Tetrahedron Letters No. 28, pp 2881 - 2884, 1972. Pergamon Press. Printed in Great Britain.

POLYMERS AS CHEMICAL REAGENTS. THE USE OF POLY(3,5 DIETHYLSTYRENE) - SULFONYL CHLORIDE FOR THE SYNTHESIS OF INTERNUCLEOTIDE BONDS (1)

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(Received in UK 31 May 1972; accepted for publication 7 June 1972)

Insoluble polymeric reagents were shown to be effective in transfer reactions of the type Ⓟ- A + B → Ⓟ + A-B. Where Ⓟ - represents a polymeric leaving group and B a nucleophile. Ⓟ- A can be taken in large excess over B, thus increasing the yield of the product A-B. Then, A-B is isolated by filtration of the polymer. This priciple was demonstrated in peptide synthesis (2).

The chemical synthesis of oligonucleotides, namely condensing monoesters of phosphoric acid with alcohols to give diesters of phosphoric acid has been effected by two methods according to the following scheme:

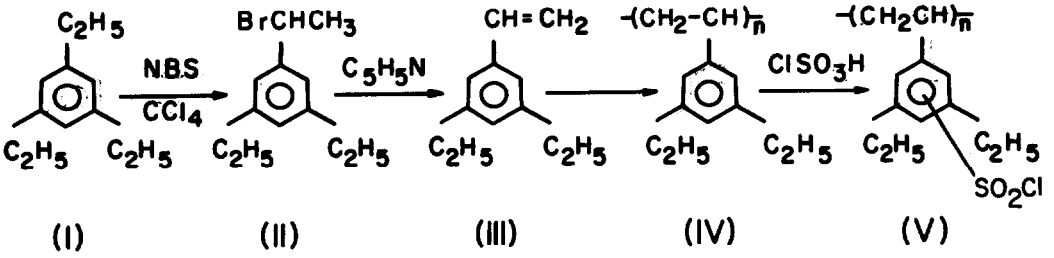
a. $ROPO_3H^- + R'OH + ArSO_2Cl \xrightarrow[2.H_2O]{1.C_5H_5N} ROPO_2^-OR' + ArSO_3H + HCl$ (3), (4).

b. $ROPO_2^-OX + R'OH + ArSO_2Cl \xrightarrow[2.H_2O]{1.C_5H_5N} ROPO(OX)OR' + ArSO_3H + HCl \xrightarrow{OH^-} ROPO_2^-OR'$ (5).

(Dicyclohexylcarbodiimide can replace $ArSO_2Cl$ in method a. but with poorer results, X in method b. represents a base labile protecting group), the activation of the phosphates in both methods is effected by sterically hindered aromatic sulfonyl chloride like mesitylenesulfonyl chloride and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS). These reagents have two disadvantages: 1. In spite of the steric hindrance, some sulfonation of R'OH does occur, leading to undesired sulfonate esters. 2. In many cases it is quite difficult to remove completely the sulfonic acid from the desired product.

It seemed to us that by using a polymeric arylsulfonyl chloride one might overcome these problems. The sulfonate esters would remain attached to the polymer and thus be easilly removable together with the polymeric sulfonic acid.

