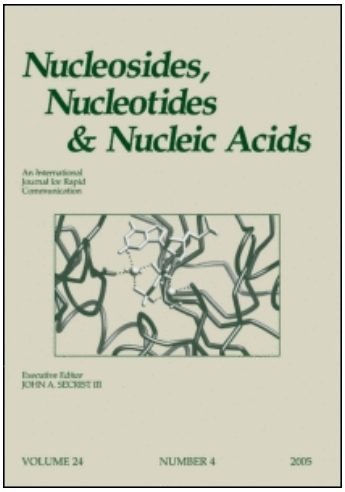

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Characterization of DNA Duplexes Containing an N³T-Ethyl-N³T Interstrand Crosslink in Opposite Orientations

Christopher J. Wilds[ab]; Anne M. Noronha[bc]; Sebastien Robidoux[a]; Paul S. Miller[b]
[a] Department of Chemistry and Biochemistry, Concordia University, Montreal, Canada [b] Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA [c] Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA

To cite this Article Wilds, Christopher J. , Noronha, Anne M. , Robidoux, Sebastien and Miller, Paul S.(2005) 'Synthesis and Characterization of DNA Duplexes Containing an N³T-Ethyl-N³T Interstrand Crosslink in Opposite Orientations', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 965 — 969
To link to this Article: DOI: 10.1081/NCN-200059329
URL: http://dx.doi.org/10.1081/NCN-200059329

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):965–969, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059329

Taylor & Francis
Taylor & Francis Group

SYNTHESIS AND CHARACTERIZATION OF DNA DUPLEXES CONTAINING AN N^3T-ETHYL-N^3T INTERSTRAND CROSSLINK IN OPPOSITE ORIENTATIONS

Christopher J. Wilds ▫ *Department of Chemistry and Biochemistry, Concordia University, Montreal, Quebec, Canada and Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA*

Anne M. Noronha ▫ *Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA and Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA*

Sebastien Robidoux ▫ *Department of Chemistry and Biochemistry, Concordia University, Montreal, Quebec, Canada*

Paul S. Miller ▫ *Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA*

▫ *DNA duplexes containing an ethyl interstrand crosslink that bridges the N^3 atoms of thymidines on the opposite strands have been synthesized using an approach that combines conventional solid phase oligonucleotide synthesis and the selective removal of protecting groups of a crosslinked thymidine dimer. This approach allows for the assembly of a crosslinked duplex directly on the solid support. Duplexes that contain a N^3T-ethyl-N^3T interstrand crosslink in a staggered orientation at either a -TA- or -AT- step in a duplex have been prepared. When placed in an -AT- step of a duplex the effect was stabilizing relative to the non-crosslinked control duplex (ΔT_m= +24°C) and this crosslinked duplex was found to efficiently form multimers in the presence of T4 ligase. In the case of the -TA- crosslinked duplex the stabilizing effect was less pronounced (ΔT_m= +6°C) and likewise did not undergo self ligation under identical conditions. Molecular modeling studies suggested that the -AT- containing lesion had little deviation in structure relative to the non-crosslinked duplex DNA control, whereas the -TA- crosslinked duplex exhibited significant buckling of the base pairs flanking the lesion.*

These studies were supported by a grant from the National Cancer Institute, CA082785. Thanks to the National Science and Engineering Research Council of Canada (NSERC) for granting a postdoctoral scholarship to AMN.

Address correspondence to Paul S. Miller, Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.

INTRODUCTION

Bifunctional alkylating agents are useful chemotherapeutics with their antitumor activity attributed to their ability to crosslink the complementary strands of DNA. The resulting bifunctional lesions can inhibit DNA replication and transcription, eventually leading to cell cycle arrest, apoptosis and inhibition of tumor growth. A problem presented in cancer treatment is the development of resistance to the effects of antitumor agents often due to enhanced repair of interstrand crosslinks.[1] If more effective treatment protocols are to be developed one needs to understand the molecular mechanisms of such repair, which unfortunately is still lacking.[1] In order to carry out various biological and structural investigations, it is necessary to have access to sufficient amounts of duplexes that contain interstrand crosslinks of well-defined structure. We recently described the syntheses and characterization of short DNA duplexes that contain both a N^4C-ethyl-N^4C[2] or a N^3T-alkyl-N^3T interstrand crosslink[3] using an appropriate crosslinked dimer phosphoramidite reagent to introduce the crosslink during automated synthesis of the duplex. This methodology has allowed the solid-phase syntheses of a number of novel crosslinked duplexes of well defined structure that have been engineered to contain an interstrand crosslink between a mismatched C—C[2] or T—T base-pair,[3] a "staggered" interstrand crosslink at a -CG- or -GC- step[4] as well as 1,3 interstrand -CNG- crosslinks containing alkyl linkages of various lengths.[5] We have continued these efforts of investigation of DNA duplexes containing a "staggered" interstrand crosslink at a -TA- or -AT- step using a N^3T-alkyl-N^3T linked dimer.

