Don Dimmel of Purdue University for the mass spectral data, and to Mr. Richard N. McCarty for carrying out some of the initial synthetic experiments.

(11) Address correspondence to the Department of Chemistry, University of Arkansas, Fayetteville, Ark. 72701.
(12) U. S. Government Grantee, 1960–1962, administered by the Institute of International Education.

Walter L. Meyer,[11] Angelo P. Lobo[12]
Department of Chemistry, Indiana University
Bloomington, Indiana
Received March 26, 1966

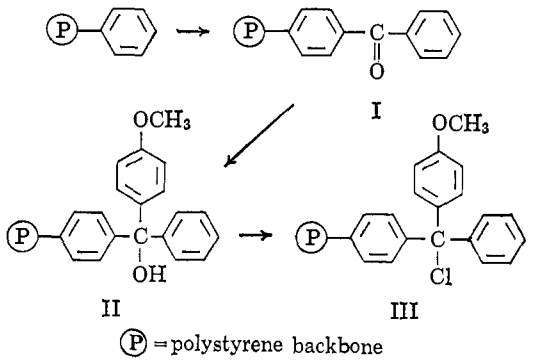
Deoxyribooligonucleotide Synthesis on a Polymer Support[1]

Sir:

The principle of carrying out reactions between reactants when one of these is provided in a readily separable form has been expressed in literature from time to time[2] and it has recently been developed with striking success by Merrifield for the synthesis of polypeptides.[3] In the Merrifield procedure, a polypeptide chain is built up in a stepwise manner from one end while it is linked by a covalent bond at the other end to an *insoluble* polymeric support. Polypeptide synthesis on a polymer support has been developed more recently in an alternative form by Shemyakin and coworkers[4] in which the polymer, polystyrene, supporting the growing peptide chain is actually soluble in the medium of reaction, and therefore the repetitive condensations are performed in completely homogeneous phase. The concept of polymer support synthesis could in principle lend itself to work in the polynucleotide field, and one synthetic approach embodying this principle has already been outlined by Letsinger and Mahadevan.[5] We have also been investigating for some time various means of carrying out deoxyribopolynucleotide synthesis on a polymer support and herein describe an approach which is among those that we have investigated.

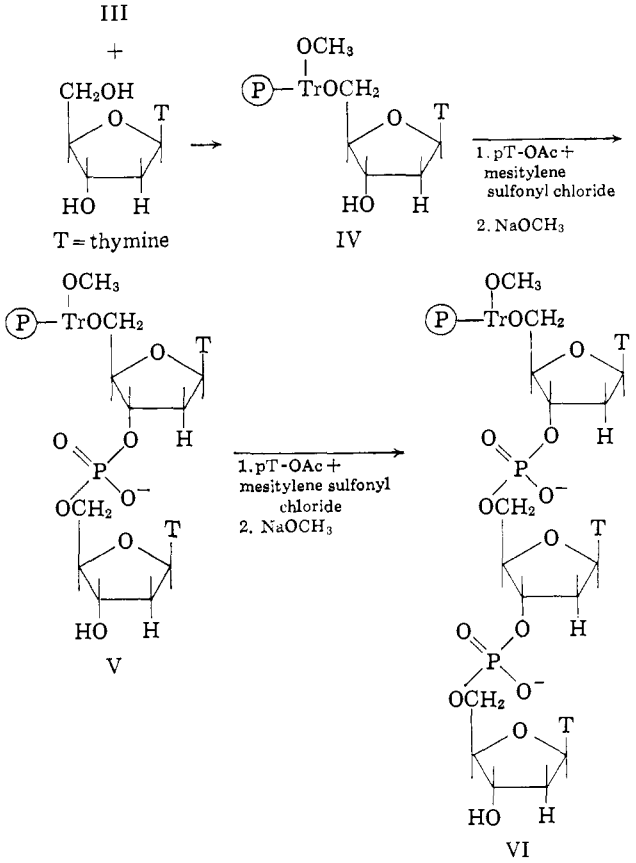
Preparation of Polymer. In concept, the approach is akin to that used previously by Shemyakin, *et al.*, in the peptide field. The starting material in our work was polystyrene of average molecular weight 270,000[6] and the steps used in preparation of the appropriate derivative are shown in Chart I. The polystyrene (10 g) was subjected to a Friedel–Crafts reaction with benzoyl chloride (51 mmoles) and aluminum chloride (51 mmoles) in carbon disulfide. The product (I; 13.2 g, λ_{max} 257 mμ in dioxane) was isolated as a yellowish resinous powder. I (2 g) was allowed to react in benzene with *p*-methoxyphenylmagnesium bromide (4 mmoles of an ethereal solution) to give the substituted trityl alcohol (II; 2 g) which gave coloration characteristic of *p*-methoxytrityl cation on addition of perchloric acid.[7] Treatment of II (1.2 g) with acetyl chloride in cyclohexane gave the monomethoxytrityl chloride (III; 1.1 g) which was isolated as a fluffy white powder. Determination of chloride ions released on treatment with methanol and pyridine showed the reactive halogen content to be 0.4 mmole/g of the derivatized polymer.

