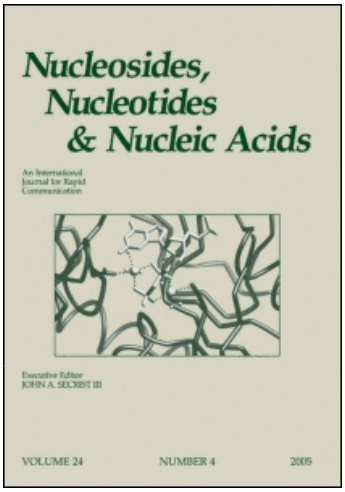

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Trifluoromethyldiazirine-Containing dUTP: Synthesis and Application in DNA/Protein Crosslinking

Galina A. Korshunova[a]; Andrey N. Topin[b]; Natalia V. Sumbatyan[b]; Olga N. Koroleva[b]; Valeriy L. Drutsa[a]
[a] A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia [b] Chemical Department, Moscow State University, Moscow, Russia

To cite this Article Korshunova, Galina A. , Topin, Andrey N. , Sumbatyan, Natalia V. , Koroleva, Olga N. and Drutsa, Valeriy L.(1999) 'Trifluoromethyldiazirine-Containing dUTP: Synthesis and Application in DNA/Protein Crosslinking', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 1097 — 1098
To link to this Article: DOI: 10.1080/15257779908041657
URL: http://dx.doi.org/10.1080/15257779908041657

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 1097–1098 (1999)

TRIFLUOROMETHYLDIAZIRINE-CONTAINING dUTP : SYNTHESIS AND APPLICATION IN DNA/PROTEIN CROSSLINKING

Galina A. Korshunova[1]*, Andrey N. Topin[2], Natalia V. Sumbatyan[2], Olga N. Koroleva[2], Valeriy L. Drutsa[1]

[1]A.N.Belozersky Institute of Physico-Chemical Biology and [2]Chemical Department, Moscow State University, Moscow, 119899, Vorobievy gory, Russia

ABSTRACT: The 5-[N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoyl)-3-aminoallyl]-2'-deoxyuridine-5'-triphosphate was synthesized via acylation of 5-aminoallyl-2'-deoxyuridine-5'-triphosphate with 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate N-hydroxysuccinimide. It was used for the preparation of 30 bp ATFMD-DNA coding for promoter sequence. UV-Irradiation (365 nm) of the specific complex of this duplex and *E.coli* RNA polymerase leads to the effective crosslinking DNA with all protein subunits.

INTRODUCTION

Photochemical crosslinking is a powerful tool for the study of nucleic acid/protein interactions. The incorporation of modified nucleotides with photoactivatable groups into the nucleic acid molecules is one of the most modern approaches: the irradiation generates highly reactive particles forming covalent bonds with adjacent proteins. Several chemical groups for DNA/protein photocrosslinking (mostly arylazides or thio-) have been used[1,2]. Photoreagents on the basis of aryl(trifluoromethyl)diazirine (ATFMD) possess some important advantages over the other photoactivatable compounds[3] but up to now there were only a few examples[4-6] of the application of ATFMD-groups for DNA/protein crosslinking.

In this work we describe the preparation and the application of 5-[N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoyl)-3-aminoallyl]-2'-deoxyuridine-5'-triphosphate (TDBAA-dUTP) - a key compound for enzymatic introduction of ATFMD-groups in DNA fragments suitable for effective DNA/protein crosslinking.

RESULTS AND DISCUSSION

We have prepared dUTP derivative, containing ATFMD-group on the maximal distance about 11-12 A from heterocycle base, by acylation of 5-aminoallyl-2'-deoxyuridine-5'-triphosphate with 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate N-hydroxysuccinimide. The acylating reagent was synthesized starting from 4-bromtoluene according procedure[7] with modifications[8]. It has been shown that most of the commonly used DNA polymerases - KF of *E.coli* DNA polymerase I, T4 and T7 DNA polymerases, *Taq* DNA polymerase - can utilize TDBAA-dUTP as a substrate in place of natural dTTP.

The TDBAA-dUTP prepared was used for the preparation of 30 bp DNA fragment, containing consensus -10 and -35 prokaryotic promoter elements, with photoactivatable groups being localized in the upper strand of -10 region. This ATFMD-containing DNA fragment can form the specific complex with *E.coli* RNA polymerase like its natural analogue[9]. The UV-irradiation of the complex at 365 nm results in the high-yield covalent DNA-protein crosslinking. Electrophoretic analysis of the reaction products has shown, that all subunits of *E.coli* RNA polymerase, including α subunit, could form covalent linkages with promoter-like ATFMD-duplex.

REFERENCES

1. Hanna, M.M. *Methods in Enzymology*, **1989**, *180*, 383-409.
2. Favre, A.in *Bioorganic Photochemistry* (Morrison, H., ed., John Wiley & Sons, New York), **1990**, 379-425.
3. Brunner, J. *Annu. Rev. Biochem.*, **1993**, *62*, 483-514.
4. Yamaguchi, T., Suyama, K., Narita, K., Kohgo, S., Tomikawa, A., Saneyoshi, M. *Nucleic Acids Res.*, **1997**, *25* (12), 2352-2358.
5. Topin, A.N., Gritsenko, O.M., Brevnov, M.G., Gromova, E.S., Korshunova, G.A. *Nucl. & Nucl.*, **1998**, *17* (7), 1163-1175 .
6. Tate, J.J., Persinger, J., Bartholomew, B. *Nucleic Acids Res.*, **1998**, *26* (6), 1421-1426.
7. Nassal, M. *Liebigs Ann. Chem.*, **1983**, 1510-1523.
8. Topin, A.N., Korshunova, G.A. *Vest. MGU, ser. khim.* (Rus.), **1995**, *36* (6), 583-587.
9. Koroleva, O.N., Shilov, I.A., Sergeev, V.N., Drutsa, V.L. *Molekuliarnaia biologiia* (Rus.), **1994**, *28* (5), 1183-1190.