The assembly of these crosslinked duplexes via solid phase synthesis requires monomer **1** shown in Figure 1. The synthetic protocol for this monomer and solid phase synthesis of the crosslinked duplexes containing a directly opposed crosslink has been described previously.[3] Short duplexes containing 8 base pairs and ligatable ends were prepared containing the N^3T-ethyl-N^3T interstrand crosslink in

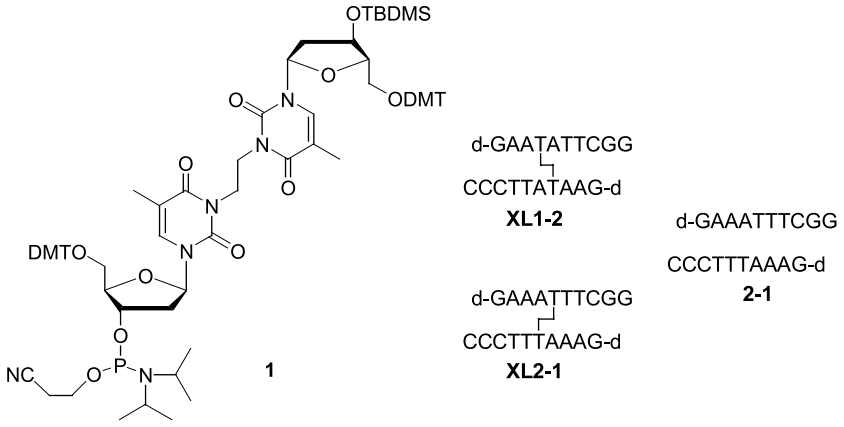

FIGURE 1 Structure of the N^3T-ethyl-N^3T phosphoramidite 1. Sequences of the cross-linked and control duplex (5′-end of each strand of the duplex is denoted by the symbol "-d").

TABLE 1

Crosslinked duplex	T_m°C[a]	Control duplex	T_m°C[a]	Δ T_m°C
XL1-2	29			+6
XL2-1	47	**2-1**	23	+24

[a]Experiments were carried out in buffer containing 2 μM duplex in 90 mM sodium chloride, 10 mM sodium phosphate, 1 mM ethylenediaminetetraacetate buffer, pH 7.0, were heated at 0.5°C/min monitoring absorbance at 260 nm.

either a -TA- (**XL1-2**) or a -AT- (**XL2-1**) orientation in a similar fashion to the directly opposed crosslinked duplexes.[3]

The results for thermal denaturation experiments for sequences **XL1-2** and **XL2-1** as well as the non-crosslinked control as shown in Table 1. In both cases, the crosslinked duplexes exhibited greater thermal stability relative to the non-crosslinked control. Duplex **XL1-2** was stabilized by about 6°C, while the duplex **XL2-1** exhibited enhanced stabilization with an increase in T_m of approximately 24°C relative to the control. As seen previously for the directly opposed N^3T-alkyl-N^3T[3] and N^4C-alkyl-N^4C,[2,4,5] the denaturation temperatures of the crosslinked duplexes are higher than those of the non-crosslinked controls, a consequence of covalently linking the two strands of the duplex. The stabilization observed for the crosslinked duplexes **XL1-2** and **XL2-1** are similar to the enhancement observed for a mispair aligned N^3T-ethyl-N^3T crosslink, which exhibited an increase in T_m of 37°C relative to the control duplex. NMR studies of the mispair aligned N^3T-ethyl-N^3T crosslink show that the ethyl linker is well accommodated between the minor and major grooves of DNA,[6] unlike the opposed N^4C-ethyl-N^4C crosslink, which resides in the major groove.[7]

In order to investigate whether insertion of the crosslink into the duplexes would distort DNA, the duplexes with self complementary ends were ligated using T4 ligase. It is known that lesions that cause bending in DNA duplexes can be easily detected as this feature manifests itself in anomalous migration relative to controls when multimerized and run on non-denaturing PAGE.[8] In Figure 2, lane 2 contains the control duplex. Lanes 1 and 3 are the crosslinked duplexes **XL1-2** and **XL2-1**, respectively. Lane 4 contains a positive control duplex containing an A-T tract (6 A-T base pairs). According to the gel, **XL2-1** appears to ligate efficiently while **XL1-2** does not. This is in stark contrast to our previous work on the N^4C-ethyl-N^4C crosslink system where duplexes with self-ligatable ends containing a -CG- staggered crosslink were capable of multimerization (with no detectable perturbation of the structure of the helix observed by electrophoresis), whereas duplexes containing a -GC- staggered crosslink did not self-ligate.[4]