Chart I. Preparation of Polystyrene-Supported *p*-Methoxytrityl Chloride



Deoxyribooligonucleotide Synthesis. The typical steps used are illustrated in Chart II. Thus trityl chloride (III), 500 mg, was allowed to react with thymidine (70 mg) in pyridine (6 ml) first at room temperature for 12 hr and then at 65–75° for 30 min. Methanol (1 ml) was then added and, after 30 min at 75°, pyridine (5 ml) was added and the reaction mixture was poured

Chart II. Deoxyribooligonucleotide Synthesis Using Polystyrene-Supported Methoxytrityl Chloride (III)



VI $\xrightarrow{\text{TFA in CHCl}_3}$ thymidylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine

(1) This work has been supported by grants from the National Science Foundation (Grant No. GB-3342), the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178), and the Life Insurance Medical Research Fund (Grant No. G-62-54).
(2) See, for example, J. J. Cebra, D. Givol, H. I. Silman, and E. Katchalski, *J. Biol. Chem.*, **236**, 1720 (1961).
(3) R. B. Merrifield, *Science*, **150**, 178 (1965).
(4) M. M. Shemyakin, Y. A. Ovchinnikov, A. A. Kinyushkin, and I. V. Kozhevnikova, *Tetrahedron Letters*, 27, 2323 (1965).
(5) R. L. Letsinger and V. Mahadevan, *J. Am. Chem. Soc.*, **87**, 3526 (1965).
(6) This preparation (Lot No. 683) and several others with different average molecular weights were kindly supplied by Dr. E. T. Dumitru of Dow Chemical Co., Midland, Mich.
(7) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3821 (1963).

into water (200 ml) with stirring. The precipitate (dry weight, 436 mg) was collected by filtration and thoroughly washed with water. The amount of thymidine in this product was determined by treatment with trifluoroacetic acid (TFA) in chloroform (1:99, v/v) at 0°, under which conditions thymidine release was complete in under 5 min. The amount of thymidine in IV was thus determined to be 6 μmoles/100 mg of IV. By a similar procedure polymer-supported 5′-O-monomethoxytrityl-N-benzoyldeoxyadenosine was prepared from III, the adenine content being 2.9 μmoles/100 mg of the product.

For internucleotide bond synthesis, IV (190 mg) was allowed to react with pyridinium 3′-O-acetylthymidine 5′-phosphate (pT-OAc, 80 mg) and mesitylene sulfonyl chloride (77 mg) in dry pyridine (2.5 ml) at room temperature for 3.5 hr. (More pyridine (2 ml) was added after 1.5 hr.) After treatment with water (0.4 ml) and pyridine (2.5 ml) at room temperature for 15 hr, the clear reaction solution was poured into 500 ml of 2% aqueous sodium chloride solution. The precipitate was collected by filtration, washed with water, and dried over phosphorus pentoxide. The 3′-O-acetyl group was removed with 1 *M* sodium methoxide in methanol–dimethyl sulfoxide–pyridine (1:5:4, v/v, 20 ml; 15 min at room temperature). The alkaline solution was poured into 500 ml of 2% aqueous sodium chloride and the resulting precipitate (178 mg) of V was collected by centrifugation followed by filtration and washing with water. Subsequent treatment with TFA–chloroform mixture followed by paper chromatographic analysis[8] showed thymidylyl-(3′→5′)-thymidine (TpT) and thymidine as the only products, the yield of TpT being 96%. In a similar manner, Ⓟ-d-TpG^{Ac}-OAc[9] and Ⓟ-d-TpC^{An}-OAc[9] were synthesized in yields of 92 and 91%, respectively.

