

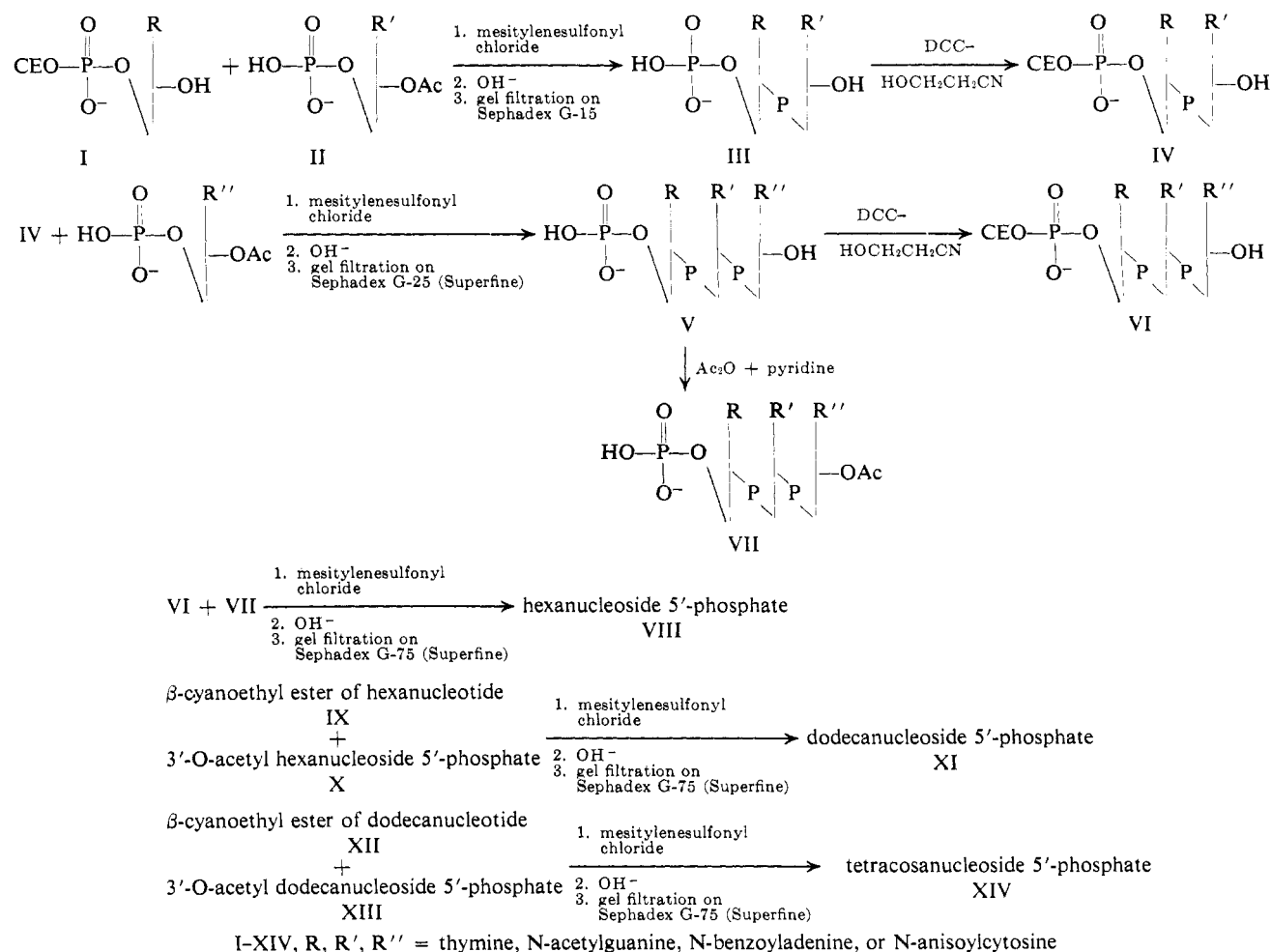
A New General Method for the Synthesis of Deoxyribopolynucleotides Bearing a 5'-Phosphomonoester End Group

Sir:

