Guanine-Thymine intrastrand cross-linked lesion containing oligonucleotides: from chemical synthesis to in vitro enzymatic replication

Sophie Bellon*, Didier Gasparutto*, Christine Saint-Pierre and Jean Cadet

Laboratoire “Lésions des Acides Nucléiques”, Service de Chimie Inorganique et Biologique UMR E3 CEA UJF, Département de Recherche Fondamentale sur la Matière Condensée, CEA Grenoble, 17 Avenue des Martyrs, F-38054 Grenoble Cedex 9, France

*Present address: Medical Research Council, Radiation and Genome Stability Unit, DNA Damage Group, Harwell, Didcot, Oxon OX11 0RD, UK

*Corresponding author:
Didier Gasparutto,
tel: +33 (0)438784548, fax: +33 (0)438785090, E-mail: didier.gasparutto@cea.fr

Running title: In vitro replication of a cross-linked tandem base lesion
Supplementary documents

**Figure S1:** ESI-MS spectrum in the negative mode of the 9-mer TSPh containing oligonucleotide.
Figure S2: MALDI-TOF mass spectrum analyses of A) mass spectrum of 9-mer G^T, B) mass spectrum of the products resulting from the digestion of the modified 9-mer that contained G^T by bovine intestinal mucosa phosphodiesterase after 1 min incubation.

5'-d (TAC CG^T GTC)-3'

5'-d (TAC CG^T)-3'

-329

-303

-T

-290

-C
Figure S3: Time course enzymatic digestion by HpyCH4 III of control 21-mer (left gel) and 21-mer G^T (right gel) on denaturing 20% PAGE.

5'-^{32}\text{P}-\text{TAC\, CG^T\, GTC\, AGA\, TAC\, GAG\, AGA} -3'  
5'-^{32}\text{P}-\text{TAC\, CGT\, GTC\, AGA\, TAC\, GAG\, AGA} -3'  
3'-\text{ATG\, GCA\, CAG\, TCT\, ATG\, CTC\, TCT} -5'

control tandem lesion

0 1h 2h 4h 0 1h 2h 4h
Figure S4: A) Structure of the 5’S and 5’R diastereomer of 5’,8-cyclodeoxyadenosine. B) CyclodA DNA template for the lesion bypass DNA polymerase assay. A 12-mer primer was 5’-end labeled and annealed with the 22-mer oligonucleotide containing the lesion (5’R)-cyclodA (lane 5 to 8) or (5’S)-cyclodA (lane 9 to 12), and the 22-mer control (lane 1 to 4). Primer extension reactions catalyzed by the translesional DNA polymerase pol IV (0, 25, 50 and 100 fmol), in the presence of 100 µM of the four dNTP. The reaction mixtures were subjected to denaturing 20% PAGE analysis and the extended products were visualized by phosphorimaging with the Image Quan T software.

![Diagram of 5',8-cyclodeoxyadenosine diastereomers](image)

![Diagram of primer extension assay](image)
Figure S5: ESI-MS and ESI-MS/MS spectra (in the negative mode) of the tandem base lesion d(G^T), resulting of the nuclease P1-mediated digestion of the modified 9-mer.
**Figure S6**: 1H NMR spectra of d[GT] (A) and d[G^T] (B) tandem lesion (in D$_2$O, 200 MHz). The latter analysis (B) clearly indicates a T5-G8 bond formation with the disappearance of the H$_8$-G and CH$_3$-T signals.