In order to understand why **XL1-2** exhibited reduced thermal stability relative to the crosslinked duplex **XL2-1**, as well as the difference in their respective abilities to undergo ligation, molecular modelling studies were carried out using Hyperchem™. The models suggest that the duplex containing the **XL1-2** crosslink is distorted around the core region with disruption of all hydrogen bonds between

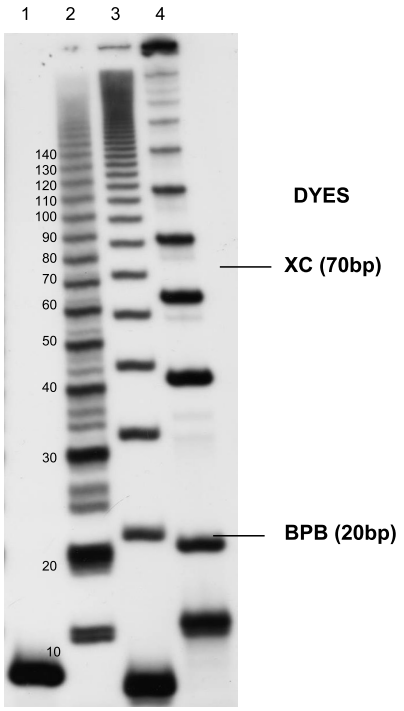

FIGURE 2 Self-ligation of crosslinked duplexes. 5′-phosphorylated duplexes (both crosslinked and controls) were ligated at 16°C in the presence of T4 DNA ligase. Electrophoresis was carried out under non-denaturing conditions (12% polyacrylamide, 4°C). The lanes show the products for ligation for (lane 1): **XL1-2**, (lane 2): control, (lane 3): **XL2-1** and (lane 4): d(GGGCAAAAAACGGCAAAAAAC)/d(CCGTTTTTTGCCGTTTTTTGC).

the flanking base pairs. In the case of the **XL2-1** duplex, there is less distortion of the core region and the lesion is tolerated in the duplex (data not shown).

Current efforts involve obtaining more structural insights about these duplexes via Circular Dichroism (CD) and NMR experiments in order to correlate their behaviour with structure.

REFERENCES

1. Dronkert, M.L.; Kanaar, R. Repair of DNA interstrand cross-links. Mutat. Res. **2001**, *486*(4), 217–247.
2. Noll, D.M.; Noronha, A.M.; Miller, P.S. Synthesis and characterization of DNA duplexes containing an N4C-ethyl-N4C inter-strand cross-link. J. Am. Chem. Soc. **2001**, *123*(15), 3405–3411.
3. Wilds, C.J.; Noronha, A.M.; Robidoux, S.; Miller, P.S. Mispair-aligned N3T-alkyl-N3T interstrand cross-linked DNA: synthesis and characterization of duplexes with interstrand cross-links of variable lengths. J. Am. Chem. Soc. **2004**, *126*(30), 9257–9265.
4. Noronha, A.M.; Noll, D.M.; Wilds, C.J.; Miller, P.S. N4C-ethyl-N4C cross-linked DNA: synthesis and characterization of duplexes with interstrand cross-links of different orientations. Biochemistry **2002**, *41*(3), 760–771.
5. Noronha, A.M.; Wilds, C.J.; Miller, P.S. N4C-alkyl-N4C cross-linked DNA: bending deformations in duplexes that contain a -CNG- interstrand cross-link. Biochemistry **2002**, *41*(27), 8605–8612.
6. Webba da Silva, M.W.; Noronha, A.M.; Wilds, C.J.; Colvin, O.M.; Miller, P.S.; Gamcsik, M.P.

Accommodation of mispair aligned N3T-ethyl-N3T DNA interstrand cross link. Biochemistry **2004**, *43*(39), 12549–12554.

7. Webba da Silva, M.W.; Noronha, A.M.; Noll, D.M.; Miller, P.S.; Colvin, O.M.; Gamcsik, M.P. Solution structure of a DNA duplex containing mispair-aligned N4C-ethyl-N4C interstrand cross-linked cytosines. Biochemistry **2002**, *41*(51), 15181–15188.
8. Koo, H.-S.; Crothers, D.M. Calibration of DNA curvature and a unified description of sequence-directed bending. Proc. Natl. Acad. Sci. U. S. A. **1988**, *85*(6), 1763–1767.