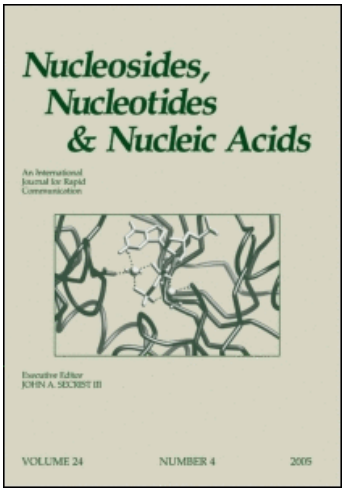


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The H-Phosphonate Method for Constructing Phosphodiester Linkages. A Progress Report

Jacek Stawiński[a]; Roger Strömberg[a]; Ingvar Lindh[a]; Tor Regberg[a]; Thomas Szabó[a]; Mats Thelin[a]; Erik Westman[a]; Per J. Garegg[a]
[a] Department of Organic Chemistry Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden

To cite this Article Stawiński, Jacek , Strömberg, Roger , Lindh, Ingvar , Regberg, Tor , Szabó, Thomas , Thelin, Mats , Westman, Erik and Garegg, Per J.(1989) 'The H-Phosphonate Method for Constructing Phosphodiester Linkages. A Progress Report', Nucleosides, Nucleotides and Nucleic Acids, 8: 5, 799 — 803
To link to this Article: DOI: 10.1080/07328318908054218
URL: http://dx.doi.org/10.1080/07328318908054218

NUCLEOSIDES & NUCLEOTIDES, 8(5&6), 799-803 (1989)

THE H-PHOSPHONATE METHOD FOR CONSTRUCTING PHOSPHODIESTER LINKAGES. A PROGRESS REPORT.

Jacek Stawiński, Roger Strömberg, Ingvar Lindh,
Tor Regberg, Thomas Szabó, Mats Thelin,
Erik Westman and Per J. Garegg*

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

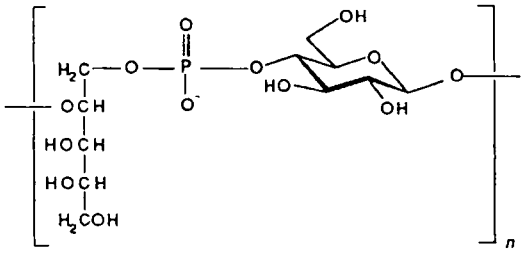
Abstract

Ongoing research into the potential of the H-phosphonate method for synthesising oligonucleotides is discussed. Examples include the synthesis of an artificial Haemophilus influenzae antigen and also efforts to extend the method into the automated solid support synthesis of long RNA oligomers.

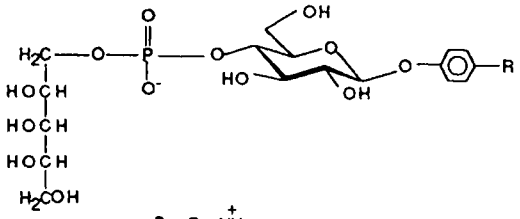
The use of hydrogenphosphonate monoesters as starting materials for oligonucleotide synthesis, first explored by Todd and coworkers,[1] has lately been re-examined and has been found to be a useful alternative method for the synthesis of DNA and also of RNA oligomers.[2-4] This is due to the relative ease of preparation of the starting materials, to the stability of the intermediate H-phosphonate diesters, which under normal condensing conditions do not form triesters, and finally to the ease of oxidation of the H-phosphonate diesters to phosphodiesters.

The method has not only been found useful in nucleotide synthesis, but also generally in the synthesis of carbohydrate phosphodiesters. Examples of exploitation of the method in the latter context include a recent synthesis of a hapten (**2**) corresponding to the repeating unit of Haemophilus influenzae a (**1**) capsular antigen.[5] The p-aminophenyl group in **2a** makes possible the attachment of the diester to a protein via a thiocarbamate linkage,[6] thereby obtaining an artificial antigen. In the synthesis of **2a**, the 4,6-benzylidene acetal **4** was regioselectively opened under reducing conditions[7] to yield the glucoside **5**, with a free 3-OH. This was now, after conversion to the corresponding p-trifluoroacetamidophenyl glucoside **6** condensed with 1,2,3,4-tetra-O-benzyl-D-ribitol-5-triethylammonium phosphonate **8**, to yield a H-phosphonate diester, from which **2** was obtained by catalytic hydrogenolysis.[5]

Other non-nucleotide examples of the use of H-phosphonates include the synthesis of phospholipids.[8]

