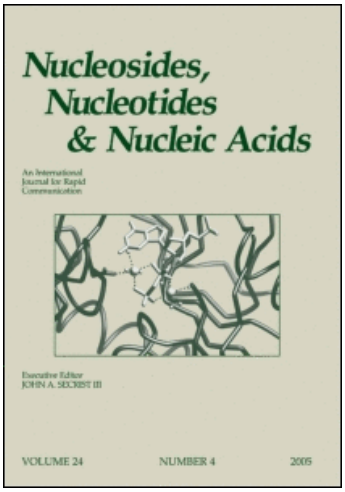

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Studies on Nucleoside Phosphonates and Their Derivatives. a Progress Report

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STUDIES ON NUCLEOSIDE PHOSPHONATES AND THEIR DERIVATIVES. A PROGRESS REPORT

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Abstract:
Some synthetic, mechanistic and structural aspects of ongoing research on biologically important phosphate derivatives and their analogues are discussed.

INTRODUCTION

Biological importance and practical significance of phosphate esters have been the major driving forces for research in various areas of synthetic organic phosphorus chemistry. The emergence of antisense[1] and antigene[2] technology for the modulation of gene expression with synthetic oligonucleotides considerably accelerated this research. Since different classes of oligonucleotide analogues may inhibit virus replications by different mechanisms and can be effective either alone or in combination with other drugs in the therapy of patients with AIDS or the AIDS-related complex, the quest for compounds with improved biological properties still continue.

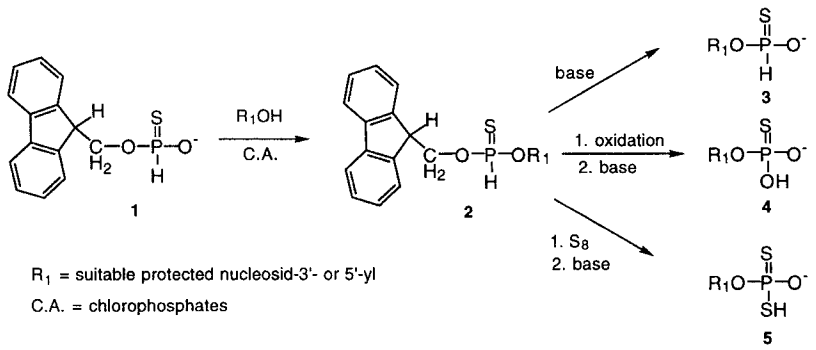
Our groups have been interested in the development of new synthetic methodologies for the preparation of biologically important phosphate esters and their analogues as well as in mechanistic and structural studies related to these compounds. In this paper a short account of some selected ongoing projects connected with these aspects of nucleic acids chemistry is given.

1. H-PHOSPHONOTHIOATES AS SYNTHETIC INTERMEDIATES

These compounds are interesting as precursors for sulfur containing phosphate analogues[3], *e.g.*, phosphorothioates, phosphorodithioates, and particularly for those which are difficult to obtain from H-phosphonates or tervalent phosphorus compounds. Recently, we have elaborated efficient synthetic methods for the preparation of nucleoside H-phosphonothioate monoesters based on sulfurization of the appropriate phosphinate intermediates[4] or involving the non-oxidative thiation of nucleoside H-phosphonate monoesters[5]. We have also shown that these compounds can be chemoselectively coupled in the presence of chlorophosphates[3,6-8] to produce H-phosphonothioate diesters, from which various phosphate analogues can be obtained[3].

1.1. 9-Fluorenemethyl H-phosphonothioate, a versatile reagent for the preparation of H-phosphonothioates and phosphate analogues.

To provide easy access to H-phosphonothioate monoesters as well as to the sulfur-containing phosphomonoester analogues, we have been searching for a versatile thiophosphonylating agent, which would allow the introduction of the thiophosphonyl group to natural product derivatives. Among various alkyl H-phosphonothioate monoesters investigated for this purpose, the most promising appeared to be 9-fluorenemethyl H-phosphonothioate **1**[9]. This compound, bearing a lipophilic, β-elimination group, was conveniently prepared[10] on a multigram scale by the reaction of 9-fluorenemethanol with triethylammonium phosphinate in the presence of a condensing agent, followed by sulfurization[4]. Its utility was assessed in the preparative syntheses of nucleoside 3'- and 5'-H-phosphonothioate **3**, nucleoside phosphorothioate **4**, and nucleoside phosphorodithioate **5** monoesters.

