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# ARYLSULFONYL CHLORIDES IN THE PRESENCE OF N-METHYLIMIDAZOLE AS EFFICIENT CONDENSING REAGENTS IN PHOSPHOTRIESTER OLIGONUCLEOTIDE SYNTHESIS

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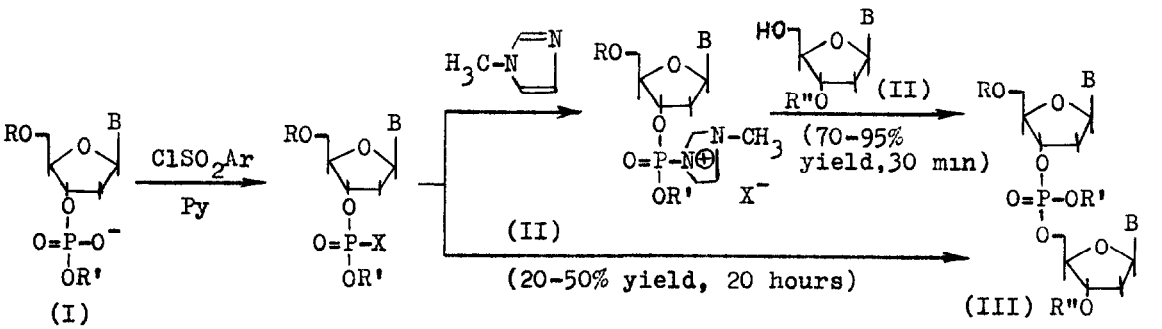
Summary: It was shown that arylsulfonyl chlorides in the presence of N-methylimidazole are powerful condensing agents for the triester internucleotide bond formation.

Nowadays the phosphotriester approach is most widely used in chemical synthesis of oligonucleotides needed for different molecular biology studies. One of the variants of this approach includes the activation of a nucleotide phosphodiester group with a suitable condensing agent and subsequent phosphorylation of hydroxy function to form a phosphotriester linkage[1]. Arylsulfonyl derivatives of azoles , such as 4-nitroimidazole, 1,2,4-triazole, 3-nitro-1,2,4-triazole and tetrazole, have been reported as the most suitable condensing agents for the triester approach[2-5]. However, none of these reagents proved to be completely satisfactory. Arylsulfonyl 4-nitroimidazoles and arylsulfonyl triazoles are rather slow in completing of the condensation reactions. Arylsulfonyl 3-nitro-1,2,4-triazoles and arylsulfonyl tetrazoles afford very rapid coupling rates, but these reagents cause various side reactions, especially modifications of guanine and other bases[4]. At the same time it was shown that arylsulfonyl chlorides are not so effective in the triester method due to long reaction times and low yield in condensation reactions[2]. In the course of our investigations on oligo- and polynucleotide synthesis we have found that arylsulfonyl chlorides are effective coupling reagents for the phosphotriester bond formation when nucleophilic catalyst, such as N-methylimidazole, is added to the reaction medium.

N-methylimidazole and 4-N,N-dimethylaminopyridine (DMAP) were used for the synthesis of short oligonucleotides with the aid of bifunctional phosphorylating reagents,namely aryl phosphoditriazolides to promote the reaction of phosphotriester bond formation[6,7]. Similarly, the addition of N-methylimidazole into a reaction mixture containing 3'-phosphodiester component (I), 5'-hydroxyl component (II) and arylsulfonyl chloride in pyridine accelerates the coupling reaction, and the reaction goes almost to a completion in 20-30 min. The yields of fully protected deoxyribodinucleotides with the use of this coupling reagent varied from 70 to 95%.

The use of arylsulfonyl chlorides in the presence of DMAP gave the same results in the synthesis of deoxyribodinucleotides with the acyl blocking group at 3'-hydroxyl terminus. Whereas the reaction of (I) with deoxyribonucleoside-3'-chlorophenyl $\beta$-cyanoethyl phosphate as a nucleoside component (II)

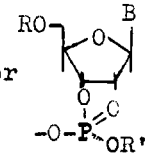



B = 6-N-benzoyladenın-9-yl, 4-N-anisoylcytosın-1-yl, 2-N-ısо-butyrylguanin-9-yl, thymin-1-yl

R = $PhC(C_6H_4OMe\text{-}p)_2$-

R'= $Cl\text{-}C_6H_4$-

R"= Bz or $ClC_6H_4O(O)POCH_2CH_2CN$

X =-$OSO_2Ar$ or 

resulted ın a partial $\beta$-elımination of cyanoethyl protecting group ( up to 40%) along with the coupling products formation.

