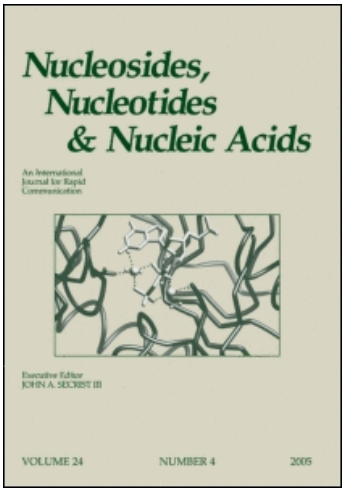

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Synthesis and Biological Evaluation of Modified DNA Fragments for The Study of Nucleotide Excision Repair in *E. coli*

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SYNTHESIS AND BIOLOGICAL EVALUATION OF MODIFIED DNA FRAGMENTS FOR THE STUDY OF NUCLEOTIDE EXCISION REPAIR IN *E. coli*

Vania Monaco[1], Karin I. van de Wetering[1], Nico J. Meeuwenoord[1], Hans A. van den Elst[1], Hanneke R. Stuivenberg[1], Rob Visse[2], Janco C. van der Kaaden[2], Geri F. Moolenaar[2], Esther E. A. Verhoeven[2], Nora Goosen[2], Gijs A. van der Marel[1], and Jacques H. van Boom[1]*.
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ABSTRACT: Three new cholesterol-containing phosphoramidites where synthesized and used in automated synthesis of modified DNA fragments. These cholesterol lesions are good substrates for the *E. coli* UvrABC endonuclease. *In vitro* they are incised from damaged DNA with higher efficiency in respect with the cholesterol lesions previously published.

To protect genetic integrity living cells need tools for repairing DNA damages. The Nucleotide Excision Repair [1] is one of the most important repair mechanisms because of its broad substrate specificity and its ability to remove bulky adducts from DNA. It is best characterized in the bacterium *Escherichia coli*. The UvrABC endonuclease [2] is responsible for the key steps of this process, which involve damage recognition and incision of the damaged strand at specific positions relative to the lesion. Other enzymes mediate the removal of the 12-13mer oligonucleotide containing the damage, DNA repair synthesis and ligation.

Recently, we have undertaken a study aimed at elucidating the UvrABC damage recognition and processing. It has been found that a specific cholesterol-DNA adduct is very efficiently recognized but poorly incised by the *E. coli* UvrABC endonuclease [3]. The same lesion was a good substrate for Human nucleotide excision repair [4]. To get a better insight into the influence of the cholesterol lesion on the actual incision process

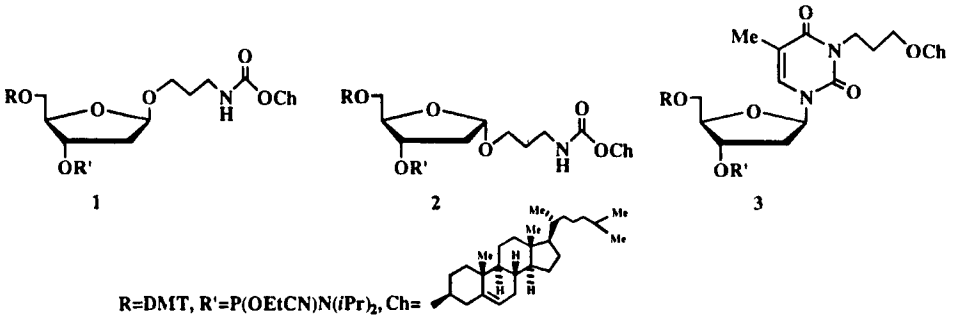

FIG. 1 Structure of the cholesterol-containing building blocks.

we designed and synthesized three new cholesterol-containing building blocks (compound 1, 2 and 3, FIG. 1) [5] to be used in automated DNA synthesis. Compound 1 and 2 were synthesized starting from 3,5-di-O-*p*-toluoyl-α-D-erythro-pentofuranosyl chloride [6] and spacer $HO(CH_2)_3NHCO_2Ch$ [7]. Compound 3 was synthesized starting from 5'-O-(4,4'-dimethoxytrityl)-3'-O-(*tert*-butyl-dimethylsilyl)-thymidine [8] and spacer $HO(CH_2)_3OCh$ [9].

Building blocks 1, 2, and 3 were used in the solid phase synthesis of DNA [5]. The modified DNA fragments (containing a single lesion on one strand in the middle of the sequence) were analyzed *in vitro* with the UvrABC proteins for damage-specific recognition and incision (results not shown). Preliminary results show that the efficiency of the incision depends upon the type of modification and, for a particular lesion, it increases with the length of the DNA fragment. DNA with these new types of cholesterol lesions is much better incised (between 20% and ~100% incision) than DNA containing the cholesterol lesion previously reported (less than 1% incision) [3]. Modification 2 is incised more efficiently than 3. The effect on the incision efficiency of the two anomers 1 and 2 is quite dramatic as incision for compound 2 (α-anomer) is much higher than for compound 1 (β-anomer) (approximately 25% for compound 1 and almost 100% for compound 2). These results strongly suggest that the orientation of the cholesterol lesion in respect with the 3D-structure of the DNA is very important in determining the efficiency of the incision.

REFERENCES

1. Sancar, A. *Annu. Rev. Biochem.*, **1996**, *65*, 43-81.
2. Van Houten, B. *Microbiol. Rev.*, **1990**, 18-51.
3. Moolenaar, G. F., Schoot Uiterkamp, R., Zwijnenburg, D. A., Goosen, N., *Nucleic Acids Res.*, **1998**, *26*, 462-468.
4. Mu, D, Hsu, D. S., Sancar, A. *Biol. Chem.*, **1996**, *271*, 8285-8294.
5. The synthesis of the cholesterol-containing building blocks and the sequences of the modified oligonucleotides used in this study will be published elsewhere.
6. Rolland, V., Kotera, M., Lhomme, J. *Synth. Commun.*, **1997**, *27*, 3505-3511.
7. Cooper, R. G., Etheridge, C. J., Stewart, L., Marshall, J., Rudginsky, S., Cheng, S. H., Miller, A. D. *Chem. Eur. J.*, **1998**, *4*, 137-151.
8. O-Yang, C., Wu, H. Y., Fraser-Smith, E. B., Walker, K. A. M. *Tetrahedron Lett.*, **1992**, *33*, 37-40.
9. Lee, E., Park, S. K., Lee, H. Y. *Bull. Korean Chem. Soc.*, **1981**, *2*, 105-112.