Synthesis of the Dinucleotide Spore Photoproduct

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In contrast to the effects of ultraviolet light on cellular DNA, where the cyclobutane photodimer **2** is the major photoproduct,[1] irradiation of DNA in the bacterial spore results in the formation of the spore photoproduct **3** as the major photolesion (Scheme 1).[2] This remarkable difference in the photochemical properties of cellular and spore DNA has been attributed to the association between spore DNA and a family of small-acid-soluble-spore proteins (SASP).[3] Efficient repair of this lesion is of considerable significance for the survival of the spore because substantial amounts of it accumulate prior to germination.[4] Two repair pathways have been identified.[5] The first involves excision of the lesion followed by fill-in of the resulting gap with DNA polymerase. The second involves the direct conversion of the spore photoproduct to two thymines. The gene coding for the spore photoproduct lyase has recently been cloned.[6]

Mechanistic studies on the spore photoproduct lyase will require the availability of the dinucleotide spore photoproduct **13a** as a likely minimal substrate for the enzyme. Three routes to spore photoproduct derivatives have previously been described. Irradiation of the DNA SASP complex results in the formation of the spore photoproduct.[7] This route is likely to result in several other types of photodamage to DNA and is not suitable for the preparation of spore photoproduct derivatives required for mechanistic studies. Irradiation of thymidine in the solid state also generates the spore photoproduct. This reaction mixture is complex, and selective functionalization for conversion to the dinucleotide spore photoproduct is problematic.[8] 5-Thyminyl-5,6-dihydrothymine can be synthesized by coupling 6-aminothymine with 5-(hydroxymethyl)uracil.[9] This route does not work for N1-substituted pyrimidines and is therefore also unsuitable for the preparation of the dinucleotide spore photoproduct. In this paper, we describe a synthetic route to the dinucleotide spore photoproduct **13a** that also has sufficient flexibility to permit the synthesis of derivatives for use as mechanistic probes.

Our strategy for synthesizing the dinucleotide spore photoproduct is summarized in Scheme 2. The key features of our synthesis involve the formation of the protected diastereomeric dinucleoside spore photoproducts **8a** and **8b** by coupling the enolate of dihydrothymidine **5** with bromomethyl deoxyuridine **7** and the selective manipulation of protecting groups at seven reactive centers.

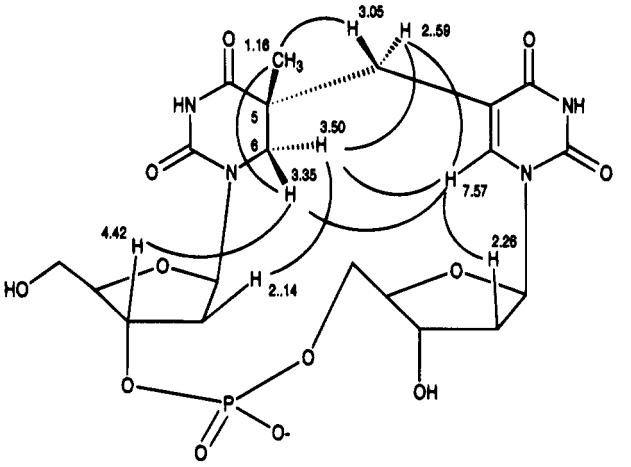
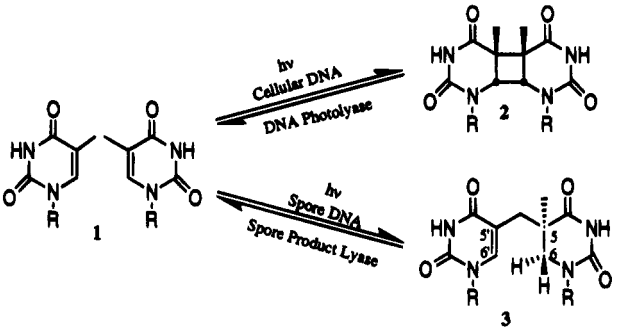


Figure 1. Key ROESY crosspeaks used for determining the stereochemistry at C5 of spore photoproduct **13a**.

Scheme 1



Protected thymidine **6** and dihydrothymidine **5** were prepared using standard reactions. The SEM group was superior to the benzyl, benzyloxymethyl, and *p*-methoxybenzyl groups for N3 protection because of its stability under the bromination conditions and its selective removal at the end of the synthesis. (Bromomethyl)deoxyuridine **7**, prepared by free radical bromination of **6**,[10] reacted with the enolate of **5** to give the protected dinucleoside spore photoproducts. Selective removal of the triethylsilyl protecting groups by treatment with HF gave **8** as a 0.45:0.55 mixture of diastereomers at C5. Dimethoxytritylation of the 5′ hydroxyl, phosphorylation of the 3′ hydroxyl, desilylation, and MSNT-mediated formation of the phosphotriester gave the fully protected spore photoproduct as a mixture of four diastereomers as indicated by ^{31}P NMR analysis (**11a′**, **11a″**, **11b′**, **11b″**). These diastereomers were separated into three components (**11a′** + **11a″**, **11b′**, and **11b″**) on TLC. The SEM and the dimethoxytrityl groups were removed from **11b″** by treatment with tin tetrachloride, and the phosphotriester was deprotected by treatment with ammonia to give spore photoproduct **13b**. Deprotection of **11b′** also gave spore photoproduct **13b**, while deprotection of **11a′** + **11a″** gave spore photoproduct **13a**.

