Synthesis and properties of DNA containing a spore photoproduct analog†

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Received (in Cambridge, UK) 13th June 2008, Accepted 18th July 2008
First published as an Advance Article on the web 30th July 2008
DOI: 10.1039/b810008j

The spore photoproduct is a unique photolesion, formed in spores upon irradiation with UV light; to investigate the properties of spore photoproduct containing DNA we have synthesized 5*S* and 5*R* lesion analogs and incorporated them into DNA.

Bacteria of the Clostridium and Bacillus species form metabolically dormant endospores in response to nutrient depletion.[1] The DNA of these dormant spores is extremely well protected against damage imposed by heat, desiccation, various chemicals, UV- and γ-irradiation.[2] Consequently, spores can store genetic material over long periods.[3] They are in every respect the most long lasting information storage systems on earth. Factors which contribute to the amazing DNA stability inside spores are the small acid soluble proteins (SASPs), which complex the DNA together with dipicolinic acid under low water conditions.[4] Although the DNA is rather stable even in the presence of UV light, UV irradiation triggers the formation of a unique DNA lesion in spore DNA, which needs to be repaired. This lesion is the spore photoproduct (SP) formed between two adjacent thymidines.[5–7] Repair of the lesion is achieved by the spore photoproduct lyase, a radical SAM enzyme active during germination.[8–13] To date, a defined synthetic system which allows monitoring of the repair process *in vitro* does not exist and the exact configuration of the lesion at C5 is not known. We were, however, recently able to show with dinucleotide lesion analogs that only one of the two possible SP-lesion diastereomers, the 5*S* isomer, is efficiently repaired by the SP-lyase.[14] We now report the incorporation of the two lesion analogs **1a** and **1b** (Fig. 1), which possess either 5*S* or 5*R* configuration at C5, into DNA in order to probe how they influence the duplex stability.[15] We show that both affect the duplex stability by different amounts with the 5*S* isomer causing the smaller destabilization. The synthesized lesion analogs only lack the central phosphodiester linkage between the two lesion substructures, which is however important to allow the needed enzymatic investigations. Studies with cyclobutane pyrimidine dimers lacking the same phosphate established that these analogs can serve as superb substrates. Since repair requires chemistry at the heterocycles of the lesion the backbone modification might influence the lesion recognition step but has likely only a limited influence on the repair process.

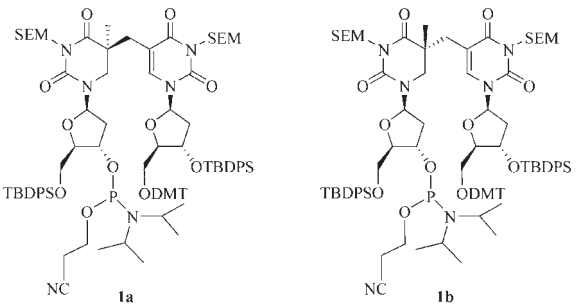

Fig. 1 The two diastereomeric phosphoramidites **1a** and **1b** prepared for the incorporation of the spore analogs into DNA. DMT = dimethoxytrityl, TBDPS = *tert*-butyldiphenylsilyl, SEM = 2-(trimethylsilyl)ethoxymethyl.

Our synthetic strategy is summarized in Scheme 1.[16] The key step of the synthesis is the formation of the diastereomeric dinucleotide spore photoproducts **2a** and **2b** by coupling of the dihydrothymidine **3** with the allyl bromide **4** followed by selective manipulations of the various protecting groups.

Protected dihydrothymidine **3** and thymidine **5** were synthesized using standard procedures. Allyl bromide **4** was prepared by Wohl–Ziegler bromination of thymidine **5**. Reaction of **4** with the enolate of **3** provided the coupled but still protected dinucleotides, which were selectively TES- and TBDMS-deprotected to give **2a/b** with pTsOH.[17] Separation of the diastereoisomers **2a** and **2b** was achieved by *np*-HPLC (120 Å, 7 μm). The configuration at C5 of **2a** and **2b** was assigned using NOESY data. As indicated in the supplementary information,† for the 5*S* isomer **2a** we measured strong NOEs between H_b and the methyl group protons and of the H_c with H_a. For the 5*R* isomer **2b**, in contrast, we determined strong NOEs between C3′-H, H_a and the methyl protons. This assignment is in agreement with previous work in our group.[16]

For the synthesis of the phosphoramidites **1a** and **1b**, we protected the free primary hydroxyl group of **2a** and **2b** with DMTOTf to obtain **6a** and **6b**.[18] Use of the triflate was essential for this conversion because of the steric bulk around the primary hydroxyl group, which did not allow any reaction with DMTCl. Transformation of the two diastereoisomers **6a** and **6b** with CEDCl furnished the needed phosphoramidites **1a** and **1b** ready for incorporation into DNA.

Having obtained both phosphoramidites it was possible to perform the DNA synthesis on a 1 μmol-scale. The coupling of