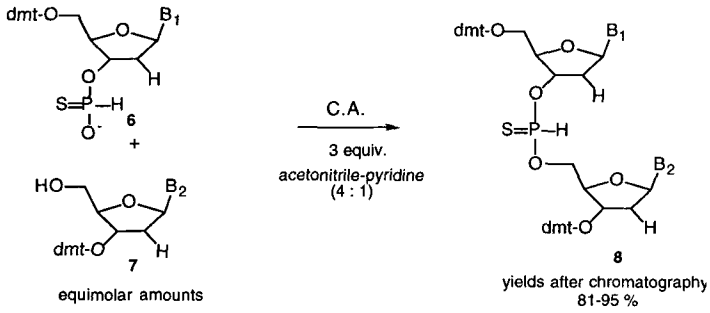


This approach involves the synthesis of the fluorenemethyl H-phosphonothioate intermediate **2** under the reaction conditions developed for dinucleoside H-phosphonothioates (*vide infra*), followed by its conversion into various products, as

shown in the Scheme above. The intermediate **2** can be isolated (if so desired) or may be used *in situ* for further transformations. To avoid the ligand exchange process[8] in **2**, the reactions should preferably be carried out with limited amounts of pyridine. The removal of the 9-fluorenemethyl group from **2** to produce H-phosphonothioates **3** can be effected with triethylamine or DBU. In other instances (*e.g.*, during the preparation of phosphorothioates **4** or phosphorodithioates **5**), using sodium hydroxide (or other alkali metal hydroxides) gave cleaner reaction mixtures.

1.2. Preparation of dinucleoside H-phosphonothioates.

The condensation of nucleoside H-phosphonothioate monoesters[3] **6** with nucleosides in the presence of various condensing agents has been studied in detail[8].



B_1, B_2 = Thy, and N-protected Ade, Cyt, Gua
C.A. = chlorophosphates, *e.g.*, diethyl or diphenyl phosphorochloridate

Among the coupling agents investigated, chlorophosphates secured both clean and chemoselective condensations (^{31}P NMR). The ligand exchange process in the produced H-phosphonothioate diesters **8** can be considerably suppressed or eliminated by reducing the amount of pyridine used as a co-solvent[8].

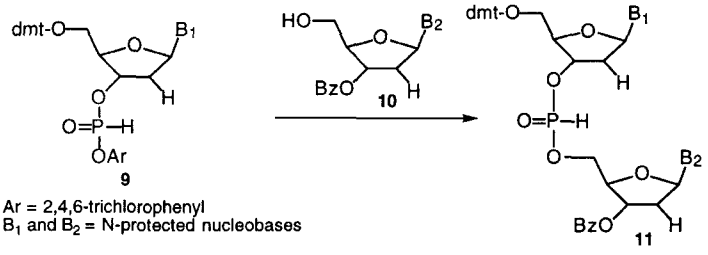
To evaluate the possibility of synthesizing oligonucleotide analogues *via* H-phosphonothioate intermediates on solid support, we carried out some preliminary studies on the preparation of oligonucleoside phosphorodithioates[11]. The corresponding thymidyl tetramers were synthesized using different reaction conditions and optimization of the synthesis is now in progress.

2. SYNTHETIC AND MECHANISTIC ASPECTS OF ARYL H-PHOSPHONATES.

Recently, we have initiated studies directed towards the assessment of aryl H-phosphonates as potential substrates for the synthesis of various nucleoside H-phosphonate derivatives[12-14]. The distinctive feature of these compounds is that they posses only one electrophilic centre, which reactivity can be controlled by substituents on the aromatic ring.

Transesterification of nucleoside aryl H-phosphonates.

We have developed a new method for internucleotide bond formation, which is based on the transesterification of aryl nucleoside H-phosphonate diesters[15] (see the Scheme below).



