Tetrahedron Letters No. 16, pp 1535 - 1538, 1972. Pergamon Press. Printed in Great Britain.

POLYMER SUPPORT OLIGONUCLEOTIDE SYNTHESIS VIII [1)]
USE OF POLYETHYLENGLYCOL

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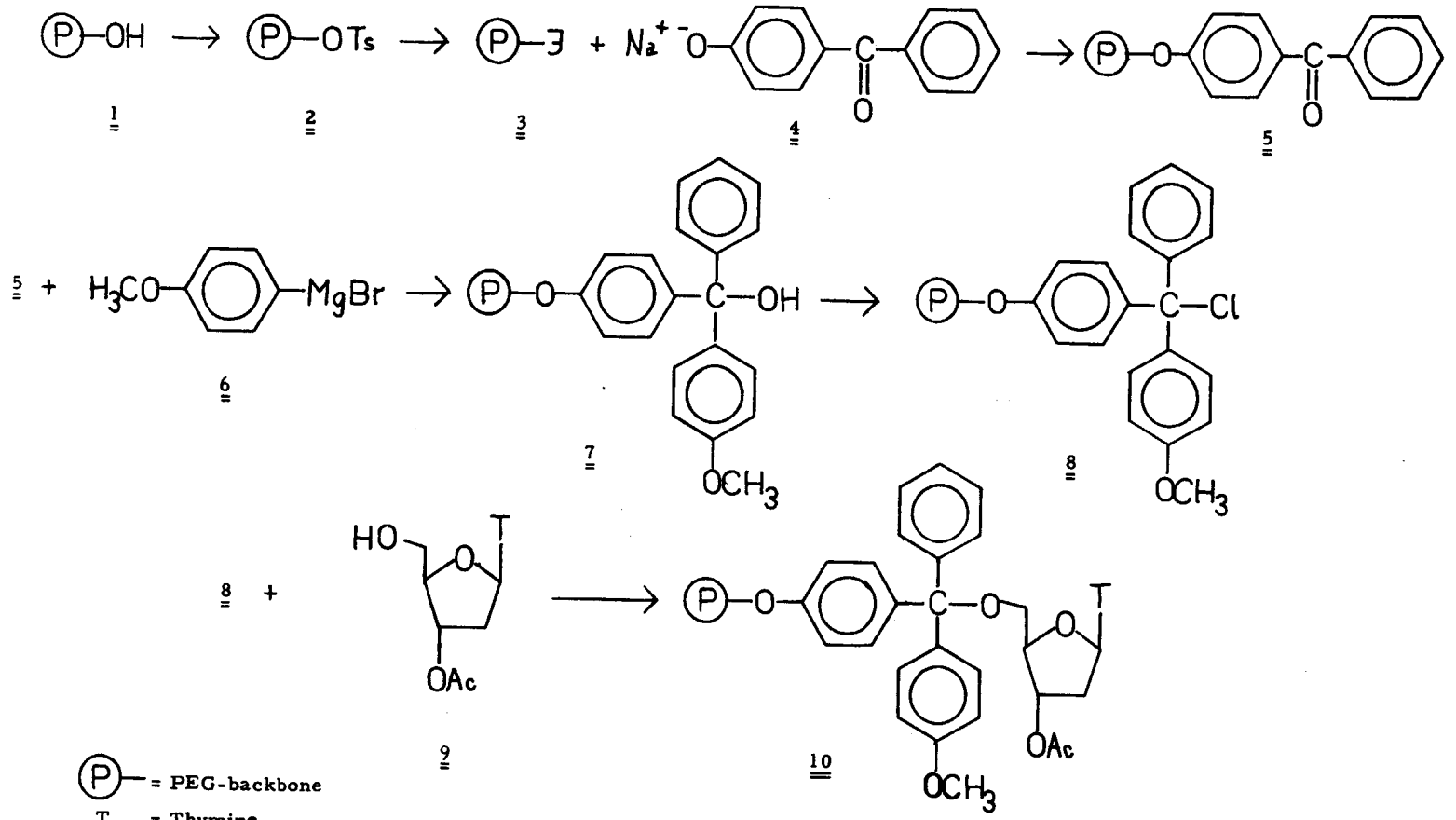
(Received in UK 28 February 1972; accepted for publication 8 March 1972)