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(5) Munakata, N.; Rupert, C. *Mol. Gen. Genet.* **1974**, *130*, 239. Van Wang, T.-C.; Rupert, C. *Photochem. Photobiol.* **1977**, *25*, 123.
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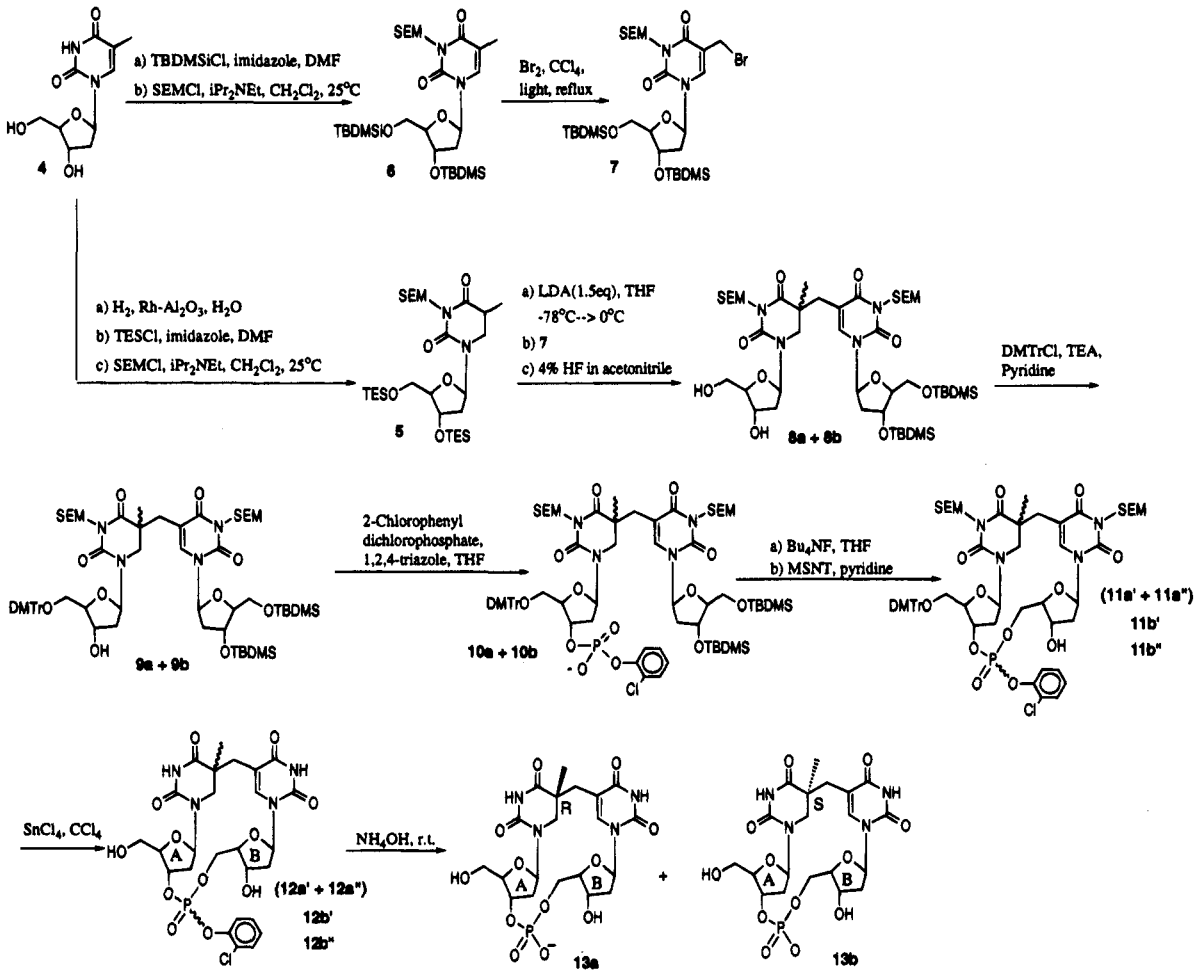
(10) This reactive compound was difficult to purify, and the yield in the subsequent coupling reaction was critically dependent on its purity.

0022-3263/95/1960-6256$09.00/0

Scheme 2



The natural isomer of the spore photoproduct will have the *R* configuration at C5 due to the constraints imposed by the DNA double helical structure. The configuration at C5 of **13a** and **13b** was analyzed using the 2-D ROESY spectrum. The pattern of ROESY crosspeaks for **13b** did not permit us to assign the configuration at C5 for this isomer because the key protons at C6 have the same chemical shift. However, the ROESY crosspeaks for **13a** (Figure 1) were consistent with the *R* and not the *S* configuration at C5 and indicated that the pyrimidines were in an extended rather than a stacked conformation. We therefore assign product **13a** as the natural isomer of the spore photoproduct.

The availability of the dinucleotide spore photoproduct will enable us to test this as a substrate for the spore photoproduct lyase and to initiate mechanistic studies on the repair of this chemically novel photolesion.

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Supporting Information Available: General experimental procedures and characterization data for all new compounds (11 pages).

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