Ar = 2,4,6-trichlorophenyl
B_1 and B_2 = N-protected nucleobases

The advantages of this methodology are that the reaction is rapid and efficient (yields after purification > 90%)[15], and it can be carried out under mild reaction conditions (RT, CH_2Cl_2/pyridine). Since its underlying principle is different from that of coupling procedures based on condensing agents, this method should expand the synthetic potential of H-phosphonate esters.

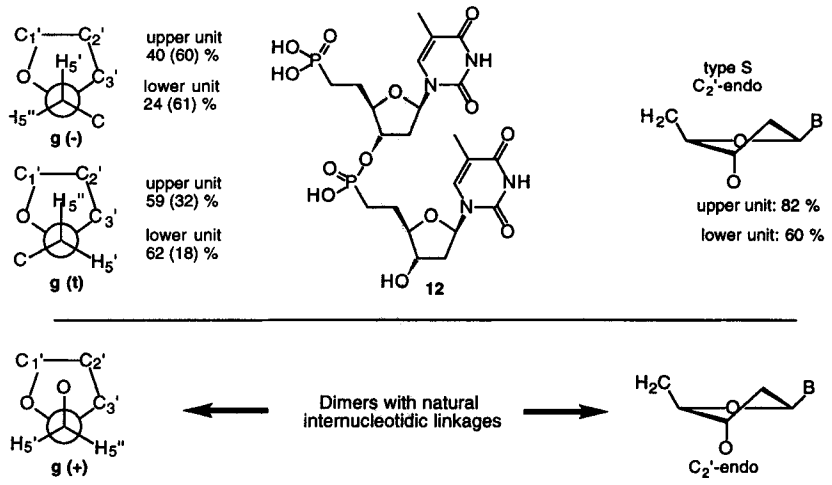
3. SYNTHETIC AND STRUCTURAL STUDIES ON 5'-METHYLENEPHOSPHONATE ANALOGUES

C-Phosphonates bearing the P-C bond in the bridging position of the phosphonate group are isosteric and isopolar analogues of oligonucleotides, potentially useful as antisense agents. A new synthetic method for the preparation of this type of compounds, based on building blocks containing an intramolecular catalytic phosphonate protecting group, was developed and evaluated in the solid phase synthesis of the thymidine oligonucleotide analogues[16].

Structural studies on 5'-deoxy-5'-methylphosphonate linked dimer[17].

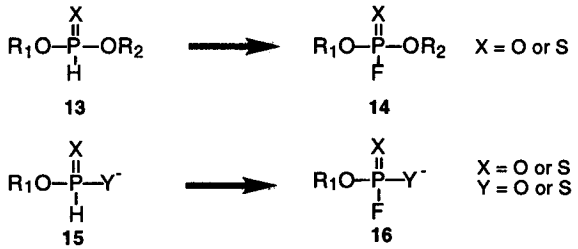
Since the replacement of a polar P-O bond at the phosphorus centre by the P-C one in nucleotides may introduce substantial electronic and conformational changes, we investigated some structural features of the dimer **12** using ^{1}H NMR spectroscopy. The results are summarized in the Scheme below.

We found that the deoxyribose moieties in **12** adopt conformations similar to those in natural nucleic acids. However, the most populated rotamers around C4'-C5' bonds are different than in the phosphodiester linked oligonucleosides, and are g(t) and g(-).



4. FORMATION OF THE P-F BOND *VIA* OXIDATIVE TRANSFORMATION OF H-PHOSPHONATES AND THEIR ANALOGUES.

A new, efficient method for the preparation of potentially useful analogues of phosphodi- and phosphomonoesters, compounds **14** and **16**, was developed[18,19].



R_1, R_2 = nucloside moieties

The transformation is effected by the oxidation of the H-phosphonate **13** (X=O) or H-phosphonothioate **13** (X=S) diesters in acetonitrile or in pyridine with iodine or with carbon tetrachloride in the presence of triethylamine trishydrofluoride (TAF) to produce the phosphorofluoridate (**14**, X=O) or phosphorofluoridothioate (**14**, X=S) diesters. In the instance of the monoesters **15**, the method involves oxidation of the appropriate H-phosphonates, H-phosphonothioates and H-phosphonodithioates with iodine in pyridine in the presence of trimethylsilyl chloride, followed by the addition of TAF. All transformations to **14** and **16** are rapid and practically quantitative.

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