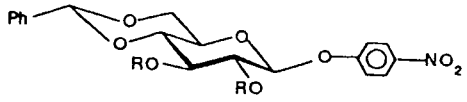


1



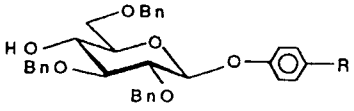
2a : R = NH_3^+

2b: R = NHCOCF



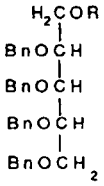
3 : R = H

4 : R = Bn

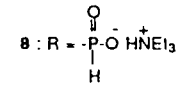


5 : R = NO_2

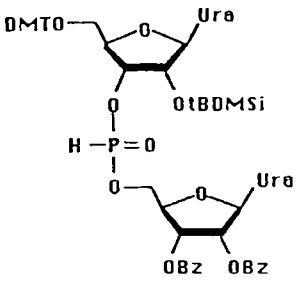
6 : R = $NHCOCF_3$



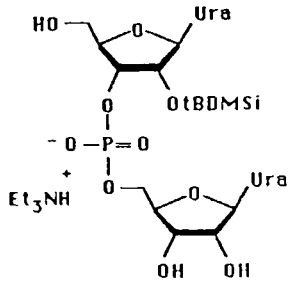
7 : R = H



8 : R = $-P(=O)(H)-O^- HNEt_3^+$



9



10

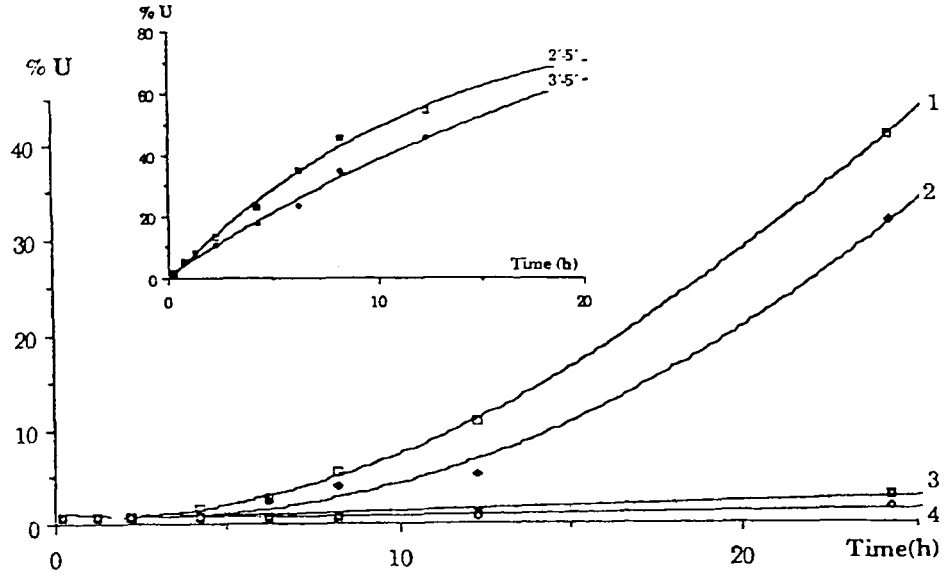


Fig. 1: Graph showing degradation of the 2'-O- silylated dimer **10** (due to loss of the t-BDMSi group) as calculated from the amount of uridine released at 55°C in different ammonia (aq) solutions. Curve 1, 25% ammonia; Curve 2, 35% ammonia; Curve 3, 25% ammonia/ EtOH (3:1, v/v); Curve 4, 35% ammonia/EtOH (3:1, v/v). Insert shows cleavage of U(2'-5')U and U(3'-5')U at 55 °C in 25% ammonia.

