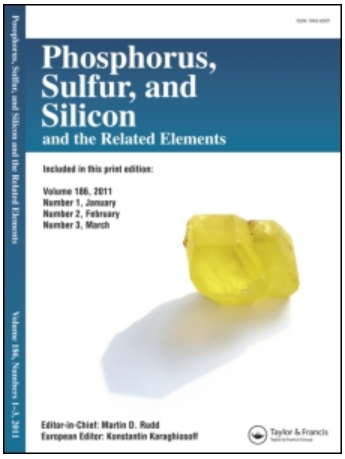

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EXPLORING REACTIONS OF NUCLEOSIDE H-PHOSPHONATES WITH BIFUNCTIONAL REAGENTS

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Abstract
Studies on reactions of nucleoside H-phosphonates with various amino alcohols showed that: (i) condensations of H-phosphonate monoesters with amino alcohols proceed with a complete chemoselectivity producing H-phosphonate diesters exclusively; (ii) H-phosphonate diesters undergo transesterification with amino alcohols and afford various products depending on the reaction conditions; (iii) the course of the oxidative coupling of nucleoside H-phosphonate diesters with amino alcohols can be controlled by protonation of the amino function, and thus the reaction can be steered to afford aminoalkyl phosphotriesters or hydroxyalkyl phosphoramidates.

INTRODUCTION

Rapid development of molecular medicinal diagnostic in recent years caused high demand for molecular probes enabling detection of specific gene sequences[1]. Due to some inherent problems connected with the handling of radioactive tracers, synthetic oligonucleotides equipped with various reporter groups detectable by fluorescent or enzymatic method are gaining interest[2]. In order to introduce to oligonucleotides a functional group amenable to the subsequent attachment of various reporter molecules, we have recently embarked on investigations of nucleotides modifications with bifunctional reagents (Fig. 1). In this paper we give a short account of our studies on reactions of amino alcohols, in which two nucleophilic centers are spaced by different numbers of methylene groups, with H-phosphonate mono- and H-phosphonate diesters.

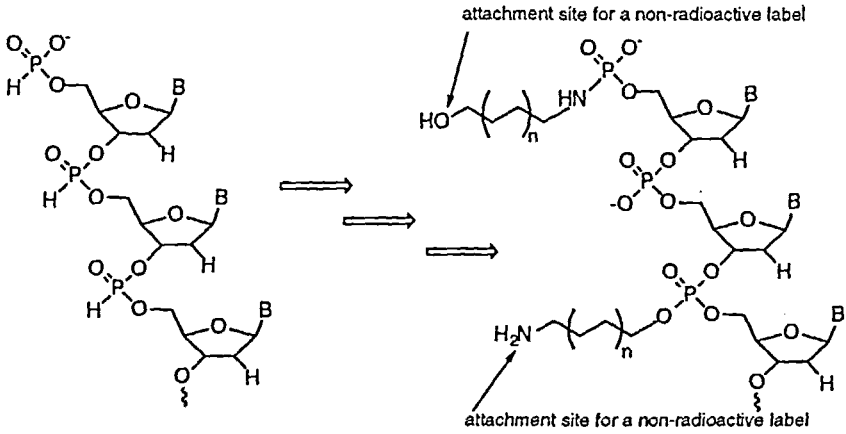


Fig. 1. Functionalization of oligonucleotides for the attachment of non-radioactive labels

RESULTS AND DISCUSSION

Condensations of nucleoside H-phosphonate monoesters with amino alcohols was investigated[3] using various condensing agents [pivaloyl chloride (PV-Cl), 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP)]. It was found, that irrespective of a coupling agent used, the reactions proceed with complete chemoselectivity affording the corresponding H-phosphonate diesters exclusively (Fig. 2). The chemoselectivity, which reflects a preferable attack of the O- *vs* N-nucleophile on the phosphorus centre of activated species derived from H-phosphonate monoesters may, in part, be enhanced due to a partial protonation of the amino group of amino alcohols under the reaction conditions. For synthesis of compounds of type **3** with a free amino function, NEP is recommended since the use of PV-Cl affords N-acylated compounds **2**. Similar results to those as in the reaction with NEP were also obtained with DCC (3 equiv.) and pyridinium chloride (3 equiv.) as a coupling system[4]. The reaction showed the same chemoselectivity, was clean and went to completion within 3 h.

Oxidation of H-phosphonate diesters **3** with iodine was studies under various experimental conditions[3]. In anhydrous media, the corresponding cyclic phosphoramidates or/and sym-pyrophosphates were formed, depending on the length of the methylene spacer in an alkyl residue. In the presence of water, oxidation of **3** (n=1-4) afforded exclusively acylic phosphodiesters with a free amino group in the alkyl chain. The exception was 2-aminoethyl nucleoside H-phosphonate **3** (n=0) which produced nucleoside 2-hydroxyethyl phosphoramidate, most likely *via* the 1,3,2-oxazaphospholidin-2-one intermediate.

