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"Base Flipping": Photodamaged DNA – RNA Duplexes Are Poor Substrates for Photoreactivating DNA-Repair Enzymes**

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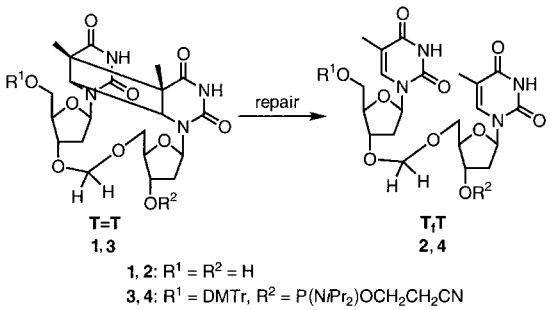
cis-syn Cyclobutane pyrimidine dimers (photodimers) are the main DNA lesions formed on irradiation of cells with UV light.[1] They are responsible for cell death, the development of various skin cancers, and therefore represent a severe threat to all organisms that are exposed to sunlight.[2] All organisms have developed DNA repair processes,[1–3] in order to remove UV-induced lesions from the genome and to overcome DNA damage. The observation that certain genome sites are repaired with greatly reduced efficiency, giving rise to mutation hot spots,[3a, 4] has shifted the investigation of the factors that determine the effectiveness of lesion recognition into the center of DNA repair research. It is currently believed that lesion-specific repair enzymes recognize structural alterations of the normal DNA duplex which are maybe caused by weakened hydrogen bonds and π-stacking interactions around a DNA lesion. Crystallographic data show that many repair enzymes subsequently "flip" the damaged base out of the DNA duplex for repair.[5] This process could be influenced by the DNA packing, which may shield DNA lesions,[6] and by the local DNA sequence and conformation.[7] A first indication that DNA repair might be influenced by the duplex conformation, stems from the discovery that dsDNA-specific repair enzymes remove lesions from DNA – RNA hybrids,[8] which are in an atypical A-like conformation, with reduced efficiency.[9]

In order to learn if and to what extent the duplex conformation is able to influence the DNA-photolyase repair process, depicted schematically in Scheme 1, we investigated the extent to which A- and B-type double strands are



Scheme 1. The starting material **1** and **2** of the phosphoamidite building blocks **3** and **4**, which were used for the synthesis of lesion-containing DNA single and DNA – DNA and DNA – RNA double strands. The repair occurs under cleavage of the cyclobutane ring catalyzed by photolyase DNA-repair enzymes.

destabilized by a photolesion, which has been incorporated site-specifically into the DNA strand. The repair was probed with a DNA-photolyase, which is believed to recognize the *cis-syn* photolesions in an extrahelical, "flipped-out" conformation.[5d] The presented thermodynamic data reveal that photodimers significantly destabilize a B-duplex but decrease the stability of an A-like duplex only to a small extent. The low destabilization was found to correlate with less efficient repair, which indicates that the local DNA conformation might modulate the DNA lesion "flipping" process.

In order to allow the preparation of oligonucleotides with a site-specific *cis-syn* photolesion in sufficient amounts for this investigation, all studies were performed with the recently introduced formacetal-linked[10] *cis-syn* photodimer analogue **1** (Scheme 1), which is readily available in gram quantities.[11] This compound was shown to be a good photolyase substrate since DNA photolyases ignore the central intradimer phosphodiester moiety during the repair process.[12] Compound **1** and the reference compound **2** were converted into the phosphoamidites **3** and **4**, respectively, and incorporated into the GC-rich 12mer and the AT-rich 14mer oligonucleotides **5** – **8** (Table 1) using previously published procedures.[11] The oligonucleotides were annealed with 1.0 equivalent (1.3 equivalents for the enzymatic studies) of the complementary DNA or RNA strand to give the DNA – DNA duplexes **9** – **12** (B-conformation) and the DNA – RNA duplexes **13** – **16** (A-like conformation).[8] The reference duplexes **9**, **11**, **13**, and **15** were prepared for comparison.

Circular dichroism (CD) studies with all duplexes were performed to investigate their conformations in solution.[13] The CD spectra were recorded at 25 °C ($c_{oligo} = 5\ \mu M$) and represent an average of two independent measurements (Figure 1). The CD spectrum of the reference DNA – DNA duplex **9** and of the photodimer-containing DNA – DNA duplex **10** are very similar and feature all the characteristics of

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Table 1. Melting temperatures T_m [°C], free energies $\Delta G^\circ_{15\,^\circ C}$ [kJ mol^{-1}],[18] enthalpies ΔH° [kJ mol^{-1}] and Entropies ΔS° [kJ K^{-1} mol^{-1}] for DNA – DNA and DNA – RNA duplex formation derived from van't Hoff plots.

