

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>

Studies on Nucleoside H-Phosphonothioates as Synthons in the Synthesis of Oligonucleotide Analogues

Rula Zain^a; Martin Bollmark^a; Jacek Stawinski^a

^a Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

To cite this Article Zain, Rula , Bollmark, Martin and Stawinski, Jacek(1997) 'Studies on Nucleoside H-Phosphonothioates as Synthons in the Synthesis of Oligonucleotide Analogues', *Nucleosides, Nucleotides and Nucleic Acids*, 16: 7, 1661 – 1662

To link to this Article: DOI: 10.1080/07328319708006250

URL: <http://dx.doi.org/10.1080/07328319708006250>

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.

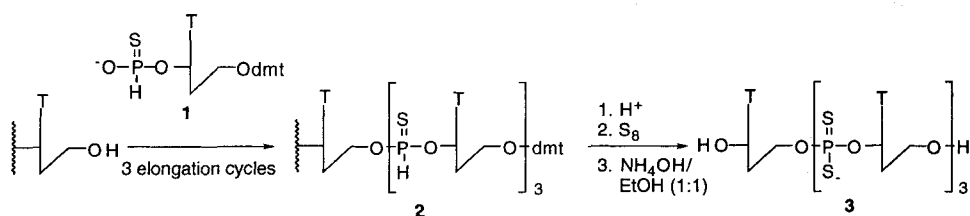
STUDIES ON NUCLEOSIDE H-PHOSPHONOTHIOATES AS SYNTHONS IN THE SYNTHESIS OF OLIGONUCLEOTIDE ANALOGUES

Rula Zain, Martin Bollmark, and Jacek Stawinski

Department of Organic Chemistry, Arrhenius Laboratory,
Stockholm University, S-106 91 Stockholm, Sweden

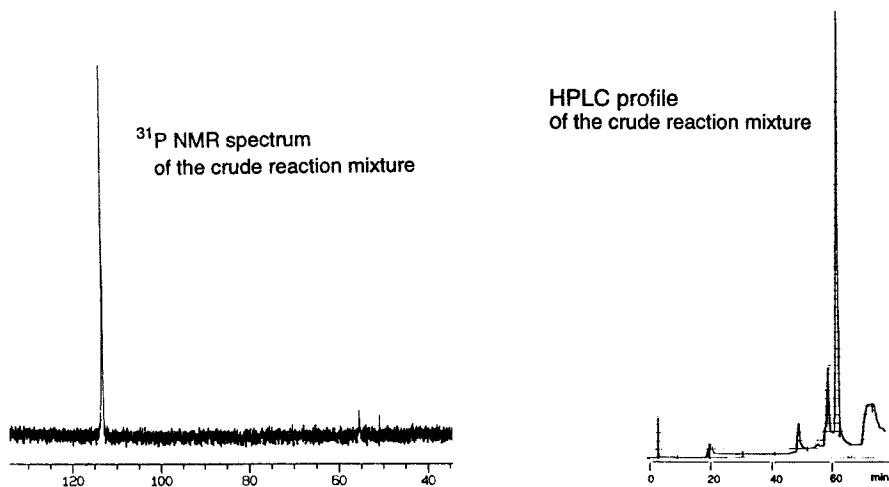
Recently, we have reported that H-phosphonothioate diesters¹ are versatile synthetic intermediates for the preparation of phosphorothioates, phosphorodithioates, or other phosphodiester analogues. The most suitable coupling agents to produce H-phosphonothioate diesters were found to be various chlorophosphates¹ [*e.g.*, 2-chloro-5,5-dimethyl-2-oxo-2 λ^5 -1,3,2-dioxaphosphinane (NEP), diphenyl phosphorochloridate]. These reagents secure the required chemoselectivity during the condensation and are unreactive towards the P-H function in H-phosphonothioate diesters^{1,2}.

To evaluate the possibility of synthesizing oligonucleotide analogues *via* H-phosphonothioate intermediates on solid support, we carried out preliminary studies on the preparation of oligonucleoside phosphorodithioates, as it is shown below.



To this end we synthesised on solid support tetrathymidine triphosphorodithioate **3** *via* the NEP promoted condensation of nucleoside H-phosphonothioate **1**³ to produce H-phosphonothioate **2**, followed by its sulfurization as the last step. The reactions were carried out in a Hamilton syringe using 30 mg of solid support (CPG 500 Å) loaded with 5'-O-(dimethoxytrityl)-3'-O-succinylthymidine. The synthetic protocol was as follows

(cycle name, reagent, time): (i) detritylation, 2% dichloroacetic acid in dichloroethane (4 x 1 mL), 4 min; (ii) washing, dichloroethane (3 x 1 mL), pyridine (3 x 1 mL), 6 min; (iii) coupling, **1** (33 mM in pyridine, 600 μ L) and NEP (100 mM in pyridine, 600 μ L), 5 min; (iv) washing, pyridine (3 x 1 mL), dichloroethane (3 x 1 mL), 6 min. In these experiments, the capping step was omitted. The coupling yield per step was ~94% as judged from the trityl assay. On completion of the cycles, the support-bound oligomer **2** containing H-phosphonothioate internucleosidic bonds was sulfurised with elemental sulfur in pyridine for 30 min and then washed successively with pyridine (3 x 1 mL) and dichloroethane (3 x 1 mL). Product **3** was cleaved from the support by treatment with 33% aqueous ammonia - ethanol (1:1, v/v) and the crude reaction mixture was subjected to HPLC [C-18 column, gradient of CH₃CN in 25 mM CH₃COONH₄ (0 to 20%)] and ³¹P NMR analyses.



The HPLC profile (see above) is consistent with the observed coupling efficiency (the major peak from **3** and some truncated sequences) and the ³¹P NMR spectrum confirms the presence of phosphorodithioate linkages in the major product ($\delta_P \sim 114$ ppm). The origin of traces of phosphoromonothioates (signals at ~ 55 ppm) is unknown. Optimization of the synthetic protocol is in progress.

Acknowledgements

The financial support from the Swedish Research Council for Engineering Sciences and the Swedish Natural Science Research Council is gratefully acknowledged.

REFERENCES

1. Stawinski, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157.
2. Zain, R.; Stawinski, J. *J. Org. Chem.* **1996**, *61*, 6617.
3. Stawinski, J.; Thelin, M.; Westman, E.; Zain, R. *J. Org. Chem.* **1990**, *55*, 3503.