In recent years two approaches have been developed for the chemical synthesis of deoxyribopolynucleotides, (1) stepwise condensation¹ and (2) polymerization.²

filtration, e.g., Sephadex gels with appropriate exclusion limits. An attractive feature of this separation technique is that the product peak emerges from the column before the peak containing starting material. (2) A minimum number of condensation steps is required (except for completely nonrepeating sequences). (3) The presence of a 5'-phosphomonoester group on each fragment offers more flexibility in extending the chain

Chart I. General Method for the Synthesis of Deoxyribonucleotides Bearing a 5'-Phosphomonoester End Group



In this communication we wish to introduce "fragment coupling," a new general approach to the synthesis of well-defined deoxyribopolynucleotides. The basic principles are that the chain length should be approximately doubled at each condensation step and that the reactants for the condensation should be suitably protected oligonucleotides with 5'-phosphomonoester end groups. This procedure has three advantages. (1) Products and reactants differ substantially in molecular weight and can be separated rapidly and quantitatively by gel

in either direction by the selective protection of 5'-phosphomonoester or 3'-hydroxyl groups at each step.

The general scheme used for the synthesis of deoxyribotetracosanucleotides is shown in Chart I.

The reactants for the condensation steps were β-cyanoethyl esters (CE)³ of the appropriate N-protected mono-, di-,⁴ tri-, hexa-, or dodecanucleotide and the corresponding 3'-O-acetyl N-protected mono-, tri-, hexa-, or dodecanucleoside 5'-phosphate.⁵ Mesitylenesulfonyl chloride (MS)³ was used as a condensing agent. To increase the rate of reaction, the reaction mixture was concentrated shortly after the addition of the condensing agent to the anhydrous pyridine solution. After each condensation step the reaction mixture was usually

(1) (a) T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, **87**, 2971 (1965); (b) S. A. Narang and H. G. Khorana, *ibid.*, **87**, 2981 (1965); (c) S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, **87**, 2988 (1965); (d) H. Kössel, H. Büchi, and H. G. Khorana *ibid.*, **89**, 2185 (1967); (e) E. Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2195 (1967).

(2) (a) E. Ohtsuka, M. W. Moon, and H. G. Khorana, *ibid.*, **87**, 2956 (1965); (b) S. A. Narang, T. M. Jacob, and H. G. Khorana, *ibid.*, **89**, 2167 (1967); (c) T. M. Jacob, S. A. Narang, and H. G. Khorana, *ibid.*, **89**, 2177 (1967).

(3) For the system of abbreviations see ref 4.

(4) S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2158 (1967).

(5) The tri-*n*-hexylammonium salt of the oligonucleotides was used.

