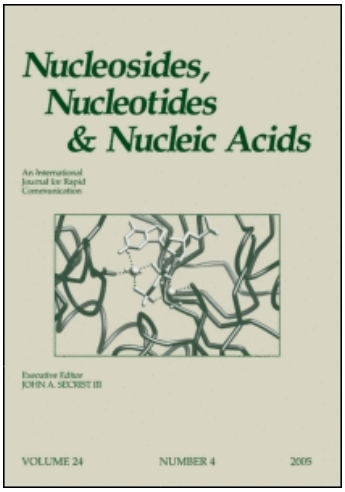

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The Application of H-Phosphonate Chemistry in the HELP Synthesis of Oligonucleotides

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THE APPLICATION OF H-PHOSPHONATE CHEMISTRY IN THE HELP SYNTHESIS OF OLIGONUCLEOTIDES.

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Abstract: The use of the H-phosphonate chemistry for its possible utilization in the liquid phase synthesis of oligonucleotides has been investigated.

The liquid phase synthesis of oligonucleotides on soluble polyethylen glycol (PEG) support has been recently developed, using both the phosphotriester[1] and the phosphoramidite[2] chemistry. This method, called HELP or High Efficiency Liquid Phase, has been proposed as an alternative to the known synthetic methods for the large scale production of oligonucleotides.

The economic considerations are clearly important in the development of a commercial production. The H-phosphonate chemistry[3] needs to be investigated for some potential advantages over the aforementioned methods. In particular, reactives and coupling agents are less expensive[4] and the H-phosphonate nucleosides are recoverable[5]. Moreover, its application to the HELP method could add a further advantage since only one single oxidation step is required at the end of the synthesis; thus, an increase in the overall speed of the process and an higher recovery of the PEG-supported product is expected owing to the reduced number of intermediate purification steps. Other interesting aspects are the reported possibility to operate without the use of the amino protecting groups[6] and the eventual substitution of the final oxidation by other chemical treatments to obtain a number of useful modified oligonucleotides[7].

For all these reasons we decided to explore the application of the H-phosphonate approach to the HELP method.

The general scheme of the proposed reactions is reported in FIGURE 1.

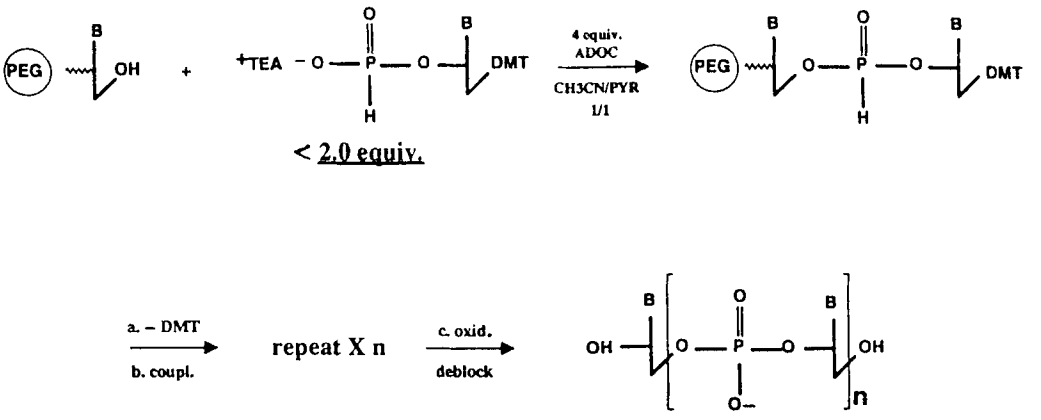

FIGURE 1

TABLE 1. Effect of reagent excess and coevaporations on the coupling yield

H-phosphonate excess	Adoc excess	n. of coevaporations	yield
2.1	10.0	3	97
1.7	12.0	3	99
1.7	4.5	3	99
1.7	3.5	1	96
1.7	3.5	=	50
1.5	3.0	3	93
1.3	6.0	3	90

The first experiment, aimed at optimising the synthetic protocol, has been performed at the dinucleotide level. In TABLE 1 are reported some data obtained by investigating the influence of the excess of the reagent and coupling agent, and of the coevaporation procedure, on the coupling yield. A 0.2 M solution of the H-phosphonate nucleosides and a 1.0 M solution of the adamantoyl chloride (Adoc) in a 1/1 mixture of pyridine and acetonitrile has been used; the PEG-supported nucleosides have been dissolved in a 10 % (w/v) solution. The time of reaction was kept to 5 minutes.