Repetition of the condensation of V (125 mg) with pT-OAc (200 mg) and mesitylenesulfonyl chloride (200 mg) in pyridine (2.5 ml) was performed at room temperature for 2.75 hr. Aqueous pyridine treatment at room temperature for 17 hr, precipitation in 2% aqueous sodium chloride, sodium methoxide treatment at room temperature for 12 min, and final precipitation in 2% aqueous sodium chloride yielded VI (105 mg). TFA–chloroform liberated mostly TpTpT (VII), some TpT, and a little T. TpTpT contained two trace contaminants (pT, 1.7%, and another nucleotide, 1.9%) as shown by subsequent electrophoresis. The yield of pure TpTpT from TpT was 87%.

All of the products, TpT, d-TpG, d-TpC, and TpTpT, were checked for their purity in paper chromatography (two solvents) and paper electrophoresis and were completely susceptible to the action of spleen phosphodiesterase, thus showing the exclusive presence of $C_{3'}$–$C_{5'}$ internucleotidic linkages in them.

The approach described incorporates all of the principles of the methods previously developed for construction of deoxyribopolynucleotide chains containing predetermined sequences. For example, the 5′-hydroxyl group of the terminal nucleoside is protected by a substituted trityl group and the chain elongation occurs by successive condensations with the terminal 3′-hydroxyl group. An important requirement is that yield at every repetition of internucleotide bond synthesis be as nearly quantitative as possible, and this appears to be the case in the several examples studied so far. Experiments on the use of this approach in the synthesis of longer oligonucleotide chains are in progress.

Hikoya Hayatsu, H. G. Khorana
Institute for Enzyme Research, University of Wisconsin
Madison, Wisconsin
Received May 31, 1966

(8) Using the solvent: 2-propanol–concentrated ammonia–water (7:1:2, v/v).

(9) Abbreviations for the protected deoxyribooligonucleotides are as defined previously: H. Shaller and H. G. Khorana, *J. Am. Chem. Soc.*, 85, 3841 (1963).

Book Reviews

An Introduction to Mathematical Crystallography. By M. A. Jaswon, M. A., Ph.D., Reader in Mathematics, Imperial College, London. American Elsevier Publishing Co., Inc., 52 Vanderbilt Ave., New York, N. Y. 1965. xi + 125 pp. 14.5 × 22 cm. $6.00.

The term "Mathematical Crystallography" in the title of this book is taken by the author to refer to the analysis of the symmetry properties of periodic structures in three dimensions, *i.e.*, the 32 point groups and the 230 space groups. In the preface the author states, "Although the theory was completed by 1890, and no new results can be expected, considerable scope still remains for fresh presentations and interpretations." He also states that, "The approach...steers a middle course between excessive formalism and excessive perspective geometry...".

In the course of 103 pages plus 21 pages of appendices, the author develops the point groups and space groups in a more or less systematic manner. After a fairly normal introduction of the point groups he develops the space groups from the "motif" point of view of Bravais. This took courage on the part of the author since it was perhaps this point of view which held back the development of the complete theory of space groups for about 40 years. It does give one a mass of material on the arrangement of point group replicas in space. From this material he develops the ideas of screw axes and glide planes, using an interesting notation for the treatment of translation operators. His discussion of the "diamond glide" space groups is very brief and probably needs a good deal of expansion to make it understandable to a student.

The chief difficulty the reviewer has had in understanding this book lies in the way that the author has chosen the "middle course" referred to above. He has given a development of the point groups "based upon intuitive geometrical considerations." This, one has to do, but to omit the basic postulates and theorems of group theory from this book on the grounds that they "may be found in numerous texts" is inexcusable.

One is grateful that the author has used the Hermann-Maugin notation for the point and space groups which has been adopted internationally for 30 years. It is however somewhat unfortunate that the operation $\bar{3}$ is first presented in this book as a sixfold rotatory reflection and not as a threefold rotatory inversion as was intended by those who developed the notation.