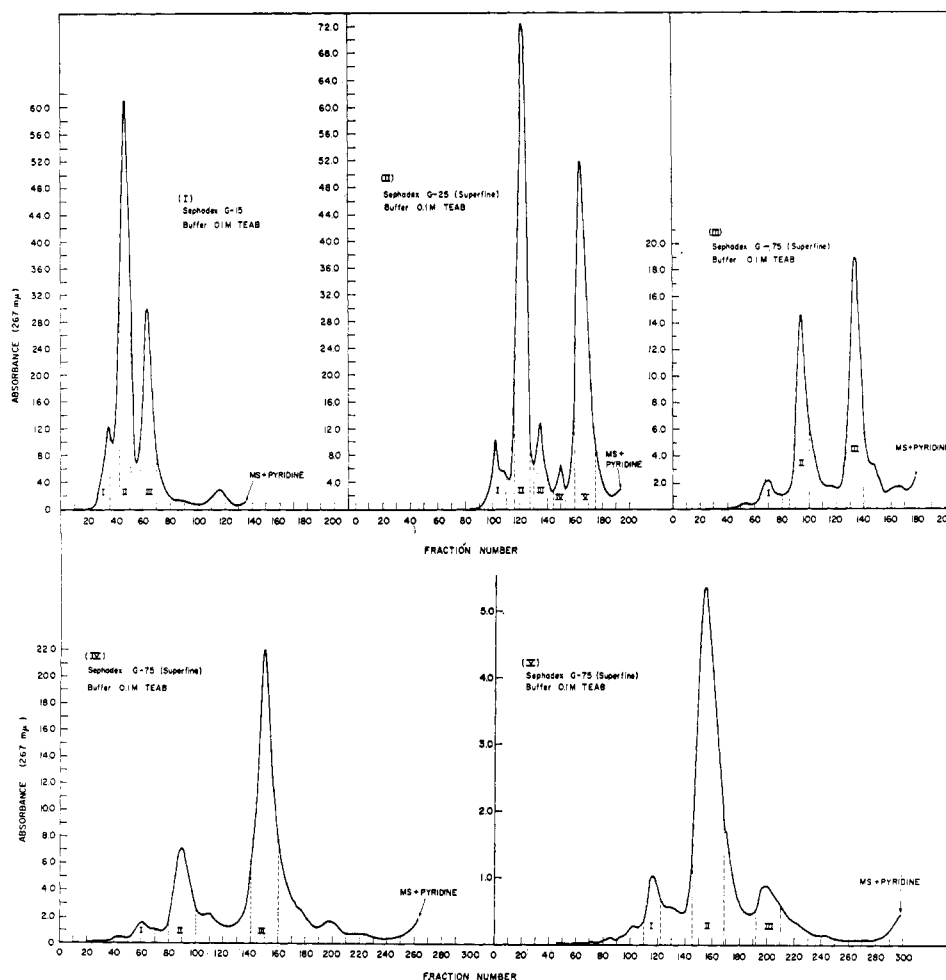


Figure 1. (I) Chromatography of the reaction mixture (one-fourth portion) on a Sephadex G-15 column (150×2.5 cm) in the preparation of $(pT)_2$. Fractions of 2 ml were collected every 20 min. (II) Chromatography of the reaction mixture (one-sixteenth portion) on a Sephadex G-25 (Superfine) column (150×2.5 cm) in the preparation of $(pT)_3$. Fractions of 2 ml were collected every 20 min. (III) Chromatography of the reaction mixture (one-ninth portion) on a Sephadex G-75 (Superfine) column (96×2.5 cm) in the preparation of $(pT)_6$. Fractions of 2 ml were collected every 20 min. (IV) Chromatography of the reaction mixture on a Sephadex G-75 (Superfine) column (96×2.5 cm) in the preparation of $(pT)_{12}$. Fractions of 2 ml were collected every 20 min. (V) Chromatography of the reaction mixture on a Sephadex G-75 (Superfine) column (96×2.5 cm) in the preparation of $(pT)_{24}$. Fractions of 1.4 ml were collected every 15 min.

Table I. Summary of Reaction Conditions, Separations,^a and Yields Obtained in Deoxyribopolynucleotide Synthesis by "Fragment Coupling" Method