Using 2,4,6-trііsopropylbenzenesulfonyl chloride (TPSCl) and N-methylimidazole as a coupling reagent, we have synthesized more than 20 deoxyribooligonucleotides of chain length between 10-16 nucleotides. The coupling reaction of 3'-phosphodiester component (1.2-1.5 mmole) with 5'-OH-component (1 mmole) in dry pyridine or dioxane[8] (10 ml per mmole) was performed in the presence of TPSCl (2.5-3.0 mmole) and Me-imidazole (5.0-9.0 mmole) during 20-30 min. After the addition of $H_2O$ the reaction mixture was evaporated to dryness, dissolved in chloroform,and the desired product was isolated by a sılica gel column chromatography using methanol concentration gradient ın chloroform as an eluent. The yıelds of coupling products on each condensatıon stage in the synthesis of these oligomers varied from 50 to 95%. The ısolation of the final oligonucleotides after removal of the protectıng groups was performed by anıon exchange chromatography. In most cases the maın peak was the desired product. The purıty of each olıgonucleotıde was checked by a hıgh pressure reversed phase chromatography. The prımary strucrure of the synthesized oligomers was analysed by a two-dımensional sequence analysıs procedure.[9]

The comparison of the 5'-hydroxyl component (II) sulfonatıon rates usıng trıisopropylbenzenesulfonyl tetrazolıde (TPSTe) or a mıxture of TPSCl and Meımidazole has shown that in the first case the reactıon proceeds faster. In normal coupling reaction condıtıons, ın the presence of a slıght excess of (I), TPSTe gave about a 5% 5'-O-sulfonated by-product ın one hour, whereas TPSCl + Me-imıdazole gave slıghtly less (2-3%). We have observed the same extent of sulfonation wıth the use of TPSCl + tetrazole (Te) mıxture whıch was recently suggested as a condensıng agent for the phosphotrıester approach[10].

In order to carry out the direct comparison of our method to the published procedures[3,10] in the synthesis of specific deoxyribonucleotide sequences we have performed synthesis of four different 16-mers: dGpTpTpCpCpApCpApApTpGpCpCpApCpG (IV), dTpGpCpApGpTpApGpTpTpCpTpCpCpApG (V), dTpCpCpGpCpTpTpCpApCpGpApCpGpCpG (VI) and dCpApCpApCpCpApGpGpTpApCpApGpApG (VII). Initially, the desired oligonucleotides were synthesized by us using TPSTe, or TPSCl + Te, as a condensing agent. Then we have obtained the same oligonucleotides with the use of TPSCl + Me-imidazole. In all the experiments the reaction conditions (starting block amounts, excess of condensing agents, reaction times, etc.) and purification procedures were similar. The oligonucleotide chains were elongated by successive addition of di-, tetra- and octanucleotide blocks to 3'-terminal dinucleoside monophosphates. The yields of phosphotriester intermediates and the fully protected desired compounds prepared by our procedure were similar to the yields of the oligonucleotides prepared by the published procedures, or slightly higher (Table 1). However, the reversed-phase HPLC analysis of the final 16-mers after removal of the blocking groups and purification by anion exchange HPLC have revealed that the compounds synthesized with the use of TPSCl + Me-imidazole were essentially higher in purity (Fig. 1)[11].

