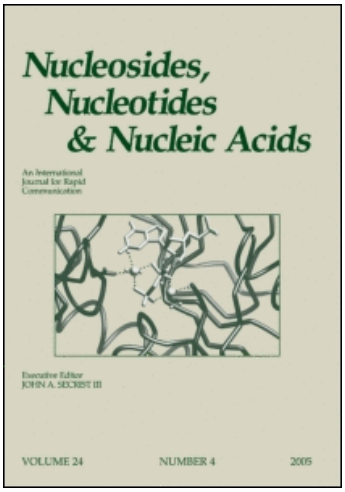


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# A Solid Supported Reagent for Internucleoside *H*-Phosphonate Linkage Formation

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# A SOLID SUPPORTED REAGENT FOR INTERNUCLEOSIDE *H*-PHOSPHONATE LINKAGE FORMATION

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▫ *A fast and convenient procedure for synthesis of dinucleoside H-phosphonates is obtained through use of the novel polystyrene supported 5-carboxy-5-methyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane reagent. Virtually quantitative H-phosphonate condensations are obtained leading to excellent isolated yields and with only a simple filtration as the purification procedure. This provides for a convenient and high-yielding procedure that should be suited for solution-phase synthesis of oligonucleotides.*

## RESULTS AND DISCUSSION

We describe the use of the novel polymer bound 5-carboxy-5-methyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane reagent[1] in synthesis of dinucleoside H-phosphonates.[2,3] This is to our knowledge the first reported solid supported chlorophosphate. This reagent is synthesized from isopropylidene-2,2-*bis*(methoxy)propionic acid, which is first coupled to aminomethylpolystyrene with HBTU/DIPEA in DMF. The isopropylidene group is removed with p-toluenesulfonic acid in $CH_2Cl_2/CH_3OH$ and the support is then treated with $POCl_3$ in $CH_3CN$/collidine. This chlorophosphate reagent was utilised in preparation of dinucleoside H-phosphonate diesters (Scheme 1).

Reactions were carried out in acetonitrile-pyridine (1:1 or 3:1 v/v, depending on solubility) using 1.78 mg of reagent/mmol of *H*-phosphonate. Virtually quantitative coupling was obtained in 1 h as judged by $^{31}P$ NMR analysis of the reaction mixtures. In the preparations a reaction time of 2 h was used. Filtering off of the polymer and washing with phosphate buffer (pH = 7.0) followed by drying and

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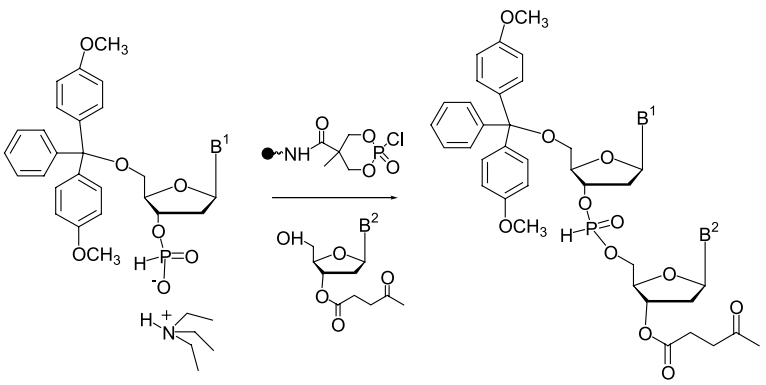

**1** $B^1$ = Thymine , $B^2$= Thymine
**2** $B^1$ = $N^6$-benzoyladenine, $B^2$= Thymine
**3** $B^1$ = $N^4$-benzoylcytosine, $B^2$= Thymine
**4** $B^1$ = $N^2$-isobutyrylguanine, $B^2$= Thymine

**SCHEME 1** Use of solid supported 5-carboxy-5-methyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane for synthesis of di (deoxynucleoside) H-phosphonates.



concentration led to isolation of the products. Only traces of remaining *H*-phosphonate monoesters were detected (primarily because of its use in slight excess) in the crude material. The material was sufficiently pure for direct use in further transformations, but the remaining monoesters were also readily removed by filtration through silica gel with 1,4 dioxane as the solvent. This procedure gave excellent yields (76–81%) of the isolated *H*-phosphonate diesters **1–4**. The products were all characterized by $^{31}$P NMR, $^{1}$H NMR, and ESI-MS analysis. In preparation of compound **4** no side product from potential reaction on the lactam function of the guanine base was detected even after keeping the reaction mixture in contact with the solid supported chlorophosphate for 8 h.

The above procedure provides for a convenient and high-yielding method for synthesis of dinucleoside H-phosphonates. Distinct advantages are that the reagent can be made in a few steps with relatively inexpensive materials and that the H-phosphonate diesters generated can be conveniently isolated in highly pure form by a simple filtration step. The compounds can then typically be used directly for further transformations to dinucleotides or different modified analogues. In addition, the procedure provides an excellent alternative for the coupling step in solution-phase synthesis of oligonucleotides. The use of the H-phosphonate method[2,4–8] especially in a modified form[9,10] has been actualised as an economic alternative for the large scale synthesis of therapeutic oligonucleotides.[11]

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