β -Cyanoethyl ester oligonucleotide component	Amount, mmoles	3'-O-Acetyl oligonucleotide component	Amount, mmoles	MS, ^e mmoles	Time, hr	Figure (fraction pooled)	Product (purity, ^b %)	Isolated yield, %
Synthesis of the Tetracosanucleotide Bearing a 5'-Phosphomonoester End Group $(pT)_{24}$								
CE-pT	0.416	pT-OAc	0.52	5.2	3	I (43-51)	$(pT)_2$ (100)	63
CE-pTpT	1.0	pT-OAc	2.0	10.0	3	II (116-127)	$(pT)_3$ (95)	65
CE-pT $(pT)_2$	0.1	$(pT)_2$ pT-OAc	0.1	1.0	3	III (85-100) ^c	$(pT)_6$ (98)	45
CE-pT $(pT)_3$	0.01	$(pT)_3$ pT-OAc	0.015	0.15	3	IV (80-100) ^c	$(pT)_{12}$ (90)	16.5
CE-pT $(pT)_{11}$	0.001	$(pT)_{11}$ -OAc	0.001	0.04	3	V (110-122) ^c	$(pT)_{24}$ (mainly)	6.5
Synthesis of the Protected Dodecanucleotide Bearing a 5'-Phosphomonoester End Group (d-pTpTpA ^{Bz} pA ^{Bz} pTpTpA ^{Bz} pC ^{An} pA ^{Bz} pA ^{Bz} pTpA ^{Bz})								
d-CE-pA ^{Bz}	0.5	d-pT-OAc	0.5	5.0	3	VI (140-245) ^d	d-pA ^{Bz} pT (81)	60
d-CE-pA ^{Bz}	4.0	d-pC ^{An} -OAc	4.0	40.0	3	VII (135-190) ^d	d-pA ^{Bz} pC ^{An} (91)	59
d-CE-pTpT	0.78	d-pA ^{Bz} -OAc	1.2	10.5	3	VIII (102-129) ^d	d-pTpTpA ^{Bz} (93)	74
d-CE-pA ^{Bz} pC ^{An}	1.37	d-pA ^{Bz} -OAc	4.0	30.0	3	IX (170-190) ^d	d-pA ^{Bz} pC ^{An} pA ^{Bz} (95)	50
d-CE-pTpTpA ^{Bz}	0.24	d-pA ^{Bz} pTpT-OAc	0.25	2.5	3	X (121-139) ^c	d-pTpTpA ^{Bz} pA ^{Bz} pTpT (95)	32
d-CE-pTpTpA ^{Bz} -pA ^{Bz} pTpT	0.03	d-pA ^{Bz} pC ^{An} pA ^{Bz} -pA ^{Bz} pTpT	0.031	0.3	3	XI (86-107) ^c	d-pTpTpA ^{Bz} pA ^{Bz} pTpT-pA ^{Bz} pC ^{An} pA ^{Bz} pA ^{Bz} pTpT-pA ^{Bz} (90)	18

^a A portion of the reaction mixture at each stage of condensation was chromatographed on a Sephadex column at 4°; for details see the legends to the figures. ^b Determined by paper chromatography and enzymic degradation. ^c Columns K25/100^c and K50/100^d (Pharmacia, Uppsala, Sweden) were used for chromatography. ^e MS, mesitylenesulfonyl chloride.