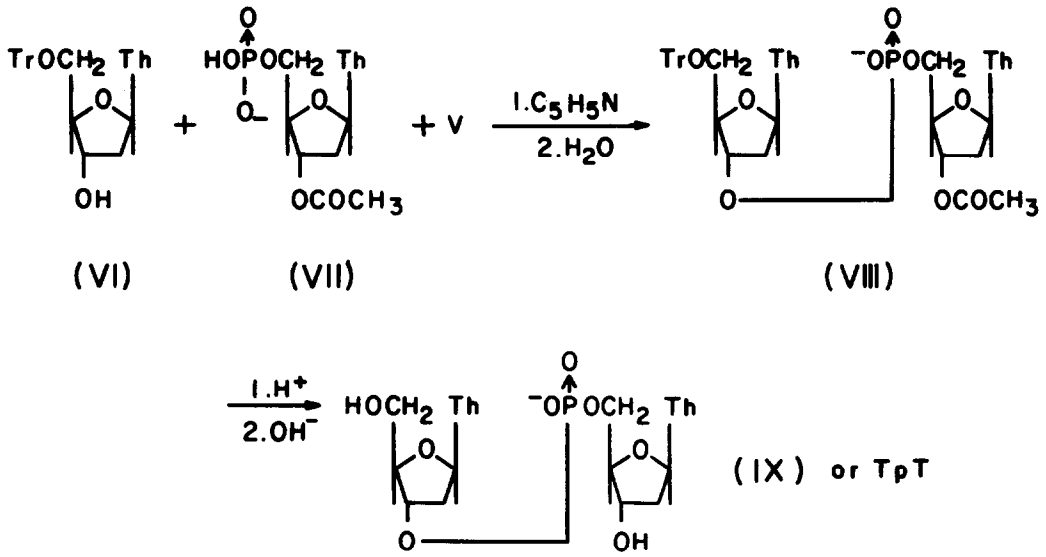
Thus the synthesis of poly (3,5 diethylstyrene) sulfonyl chloride was undertaken. When sym. triethylbenzene (I) was treated with N-bromosuccinimide (NBS), α bromoethyl 3,5 diethylbenzene (II) was formed. (B.P. 106°/1.5 mm. NMR δ 1.2, 2.6 for ethyl protons, 2.0 for methyl protons α to the bromine, 5.0 for the proton vicinal to the bromine and 6.85, 6.95 for the aromatic protons. Anal. calcd. for $C_{12}H_{17}Br$: Br. 33.3%. Found: Br. 33.0%). The bromide (II) was refluxed in pyridine to give 3,5 diethylstyrene (III). (B.P.85°/4mm. NMR δ 5.4, 6.6 for vinyl protons, the absorbtions of the ethyl and aromatic protons were the same as in II). The styrene (III) was copolymerised with 5% divinylbenzene as a cross linking agent. A 0.25% aqueous solution of polyvinylalcohol was used as a medium for the bead polymerisation. The cross linked polymer was obtained in bead form and was chlorosulfonated by chlorosulfonic acid in chloroform to give the totally chlorosulfonated polymer (V). (Anal. calcd. for $(C_{12}H_{15}SO_2Cl)_n$: S.12.3%, Cl 13.7%. Found: S.12.1% Cl. 13.2%). No attempt was made to determine the position of the SO_2Cl group on the aromatic ring. However in both possibilities the SO_2Cl group is sterically hindered.



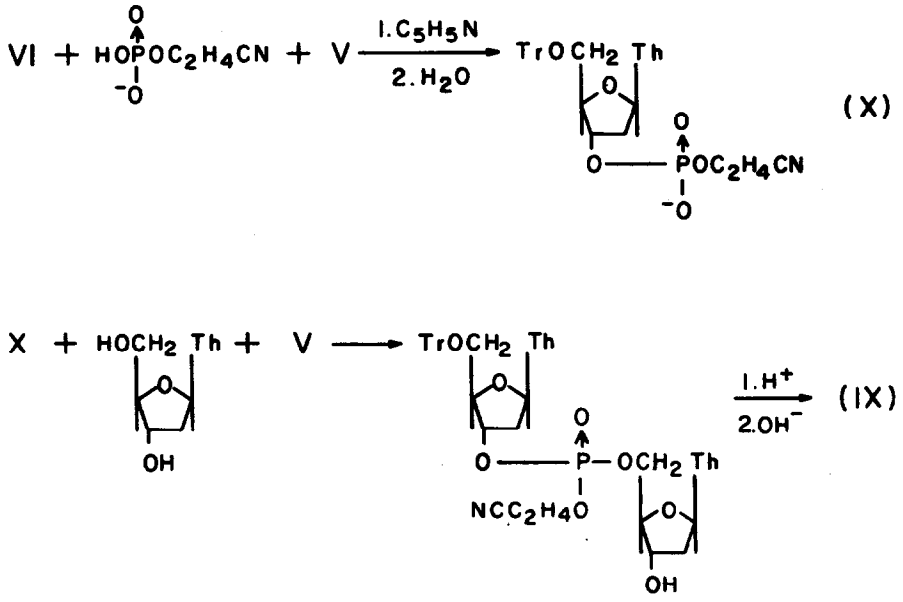
Scheme 1. The preparation of poly (3,5 diethylstyrene) sulfonyl chloride.