	Sequence	T_m [°C][a]	$\Delta G^\circ_{15\,^\circ C}$ [kJ mol^{-1}][b]	ΔH° [kJ mol^{-1}]	ΔS° [kJ K^{-1} mol^{-1}]
5	5′d(CGACG**T**$_f$**T**GCAGC)3′	–	–	–	–
6	5′d(CGTAT**T**$_f$**T**ATTCTGC)3′	–	–	–	–
7	5′d(CGACG**T**=**T**GCAGC)3′	–	–	–	–
8	5′d(CGTAT**T**=**T**ATTCTGC)3′	–	–	–	–
B-type DNA duplexes					
9	5′d(CGACG**T**$_f$**T**GCAGC)3′				
	3′d(GCTGCA ACGTCG)5′	58.2	– 81	– 367	– 0.99
10	5′d(CGACG**T**=**T**GCAGC)3′				
	3′d(GCTGCA ACGTCG)5′	51.0	– 69	– 321	– 0.88
11	5′d(CGTAT**T**$_f$**T**ATTCTGC)3′				
	3′d(GCATAA ATAAGACG)5′	46.8	– 78	– 458	– 1.32
12	5′d(CGTAT**T**=**T**ATTCTGC)3′				
	3′d(GCATAA ATAAGACG)5′	41.2	– 68	– 417	– 1.21
A-type DNA duplexes					
13	5′d(CGACG**T**$_f$**T**GCAGC)3′				
	3′r(GCTGCA ACGTCG)5′	54.9	– 78	– 371	– 1.02
14	5′d(CGACG**T**=**T**GCAGC)3′				
	3′r(GCTGCA ACGTCG)5′	51.5	– 72	– 344	– 0.95
15	5′d(CGTAT**T**$_f$**T**ATTCTGC)3′				
	3′r(GCATAA ATAAGACG)5′	46.3	– 75	– 427	– 1.22
16	5′d(CGTAT**T**=**T**ATTCTGC)3′				
	3′r(GCATAA ATAAGACG)5′	45.6	– 73	– 412	– 1.18

[a] Conditions: 150 mM NaCl, 10 mM Tris/HCl,[18] pH 7.4, $c_{oligo} = 4.0\,\mu M$. Error in T_m: $\pm 0.3\,^\circ C$. [b] Obtained by plotting $1/T_m$ vs ln c_T. Data from at least five concentrations and of two to three independent measurements were used; error estimate at $\pm 5\,\%$.

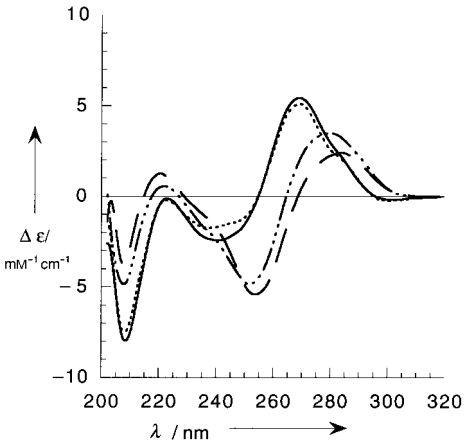

Figure 1. CD spectra of the oligonucleotide at 25 °C in 150 mM NaCl, 10 mM Tris/HCl (Tris = tris(hydroxymethyl)aminomethane),[18] pH 7.4, $c_{oligo} = 5\,\mu M$. – – – **9**, — ••• **10**, •••• **13**, —— **14**. Similar results were obtained for the series **11, 12** and **15, 16**.

a B-type double helix. Incorporation of the photodimer analogue **1** does consequently not affect the overall B-structure. The CD spectra of the DNA – RNA duplexes **13** and **14** reveal an A-type duplex structure.[8, 13] Both CD spectra of **13** and **14** were found to be mostly identical, indicating that the photolesion does not affect the A-like conformation as well.

Melting temperature studies were carried out to quantify the destabilizing effect of a photodimer lesion in an A- and B-type duplex environment (Table 1). Thermodynamic parameters (15 °C) were determined from van't Hoff plots with at least five different concentrations (0.5 – 8.0 μM) each.[14] Under the described conditions, the two reference DNA – DNA duplexes **9** and **11** have a T_m (4 μM) of 58.2 °C and 46.8 °C (Table 1). Incorporation of the photodimer model results in a significant destabilization of the duplexes **10** and **12**, which possess T_m values (4 μM) of 51.0 °C (**10**) and 41.2 °C (**12**). Such a destabilization has been previously ascribed to a local disruption of the π-stacking and hydrogen-bonding interactions of the dimer in the DNA duplex,[15] although NMR investigations[15c, 16] and calculations[17] suggest that the dimer lesion is still positioned within the duplex.