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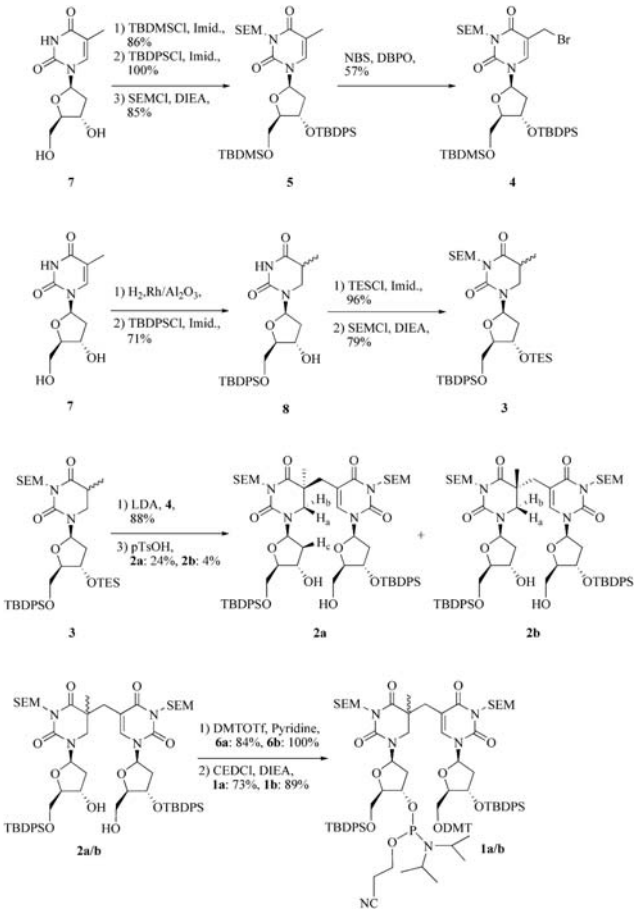
† Electronic supplementary information (ESI) available: Synthesis of the SP-isomers **1a** and **1b**, HPLC chromatograms of the separated isomers **2a** and **2b**, ^{1}H-NMR spectra from **2a** and **2b** and NOE data for the isomers **2a/b**, DNA melting curves. See DOI: 10.1039/b810008j



Scheme 1 Synthesis of the spore photoproduct lesion starting from thymidine **7** and 5′-TBDPS-protected dihydrothymidine **8**. TBDPSCl = *tert*-butyldiphenylsilyl chloride, TBDMSCl = *tert*-butyldimethylsilyl chloride, SEMCl = 2-(trimethylsilyl)ethoxymethyl chloride, DIEA = diisopropylethylamine, NBS = *N*-bromosuccinimide, DBPO = dibenzoyl peroxide, TESCl = triethylsilyl chloride, LDA = lithium diisopropylamide, pTsOH = *p*-toluenesulfonic acid, DMTOTf = dimethoxytrityl triflate, CEDCl = 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite.

the natural nucleotides was carried out with a standard protocol. Coupling of the dinucleotide spore photoproduct analogs, however, required modification of the protocol. We had to prolong the coupling time of **1a** and **1b** and in some cases double coupling was necessary to achieve a sufficiently high reaction yield on the synthesizer as needed particularly for the synthesis of longer oligonucleotides. Complicated, however, was the cleavage of the SEM-groups of **1a** and **1b** after full DNA assembly. All the standard methods based *e.g.* on treatment of the compounds with fluorides,[19–21] bases[22] or acids[23,24] failed. However, we were finally able to cleave the SEM groups in the presence of DNA directly on the solid support with a 1 M $SnCl_4$-solution in CH_2Cl_2, which was slowly rinsed through the cartridge containing the solid phase material with the DNA attached.[25] After subsequent cleavage of the DNA from the solid phase with NH_3–ethanol we finally cleaved the TBDPS-groups with TBAF.

The completely deprotected oligonucleotides were purified by *rp*-HPLC (100 Å, 3 μm, C18) and desalted with *SepPak*™ C18-columns. The synthesized and purified oligonucleotides

Table 1 DNA sequence, name and melting temperature for the corresponding duplex. ASA/CSA synthesized with **1a**, ASB/CSB synthesized with **1b**

Name	Sequence	Melting temperature T_m/°C
AS0	5′-ATC GGC TTC GCG CA-3′	65–66
ASA	5′-ATC GGC T^TC GCG CA-3′	45–46
ASB	5′-ATC GGC T^TC GCG CA-3′	42–43
CS0	5′-T ATT GCA TCA TGC-3′	51–52
CSA	5′-T AT^T GCA TCA TGC-3′	43–44
CSB	5′-T AT^T GCA TCA TGC-3′	41–42

are listed in Table 1. In order to determine how much the various stereoisomers influence the stability of the duplex we prepared two series of oligonucleotides: one in which we inserted the spore lesion analogs in the middle of the duplex (AS-series) and one in which the lesion is placed close to the 5′ end (CS series). These latter strands are typical substrates for primer extension studies.

The DNA strands were hybridized with the appropriate counter strands and the melting points of the duplexes were measured in comparison to the unmodified DNA (CS0 and AS0). The obtained data are compiled in Table 1. The first result of the study is that the spore lesion dramatically reduces the stability of the duplex. Important is a direct comparison between the 5*S* (CSA and ASA) and the 5*R* (CSB and ASB) isomers. Here the data show that the *R* isomer reduces in both cases the duplex stability (45 °C and 43 °C) by an additional 2–3 °C, in comparison to the *S* isomer (42 °C and 41 °C).

The amount of destabilization is clearly detectable even if one assumes a rather large experimental error of about 1 °C. It is therefore clear that the melting point decrease is much stronger for duplexes with ASB and CSB. In other words the lesion with 5*R* configuration gives a less stable DNA. This would suggest that in nature the *S* isomer might be formed to a larger extent. This result together with the observation that only the 5*S* isomer is repaired by spore photoproduct lyases indicates that it is the 5*S* isomer which is predominantly formed in spores. More important, however, is that we achieved for the first time the synthesis of pure oligonucleotides containing repairable, site specific spore photoproduct lyase substrates in sufficient amounts for future biochemical studies.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG: SFB 749 and Excellence cluster CiPS^M) and the Fonds der Chemischen Industrie (pre-doctoral fellowship to E.B.). Support by Novartis is gratefully acknowledged.

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