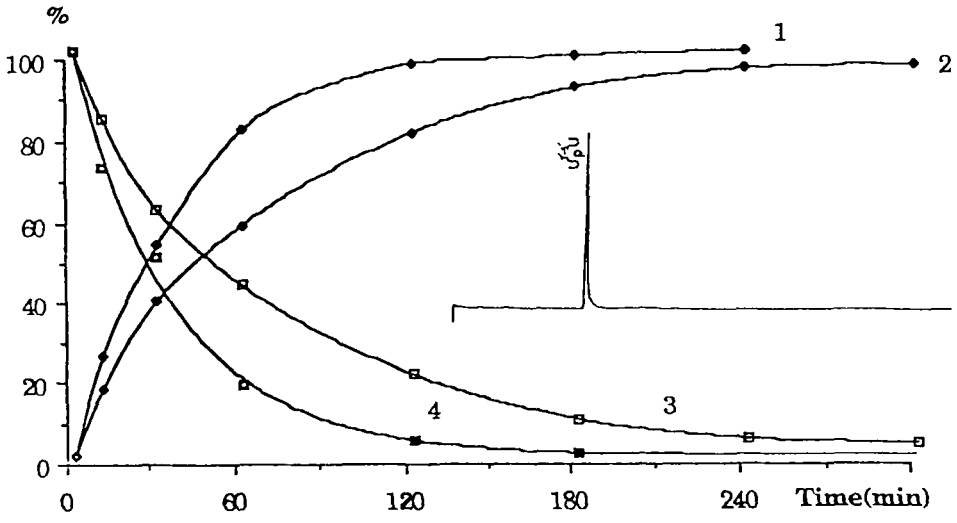


Fig. 2: Graph showing decrease of **10** and increase of U(3'-5')U with time during treatment with TBAF in THF. Curves 1 and 4, 1.0 M TBAF; curves 2 and 3, 0.1 M TBAF. Insert shows h.p.l.c. profile after 24 hours treatment of **2** with 1.0 M TBAF.

In automated solid phase synthesis of RNA oligomers, we have so far used t-butyldimethylsilyl (t-BDMSi) groups for persistent 2'-O-protection, di-p-methoxyltrityl (DMT) groups for transient 5'-O-protection, benzoyl for N-protection of adenine and cytosine groups, and isobutyryl for N-protection of guanine groups. In recent studies, a critical evaluation of the use of t-BDMSi for 2'-O-protection for the synthesis of long RNA oligomers (above fifty units) has been carried out. The findings were as follows:

1. Difficulties in preparing the starting materials have not been encountered. 2'-O-t-BDMSi and 3'-O-t-BDMSi ethers of the various (N-protected) 5'-O-DMT ethers are easily separated and their regioisomeric purity ascertained by chromatography.
2. The 2'-O-t-BDMSi groups are stable under detritylating conditions. Thus, during treatment of the diester **9** with 2.5% dichloroacetic acid at room temperature for 16 h, no desilylation or silyl migration was observed. (TLC, ^{31}P NMR). This corresponds to 480 detritylations in automated solid phase synthesis.
3. The results of base treatment, corresponding to conditions used for removal from the polymer and N-deacylation, using **10** as a model compound, is shown in **Fig. 1**. The best results were obtained using a 35% ammonia/ethanol solution at 55 ^{o}C, in which a loss of only 4% of the silyl groups and undetectable phoshodiester cleavage was observed after 8 h, corresponding to complete deprotection of 20-40 mers. However, for the synthesis of longer oligomer, for which the deprotection times will have to be increased, the present base protecting groups seem less suitable in conjunction with 2'-O-t-BDMSi protection. On the other hand, removal of the oligomer from the solid support only requires 2 h at room temperature in 35% ammonia/ethanol.
4. The results of treatment of **10** with tetrabutylammonium fluoride (TBAF) are shown in **Fig. 2**. Extended treatment with TBAF in THF gave U(3'-5')U as the only product (HPLC).

We are currently evaluating the phenoxyacetyl group as N-protecting group for adenine and guanine, and propionyl for N-protection of cytosine.[9]

Acknowledgement

We are indebted to the Swedish Natural Science Research Council and to the Swedish National Board for Technical Development for financial support.

References

1. Hall, R. H., Todd, A. , Webb, R. F. J. Chem. Soc., **1957**, 3291.
2. Garegg, P. J., Regberg, T., Stawinski, J., Strömberg, R. Chemica Scr., **1985**, 25, 280; **1986**, 26, 63.
3. Froehler,B. C., Matteucci, M. D. Tetrahedron Lett., **1986**, 27, 469.
4. Garegg, P. J., Lindh, I., Regberg, T., Stawinski, J., Strömberg, R., Henrichson, C. Tetrahedron Lett., **1986**, 27, 4051.

5. Classon, B., Garegg, P. J., Lindh, I. Carbohydr. Res., **1988**, 179, 31.
6. Buss, D. H., Goldstein, J. J. Chem. Soc., **1968**, 1457.
7. Garegg, P. J., Hultberg, H. Carbohydr. Res. **1981**, 93, c10.
8. Lindh,I.; Stawinski, J. Nucl. Acids Res. sym. ser., **1987**, 189.
9. Schulhof, J. C., Molko, D., Teoule, R. Nucl. Acids. Res., **1987**, 15, 397.