

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

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

To cite this Article Brown, C. A. , Barnes, C. L. and Serafinowski, P. J.(1999) 'Synthesis and Properties of Some (2'-5') Linked Dinucleoside Monophosphates Modified with 3'-Difluoromethylene Groups', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1249 – 1250

To link to this Article: DOI: 10.1080/07328319908044680

URL: <http://dx.doi.org/10.1080/07328319908044680>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

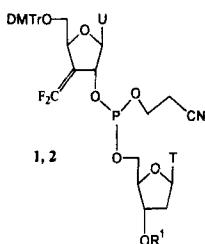
The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

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

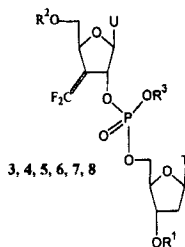
C. A. Brown, C. L. Barnes and P. J. Serafinowski*
CRC Centre for Cancer Therapeutics, Institute of Cancer Research,
15 Cotswold Road, Sutton, Surrey SM2 5NG, UK

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 groups¹. 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 R¹ = acetyl
2 R¹ = levulinyl



- 3 R¹ = acetyl R² = dimethoxytrityl R³ = cyanoethyl
4 R¹ = levulinyl R² = dimethoxytrityl R³ = cyanoethyl
5 R¹ = acetyl R² = dimethoxytrityl R³ = H
6 R¹ = acetyl R² = H R³ = cyanoethyl
7 R¹ = levulinyl R² = dimethoxytrityl R³ = H
8 R¹ = H R² = dimethoxytrityl R³ = 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.

These investigations were supported by the Cancer Research Campaign.

REFERENCE

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