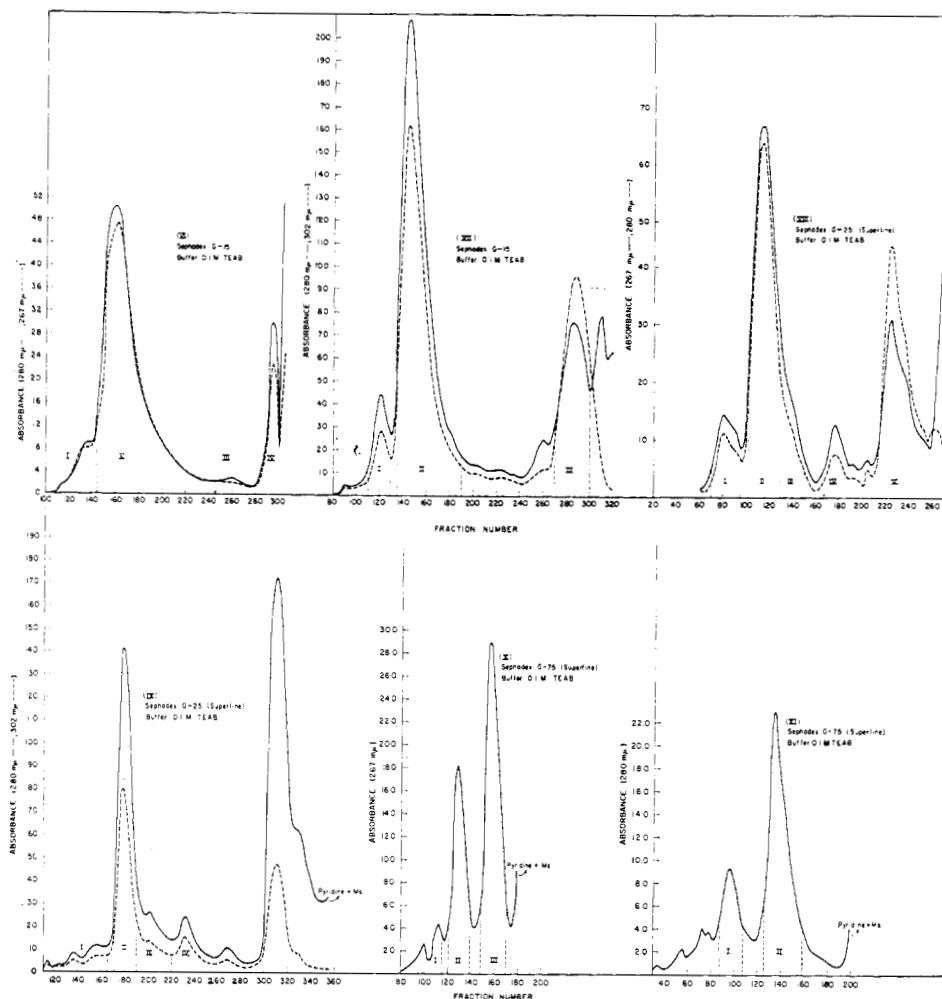


Figure 2. (VI) Chromatography of the reaction mixture on a Sephadex G-15 column (96×5 cm) in the preparation of $d\text{-pA}^{Bz}pT$. Fractions of 4 ml were collected every 6 min. (VII) Chromatography of the reaction mixture (one-fourth portion) on a Sephadex G-15 column (96×5 cm) in the preparation of $d\text{-pA}^{Bz}pC^{An}$. Fractions of 4 ml were collected every 6 min. (VIII) Chromatography of the reaction mixture (one-third portion) on a Sephadex G-25 (Superfine) column (96×5 cm) in the preparation of $d\text{-pTpTpA}^{Bz}$. Fractions of 4 ml were collected every 6 min. (IX) Chromatography of the reaction mixture (one-third portion) on a Sephadex G-25 (Superfine) column (96×5 cm) in the preparation of $d\text{-pA}^{Bz}pC^{An}pA^{Bz}$. Fractions of 4 ml were collected every 6 min. (X) Chromatography of the reaction mixture (one-ninth portion) on a Sephadex G-75 (Superfine) column (96×2.5 cm) in the preparation of $d\text{-pTpTpA}^{Bz}pA^{Bz}pTpTpA^{Bz}pC^{An}pA^{Bz}pA^{Bz}pTpA^{Bz}$. Fractions of 2 ml were collected every 15 min. (XI) Chromatography of the reaction mixture (one-third portion) on a Sephadex G-75 (Superfine) column (96×2.5 cm) in the preparation of $d\text{-pTpTpA}^{Bz}pA^{Bz}pTpTpA^{Bz}pC^{An}pA^{Bz}pA^{Bz}pTpA^{Bz}$. Fractions of 2 ml were collected every 15 min.

given a prolonged treatment with aqueous pyridine-triethylamine followed by an alkali treatment to remove the acetyl and cyanoethyl groups. The products were fractionated on columns of an appropriate Sephadex. The separations were rapid and excellent. Figures 1 and 2 show the elution pattern for each step. A summary of the pertinent reaction conditions and separations used in the syntheses of $(pT)_{24}$ and $d\text{-pTpTpA}^{Bz}pA^{Bz}pTpTpA^{Bz}pC^{An}pA^{Bz}pA^{Bz}pTpA^{Bz}$ is shown in Table I.

Characterization of all the protected and unprotected intermediate compounds was accomplished by paper chromatography. The final compounds were further characterized by degradation with spleen and venom phosphodiesterase treatment after the enzymic removal of the phosphomonoester group. Enzymic degradation proceeded to completion and the hydrolyzed products (nucleotide and nucleoside) were produced in the expected molar proportions.

This methodology was developed as a necessary preliminary step toward the DNA-directed synthesis of

bovine insulin chain A. Further work in this direction will be the subject of forthcoming papers.

(6) To whom all inquiries should be made.

(7) National Research Council of Canada Postdoctorate Fellow, 1967-1968.

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Purification and Structure of Porcine Calcitonin-1

Sir:

We wish to report the isolation in pure form of a porcine calcitonin (PC-1) and the elucidation of its structure.¹

(1) We also wish to acknowledge the valuable assistance of C. Pidacks for chromatography development work, as well as E. Lindemann and H. Falk for biological assay data.