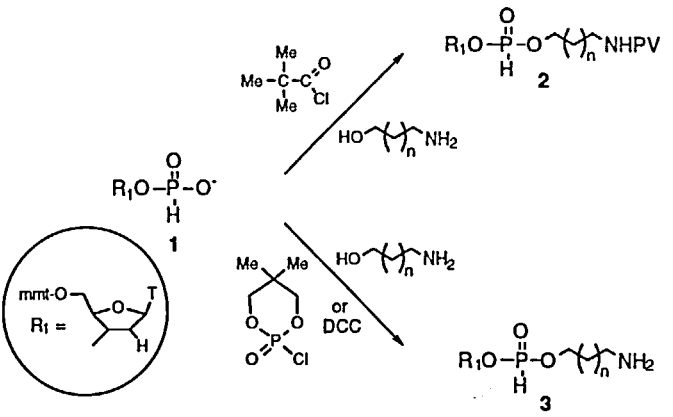
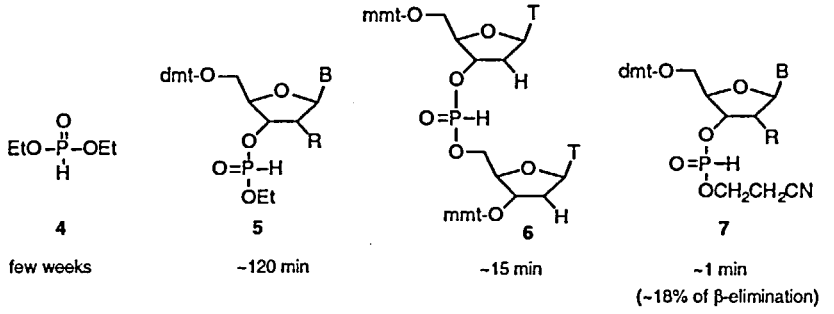


Fig. 2. Reactions of nucleoside H-phosphonate monoesters with amino alcohols

Reactivity of the -OH *vs* $-NH_2$ groups of amino alcohols toward the phosphorus centre in H-phosphonate diesters also was investigated[5,6]. The primary products of the reactions were found to be the mixed and the symmetrical H-phosphonate diesters. No evidence for the attack of the phosphorus centre by the amino group, which should result in the formation of H-phosphonamidates, was obtained. Since under these conditions mixture of products usually are formed, the reactions seem of less synthetic value for functionalization of oligonucleotides.

It is worth mentioning that among H-phosphonate diesters there are often significant differences in rates of transesterification with amino alcohols[6] (Fig. 3).



Reaction conditions: 10 equiv. of ethanoloamine in pyridine, RT. Time refers to a complete disappearance of the starting H-phosphonate diesters.

Fig. 3. Rates of transesterification of various H-phosphonate diesters with ethanoloamine

These one should bear in mind when oligonucleotides containing H-phosphonate bonds are subjected to various reactions on a solid support.

Our studies on the iodine promoted oxidative coupling of H-phosphonate diesters[7] with amino alcohols showed that in this reaction chemoselectivity (P-O *vs* P-N bond formation) can be controlled by protonation of the amino function (Fig. 4). This phenomenon can be exploited for functionalization of oligonucleotides by converting

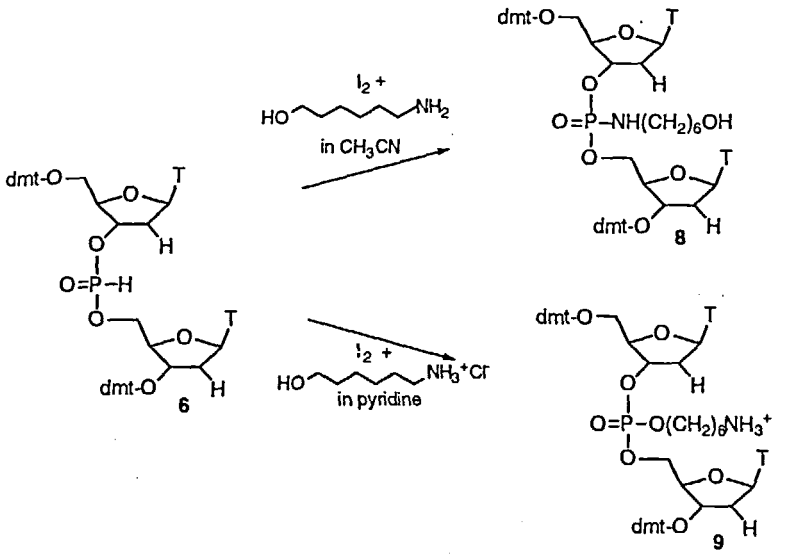


Fig. 4. Oxidative coupling of H-phosphonate diesters with amino alcohols

an H-phosphonate diester function into hydroxyalkyl phosphoramidate (**8**) or aminoalkyl phosphotriester (**9**). The transformation seems to be fully compatible with a machine-assisted solid phase synthesis of oligonucleotides and can be implemented as an optional step into the standard protocol.

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