In our previous paper [1, 2)] we described the use of hydrophylic polymeric carriers for oligonucleotide synthesis on polymer supports which are completely insoluble. In these kinds of polymers which are swellable [1)] or of permanent porosity [2)], diffusion problems might occur. Besides this - on steric reasoning - the yield of a condensation reaction might be reduced in consequence of the bulkiness of the phosphorylating species. The same holds for the stepwise enzymatic synthesis on polymeric carriers [3, 4)], where the active centre of the enzyme has to come into correct contact with one substrate being bound to the polymeric carrier. In order to circumvent this problems we selected polyethylene glycol (PEG) of molecular weight 20 000 as carrier acting as a macromolecular blocking group. Very recently PEG was introduced for the solid phase peptide synthesis, but only a few experimental details were given [5)]. For the synthesis of oligonucleotides this carrier has the advantage that it is soluble in water, pyridine and dioxan. Because of this the condensation reaction and all selective deprotections of blocking groups can be done in homogeneous reactions and the yield of the condensation reaction can be quickly measured by simply dissolving the polymeric carrier with its bound oligonucleotide chain in water or buffer and measuring the absorbance in a quartz vessel. The yield of the synthesis for short oligonucleotides , using all the four nucleotides occuring in DNA can be calculated by the relation of the different extinction coefficients at the λ_{max} of the synthesized chain. Thus loss of material for the determination of the yield in a given condensation reaction can be avoided No time consuming cleavage of nucleotidic material is necessary

An excess of the low-molecular-weight component can be used in order to drive the reactions to completion, as it can be removed by dialysis under very mild conditions; the condensation product which is bound to the carrier will remain in the dialysis tube, while the low-molecular-weight material will penetrate to the surrounding liquid. As dialysis can be done in buffer systems at low temperature very sensitive oligonucleotides can be synthesized without any damaging effects.

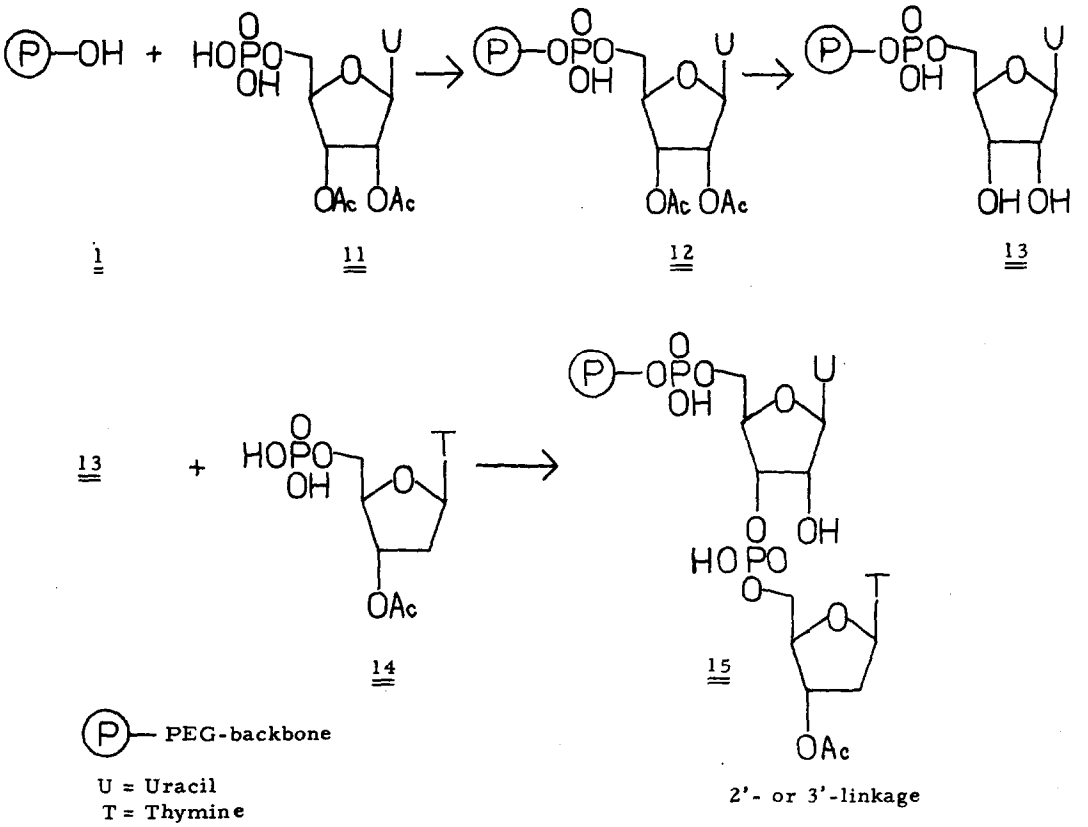
In order to anchor the oligonucleotide chain to PEG we first tried two methodes for the chemical synthesis (for enzymatic synthesis, see [3, 4)])



