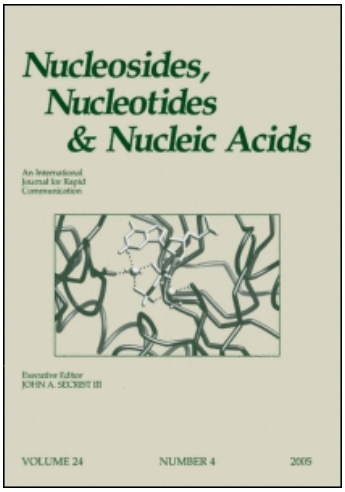

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Synthesis and Properties of Some (2'-5') Linked Dinucleoside Monophosphates Modified with 3'-Difluoromethylene Groups

C. A. Brown[a]; C. L. Barnes[a]; P. J. Serafinowski[a]
[a] CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey, UK

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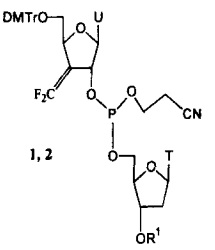
SYNTHESIS AND PROPERTIES OF SOME (2'-5') LINKED DINUCLEOSIDE MONOPHOSPHATES MODIFIED WITH 3'-DIFLUOROMETHYLENE GROUPS

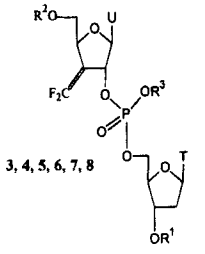
C. A. Brown, C . L. Barnes and P. J. Serafinowski*
CRC Centre for Cancer Therapeutics, Institute of Cancer Research,
15 Cotswold Road, Sutton, Surrey SM2 5NG, UK

ABSTRACT: The title dimers were prepared to investigate conditions required for the synthesis of 3'-difluoromethylene modified oligonucleotides on solid support. As a result a new synthetic cycle was developed that enabled the solid phase synthesis of the modified oligonucleotides.

As part of our research programme on antisense oligonucleotides we prepared 3'-deoxy-3'-difluoromethylene-5'-O-dimethoxytrityluridine-2'-O-phosphoramidite and 2'-deoxy-2'-difluoromethylene-5'-O-dimethoxytrityluridine-3'-O-phosphoramidite envisaged as precursors for the automated synthesis of oligonucleotides modified with 2'- or 3'-difluoromethylene groups[1]. Attempts at the incorporation of these phosphoramidites into oligonucleotide sequences, using a standard solid phase synthetic cycle, resulted in low yields of the target oligonucleotides. Therefore, studies in solution had to be carried out to establish which step of the solid phase synthetic cycle was responsible for the low overall yield.



1 R^1= acetyl
2 R^1= levulinyl



3 R^1= acetyl R^2= dimethoxytrityl R^3= cyanoethyl
4 R^1= levulinyl R^2= dimethoxytrityl R^3= cyanoethyl
5 R^1= acetyl R^2= dimethoxytrityl R^3= H
6 R^1= acetyl R^2= H R^3= cyanoethyl
7 R^1= levulinyl R^2= dimethoxytrityl R^3= H
8 R^1= H R^2= dimethoxytrityl R^3= H

Modified dinucleoside monophosphates **3** and **4** were selected as model compounds in these studies. Condensation of 3'-deoxy-3'-difluoromethylene-5'-O-dimethoxytrityluridine-2'-O-phosphoramidite with 3'-O-acetyl or 3'-O-levulinyl-thymidine in the presence of various condensing agents including tetrazole, gave the expected dinucleoside monophosphites **1** and **2**. Oxidation of compound **1** with iodine gave the expected dinucleoside monophosphate **3** in only 12% yield owing to the S_N2' substitution at the difluoromethylene group. However, oxidation of both **1** and **2** with t-butylhydroperoxide gave nearly quantitative yields of dimers **3** and **4**.

The dimers proved stable under the acidic conditions required for removal of the 5'-O-dimethoxytrityl group and the 5'-deprotected dimer **6** was isolated in 90% yield.

Treatment of **3** or **4** with concentrated ammonia, required for the cleavage from succinyl modified solid support, resulted in a complex mixture of products. Therefore, various bases were tried in order to find conditions for selective deprotection of the cyanoethyl and acyl groups without the degradation of the internucleotide bond. It was found that 5% triethylamine in water/dioxane (9:1) or triethylamine/pyridine/water (1:3:1) caused only negligible breakdown of the internucleotide bond while removing the cyanoethyl and/or 3'-O-levulinyl groups to give dimers **5**, **7** and **8**.

The findings of this study were subsequently applied to the synthesis of the modified oligonucleotides on the solid support. Thus, iodine was replaced with t-butylhydroperoxide in the oxidation step and the succinyl linker was replaced with the oxalyl linker allowing cleavage from the support under mild alkaline conditions. Conditions for the cleavage were optimised for each individual oligonucleotide. As a result, solid phase synthesis of several alternating (2'-5') linked oligonucleotides, with 3'-difluoromethylene group, using the modified cycle, could be carried out. The oligonucleotides obtained were purified by HPLC and characterised by their mass spectra although some problems occurred with longer sequences.

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1. Serafinowski, P.J. ; Barnes, C.L. *Tetrahedron*, **1996**, *52*, 7929-7938