The synthesis of a dinucleoside phosphate by this polymer was then undertaken and compared with the conventional methods. 5' Trityl thymidine (1 eq.) and 3' acetyl thymidine 5' phosphate (2 eq.) were treated with 3 eq. of polymer V in dry pyridine overnight. Water then was added and after 24 hrs. the polymer was filtered. The protecting groups were removed according to the literature, the reaction mixture was chromatographed and the yield of TpT in the condensation step was 90% based on VI as determined spectrophotometrically. The synthesis is described in scheme 2.

TpT was obtained also by method b. in a two stage synthesis: VI (1 eq.) and pyridinium β cyanoethyl phosphate were treated with polymer V (3 eq.) in the usual manner. X was isolated by filtration, extracted with $CHCl_3$ and reacted in the second stage, with Thymidine (2 eq.) and polymer V (3 eq.) in dry pyridine overnight. The yield of TpT based on X was 70%, determined as before. The synthesis is described in scheme 3. TpT was degradable by snake venom phosphodiesterase to thymidine and 5' TMP; T/pT=1.03 .



Scheme 2. The synthesis of TpT by polymeric reagent according to method a.
(Tr = trityl, Th = Thymin)

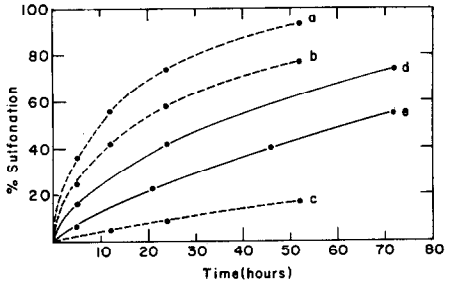


Scheme 3. The synthesis of TpT by polymeric reagent according to method b.

The results were compared with those of the conventional methods. The yields and rate of the reactions were found to be similar, but when TPS was used, triisoprophylbenzenesulfonic acid contaminated the products, as could be seen by TLC and spraying with an acid base indicator.

The sulfonic acid. owing to its detergency, caused stable emulsions with chloroform when the later was used for extracting the products. These problems were completely eliminated when polymer V was used instead of TPS. Moreover, contrary to the TPS procedure, the reaction mixtures described in scheme 3, remained colourless and no sulfonate esters or other impurities were detected on TLC.

Finally. the rate of sulfonation of primary alcohols by polymer V was measured and compared with other arylsulfonyl chlorides. The results of the sulfonation are summarised in Fig. 1.



a. Tosyl chloride. b. Mesitylenesulfonyl chloride. c. TPS. d. Copolystyrene, 2% Divinylbenzenesulfonyl chloride. e. V.

Fig. 1. The rate of sulfonation of thymidine and 2'3'dibenzoyluridine (broken lines) by different arylsulfonyl chlorides. The conditions and results with 2'3'dibenzoyluridine are taken from (3).

The rate of sulfonation by polymer V was close to that of TPS. Therefore the polymer is suitable for oligo nucleotide synthesis. The synthesis of longer oligo nucleotides by this approach is under investigation.

References

1. This work has been supported by Grant No. AM 05098 from the National Institute of Health.
2. M. Fridkin, A. Patchornik and E. Katchalsky, J. Amer. Chem. Soc., 90, 2953 (1968).
3. R. Lohrmann and H.G. Khorana, J. Amer. Chem. Soc., 88, 829 (1966).
4. H.G. Khorana et, al., Nature, 227, 27 (1970) and references cited in.
5. R.L. Letsinger and K.K. Ogilvie, J. Amer. Chem. Soc., 91, 3350 (1969).