P— = PEG-backbone
T = Thymine
Ac = Acetyl
Ts = Tosyl

a) Monomethoxytrityl group as anchor : The monomethoxytrityl group was introduced in a reaction sequence which is shown in the accompanying chart. 1 could be tosylated in quantitative yield (2 g 1, 4 mmol tosyl chloride, 2 hrs , 22°) ; this could be established by elementary analysis and the resistance of the product towards acetylation (acetylation can easily be detected by infrared spectroscopy). 2 could be converted into 3 with an excess of sodium iodide in acetone (24 hrs., reflux). 3 is dissolved in tetrahydrofuran (THF) and can be coupled with the sodium salt of p-hydroxy-benzophenone (4), which is freshly generated with sodium methoxide in absolute methanol (18 hrs., reflux ; C = O 1660 cm^{-1}). The usual work up is to precipitate all PEG-bound material and inorganic salts with ether, chloroform, benzene or petrolether in the cold, to remove low-molecular-weight organic molecules and to dialyze the precipitate against water or buffer. The content of the dialysis tube is then lyophylized and is ready for the next reaction. 5 is now converted in a Grignard reaction with p-bromoanisol in THF (2 hrs., reflux) to give 7 The conversion can be shown by the absence of the C=O band at 1660 cm^{-1} and by the color test with 10% perchloric acid. All trityl groups are bound to the polymeric carrier, as can be shown by tlc on silica gel (chloroform/methanol = 9 : 1, v/v); the only trityl positive spot remains at the origin. Conversion of 7 to 8 is possible using benzene/acetic acid chloride (6 hrs., 70°) and by precipitating 8 with petrol ether (60 - 70°). 3'-O-Acetyl-thymidine could be bound to 8 in a yield of 28.7 µmol/g (10). After dialysis all UV-absorbing material remains at the origin using the above mentioned chromatographic system.

b) Uridine as anchor : The use of uridine as anchor has already being described using Sephadex LH 20 as carrier [1]. It is introduced in a condensation reaction between 1 and 2', 3'-O-acetyl-uridine-5'-phosphate (11) using triisopropylbenzene sulfonylchloride (TIPS) as condensing agent (1 g 1, 0.2 mmol 11, 0.5 mmol TIPS, anhydrous pyridine, 20 hrs., 22°). After extensive dialysis a sample was dissolved in water and the extinction determined : 78.2 µmol 11 is bound/g carrier. In order to protect all OH-groups which might not have reacted in the condensation reaction, 12 is reacted with an excess of ethyl vinylether to give an alkali stable but acid labile blocking group [6], and which will alter the properties of the carrier only to a very slight extent. The 3'-O-acetyl groups were removed by dissolving 12 in 0.1 N sodium hydroxide (1 hr., 0°); this reaction can be followed qualitatively by infrared spectroscopy. In a subsequent condensation step the first deoxynucleotide 3'-O-acetyl-thymidine-5'-phosphate (14) (0.1 g 13, 0.2 mmol 14, 0.5 mmol TIPS, 4 hrs., 22°) can be coupled to the carrier. After dialysis and lyophyllization a sample is dissolved in trisbuffer 0.1 M pH 7.5 which leads to 127.5 µmol nucleotidic material/g (λ_{max} = 265 nm) ; from this result a greater than 98% conversion of 13 to 15 can be calculated (ε_{265} of uridine : 8200, ε_{265} of thymidine : 9400). After protection of the free OH-group of the uridine moiety with ethyl vinylether, the 3'-O-acetylgroup can be removed and the next condensation step can take place.



The chemical and enzymatic synthesis of oligonucleotides using this carrier is now under extensive investigation in this laboratory [3, 4].

Acknowledgement : The author wishes to thank Professor K. Heyns for his interest and the Deutsche Forschungsgemeinschaft for financial support. The excellent technical assistance of Mrs. C. Woldtmann is gratefully acknowledged.

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