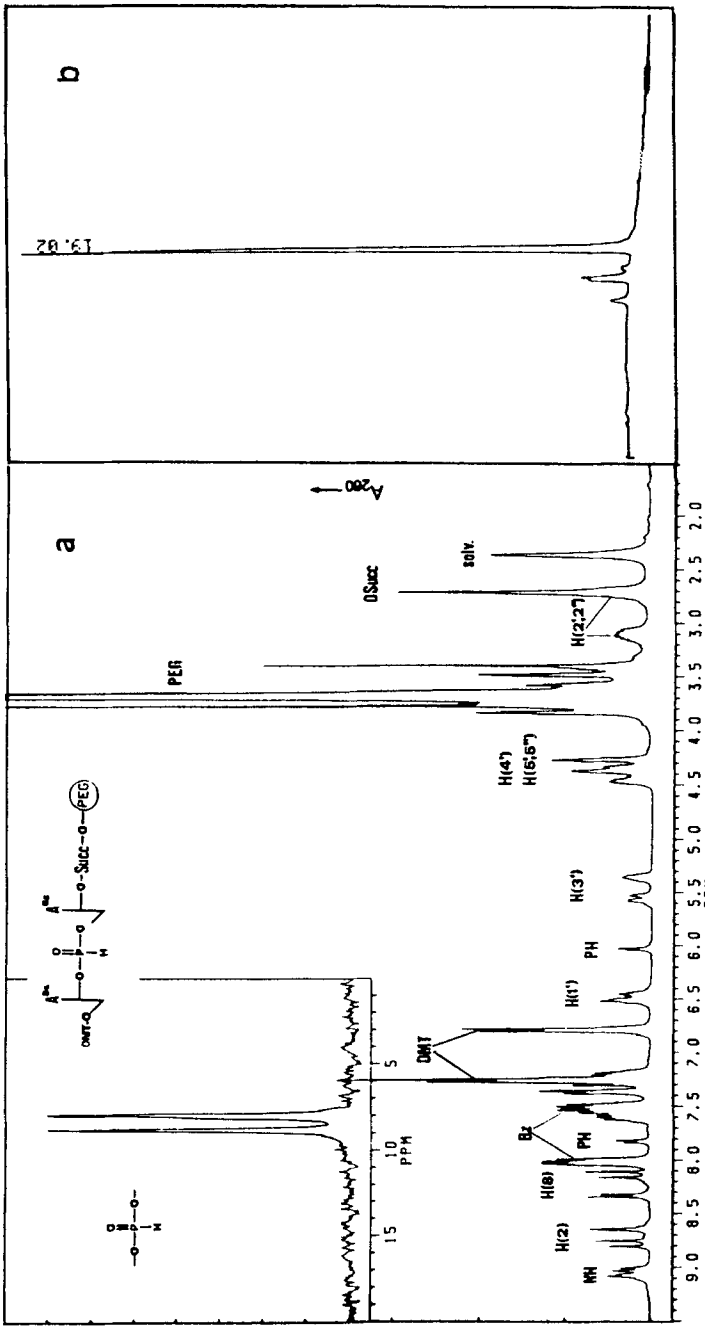

FIGURE 2. a. ^{1}H NMR of PEG-[dA(Bz)]$_2$-DMT H-phosphonate (^{31}P NMR in the insert).
b. RP HPLC of crude OH-(dA)$_2$-OH released from PEG

The best coupling conditions have been applied to all the natural nucleosides; similar yield values have been always obtained with less than 2.0 equivalents of H-phosphonate nucleoside.

The oxidation procedure has been investigated by comparing two different I2 solutions: a low-content (0.01 M), as commonly used in the commercial synthesisers, and an high-content one (0.1 M), as suggested for the H-phosphonate approach[8]. The NMR analyses indicated that a better product was obtained with the low-content I2 solution.

In FIGURE 2, the RP HPLC of a crude dinucleotide and the NMR spectra of the fully protected, PEG-supported H-phosphonate dinucleotide are reported.

From the first experiments some disadvantages also emerged. First, the H-phosphonate nucleosides are poorly soluble in the solvents used for the purification steps. This drawback could be resolved by a different purification procedure, as the ultrafiltration or the molecular sieving chromatography, or by the production and use of more "soluble" H-phosphonates. Further disadvantages seem derived from a possible lability of the succinate bond between the PEG and the oligonucleotide, as suggested[9], and from the low stability of the internucleotide H-phosphonate diester.

A detailed study of these problems, together with the synthesis of longer oligonucleotide chains, is under investigation.

REFERENCES.

1. Bonora, G.M., Scremin, C.L., Colonna, F.P. and Garbesi, A. (1990) *Nucleic Acids Res.* 18, 3155-3159.

2. Bonora, G.M., Biancotto, G., Maffini, M. and Scremin, C.L. (1993) *Nucleic Acids Res.* 21, 1213-1217.

3. a) Froheler, B.C and Matteucci, M.D. (1986) *Tetrahedron Lett.* 27, 4069-4072; b) Garegg, P.J., Lindh, I., Regberg, T., Stawinski, J. and Stromberg, R. (1986) *Tetrahedron Lett.* 27, 4051-4054.

4. Gaffney, B.I. and Jones, R.A. (1988) *Tetrahedron Lett.* 29, 2619-2622.

5. Seliger, H. and Roesch, R. (1990) *DNA and Cell Biology* 9, 691-696.

6. Kung, P.-P. and Jones, R.A. (1992) *Tetrahedron Lett.* 33, 5869-5872.

7. Froheler, B.C. (1986) *Tetrahedron Lett.* 27, 5575-5578.

8. Froheler, B.C. and Matteucci, M.D. (1986) *Nucleic Acids Res.* 14, 5399-5407.

9. Efimov, V.A., Kalinkina, A.L. and Chakhmakhcheva (1993) *Nucleic Acids Res.* 5337-5344.