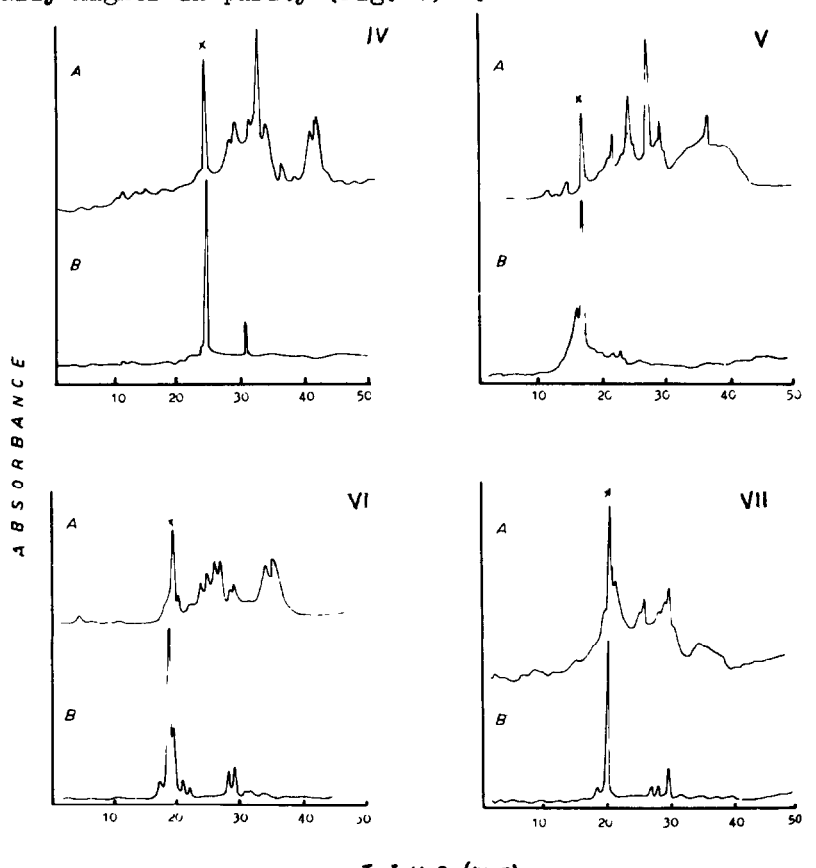



Figure 1.
Reversed-phase HPLC analysis of hexadecanucleotides IV - VII on Zorbax C-8 column. Column was eluted at flow rate of 1ml/min with 5%-35% methanol gradient in 0.1 M ammonium acetate at 50°. Peaks corresponded to the desired 16-mers are indicated by (x). (A)- Coupling agent TPSTe (V,VII) or TPSCl + Te (IV,VI). (B)- Coupling agent TPSCl + Me-imidazole.

Table 1. Overall yields of hexadecadeoxyribonucleotides [a].

| Oligonucleotide | Yield before deprotection,%[b] | | | Yield after deprotection and reversed-phase HPLC,%[b] | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| IV | | 43 | 40 | | 1.8 | 14 |
| V | 38 | | 42 | 1.3 | | 10 |
| VI | | 20 | 25 | | 0.7 | 5.5 |
| VII | 21 | | 28 | 1.0 | | 6 |

[a] Based on 3'-terminal dinucleoside monophosphate.
[b] Coupling agent (1)- TPSTe, (2)- TPSCl + Te, (3)- TPSCl + Me-imidazole.

The results obtained in this work indicate that TPSCl in combination with N-methylimidazole are highly effective condensing reagent for the triester approach with respect to the time of reaction and yields. It must be noted that, in contrast to TPSTe, both TPSCl and N-methylimidazole are stable and commercially available substances which can be stored for a long time at room temperature. Furthermore, the oligonucleotides obtained using TPSCl + Me-imidazole contain a small amount of by-products, especially of modifications of guanine and other bases. As a result the overall yields of the oligonucleotides synthesized by this method are higher than those with TPSTe, or TPSCl + Te (Table 1). The method appeares extremely promising for the synthesis of oligonucleotides with chain length of more than 12 monomeric units.

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When a 1:3 mixture of TPSCl and tetrazole in dry pyridine was used as a coupling reagent, we observed the in situ formation of TPSTe with the yield of 10-15%.
11. As it was shown by two-dimensional sequence analysis, the substances eluted from Zorbax C-8 column after the desired oligonucleotide represent oligomers of the same chain length containing various modifications of heterocyclic bases.

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