The control DNA – RNA heteroduplexes **13** and **15** melt at $T_m = 54.9\,^\circ C$ and 46.3 °C, respectively (4 μM). Incorporation of the photodimer unit in these A-like duplex structures causes a significantly smaller destabilization of the duplex: the melting points decrease to a much smaller extent yielding a T_m value of 51.5 °C for **14** and of 45.6 °C for **16** (4 μM). These values are only 3.4 °C and 0.7 °C lower than those for the reference compounds **13** and **15**, respectively. The diminished destabilization of the damaged DNA – RNA hybrids is supported by the thermodynamic data (Table 1). Incorporation of the dimer unit into the DNA – DNA duplexes (**10**, **12**) reduced their stability by +12 and +10 kJ mol^{-1} compared to that of **9** and **11**. Within the A-type DNA – RNA series, however, the reduction of the stability is two to four times smaller, yielding $\Delta(\Delta G) = +6$ kJ mol^{-1} between **13** and **14**, and only +2 kJ mol^{-1} between **15** and **16**.

If the DNA lesion recognition process is influenced by the duplex conformation and the amount of duplex destabilization, we would expect an increased repair rate for **1** in the strongly destabilized B duplex. In order to test the hypothesis we compared the repair efficiency of **1**-containing DNA – DNA and DNA – RNA duplexes using a DNA-photolyase (*A. nidulans*).[19] Footprinting data showed that these enzymes recognize only a few phosphodiester groups directly adjacent to the dimer unit, almost exclusively in the lesion-containing

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strand.[12] In order to verify this result we initially investigated the repair of DNA single strands (**7** and **8**) and DNA–DNA double strands (**10** and **12**). We observed, however, identical repair efficiencies, which underlines that photolyases repair *cis-syn* photolesions, at the used concentrations, largely independent of the counter strand (Figure 2).[3d] The final

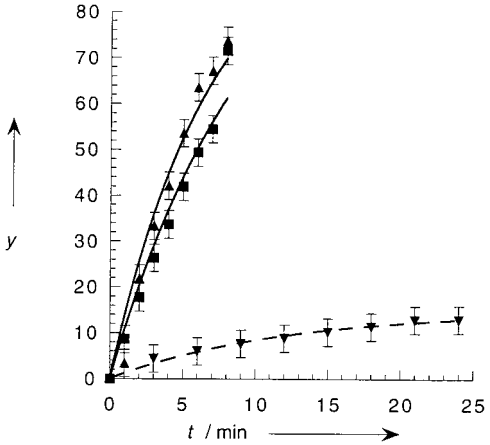

Figure 2. The repair kinetics measured with the lesion-**1**-containing single strand (**7**, ■), the lesion-**1**-containing DNA–DNA duplex (**10**, ▲), and the DNA–RNA duplex (**14**, ▼). Similar results were obtained for the series **8**, **12**, and **16**. All photolyase measurements were performed at least twice with two different oligonucleotide concentrations ($c_{oligo} = 10^{-6}$ M and 10^{-7} M, $c_{enzyme} = 5 \times 10^{-8}$ M). *y*: Amout of repaired oligonucleotide in %.

repair data obtained for the DNA–DNA and DNA–RNA duplexes are presented in Figure 2 for the oligonucleotide series **7**, **10** and **14**. Measurement of the dimer repair in the A-like DNA–RNA environment (**14** and **16**) revealed in agreement with the hypothesis dramatically reduced repair efficiencies (Figure 2). Under all circumstances we observed a repair rate that was reduced by a factor of almost 10(!). Addition of noncomplementary RNA during repair experiments did not influence the repair efficiency, which excludes the possibility that RNA inhibits the photolyase enzyme. Although we cannot fully exclude that the 2′OH group of the RNA in the DNA–RNA duplexes affects the lesion recognition step, the RNA control experiments and the single strand results indicate that the influence of the 2′OH group is limited.

