (Scheme 3). Only a trace

Scheme 3. Spore Photoproduct Fragmentation



of **13** was detected in the reaction mixture. This suggests that the C6 radical of the spore photoproduct can undergo a facile β-scission reaction and is a likely intermediate for the enzymatic repair reaction.[5,6]

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Supporting Information Available: Synthetic procedures for the formation of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) For a review, see: Frey, P. A. *Comprehensive Natural Products Chemistry*; Poulter, C. D., Ed.; Elsevier Science Ltd.: New York, 1999; Vol. 5, pp 205−223.

(5) An alternative mechanism for the cleavage reaction involving the addition of a putative active site cysteine to C6′ of **2** followed by a retro-Michael reaction and a formal hydride transfer is unlikely because treatment of **13** with cysteine and base under conditions known to result in facile H/D exchange at C5 of uracil did not result in cleavage of the spore photoproduct. Mehl, R. A.; Nicewonger, R.; Begley, T. P. Unpublished results.

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