The thermodynamic data show that one of the major environmentally induced DNA photolesions destabilizes a duplex in an A-like conformation to a significantly smaller extent compared to a duplex in standard B-conformation. The DNA-photolyase-catalyzed repair of the same lesion-**1**-containing DNA strand, if paired with a RNA counter strand (DNA–RNA hybrid), is strongly reduced in comparison to the corresponding DNA–DNA duplex. Although neither the supposed photolyase-induced "lesion-flipping" nor the question to which extent the 2′OH groups of the RNA counter strand effects the photolyase binding are yet fully understood our data provide clear evidence that conformational factors modulate the destabilization affect of DNA lesion and influences the DNA repair efficiency. Based on our data and the knowledge that photolyases approach the DNA duplex through the major groove we argue, that the special A-like conformation, with its narrow and deep major groove, hinders the "flipping" of lesions out of the A-like duplex.

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A Novel, Highly Enantioselective Ketone Alkynylation Reaction Mediated by Chiral Zinc Aminoalkoxides

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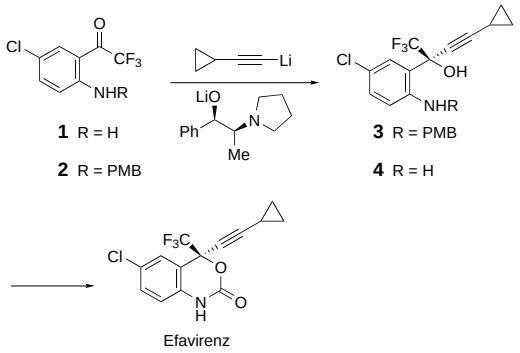
Stereocontrolled nucleophilic addition to carbonyl compounds is an important synthetic method. While the enantioselective alkylation of carbonyl compounds has been widely studied,[1] nucleophilic alkynylation has enjoyed only very limited success. A few examples of enantioselective alkynylation of aldehydes by organometallic compounds in combination with chiral modifiers have been reported.[2, 3] For example, Soai and Niwa showed that the addition of dialkynylzinc and alkylalkynylzinc reagents to benzaldehyde in the presence of amino alcohols provides propargyl alcohols with an *ee* of less than 50 %.[2c] Recently, Corey and Cimprich reported the addition of alkynylboranes to aldehydes with promotion by substoichiometric quantities of proline-derived oxazaborolidines to give propargyl alcohols with up to 97 % *ee* at low temperature.[3] We report here a novel, highly enantioselective, and practical alkynylation (up to 99.2 % *ee*) of a prochiral ketone by alkynyllithium and alkynylmagnesium reagents with mediation by chiral zinc aminoalkoxides.

Efavirenz is a potent nonnucleosidal HIV reverse transcriptase inhibitor which has just been approved by the US FDA for treatment of AIDS.[4] The importance of this compound prompted us to seek an efficient and scaleable synthesis that would allow the installation of the quaternary carbon atom with absolute stereocontrol. A recently reported asymmetric synthesis of this compound is based on a highly enantioselective addition of lithium cyclopropylacetylide to the PMB-protected ketoaniline **2** (Scheme 1).[5] The reactive



Scheme 1. Synthesis of efavirenz. PMB = *p*-methoxybenzyl.

species responsible for the strong chiral induction in this reaction was well characterized on the basis of ^{6}Li NMR data.[6] The chiral addition step, which proceeds with greater than 98 % *ee*, requires the use of 2.2 equivalents of lithium cyclopropylacetylide, 2.2 equivalents of (1*R*,2*S*)-*N*-pyrrolidinylnorephedrine alkoxide as chiral controller, and low temperatures (−60 °C). In addition, the success of the reaction relies on the protection of the aniline moiety, and this makes a protection/deprotection step necessary. The most straightforward and efficient asymmetric synthesis of efavirenz would involve the direct enantioselective alkynylation of the unprotected ketoaniline **1**[5] to afford amino alcohol **4**. Addition of lithium cyclopropylacetylide to **1** by the reported method,[5] however, suffered from low conversion and low enantioselectivity. Furthermore, the strongly basic conditions eventually led to decomposition of the product.

We reasoned that the inefficiency of the reaction between lithium cyclopropylacetylide and **1** is due to the strong basicity of the lithium reagent, which deprotonates the aniline group. It was proposed that complexation of a zinc alkoxide $Zn(OR)_2$ with the lithium acetylide would lower the basicity while maintaining the nucleophilicity of the acetylide. In addition, a chiral alkoxide could serve as a mediator for asymmetric induction. This conceptually simple approach proved to be highly effective for the asymmetric alkynylation of the unprotected ketoaniline **1**. The reaction of dimethylzinc with one equivalent of (1*R*,2*S*)-*N*-pyrrolidinylnorephedrine (**5**)[7] followed by one equivalent of methanol generated the chiral zinc alkoxide **6** (Scheme 2).[8a] The zinc reagent **6** was then treated with lithium cyclopropylacetylide, which presumably generates the zincate **7**.[8] Reaction of **7** with **1**

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