CHEMICAL SYNTHESIS OF THE "STICKY END" OF LAMBDA PHAGE DNA r-STRAND*.

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Summary: The chemical synthesis of a dodecadeoxyribonucleotide, d(pA-G-G-T-C-G-C-C-G-C-C-C), constituting the 5'-terminus of the r-strand of λ-phage DNA, is described. Using a phosphorothioate anchoring group throughout, fragment condensation is carried out by the general methodology of Khorana. Final deprotection utilizes mild oxidative hydrolysis.

The occurrence in nature of certain bacteriophages possessing cohesive ends at the termini of their DNA (2) has been utilized in sequencing the fine structure of these "sticky ends". Recently, complete sequences of the two cohesive ends of bacteriophage λ DNA have been determined by enzymatic means (3). These turn out to be dodecadeoxyribotides very rich in guanosine and cytosine, a useful property for insuring proper cohesion. We now report the chemical synthesis of one of these dodecamers - the one constituting the 5'-terminus of the r-strand (4), i.e., the strand terminating in deoxy adenylate.

Materials and Methods: Deoxyribonucleotide monomers were purchased from commercial sources. Condensations followed the procedures of Khorana closely, and have been detailed in earlier papers (5). The following compounds** were prepared according to the literature: d(Et_s-bzA), (7); d(pT-anC(OAc)), (8); d(panC-anC(OAc)), (9); d(pibuG-anC), modified after (10). Conversion to more or less highly protected species is also found in these references.

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* This publication constitutes Paper VIII in a series entitled Nucleoside Phosphorothioates. For Paper VII, see (1).

** Abbreviations are in strict accordance with the 1970 IUPAC-IUB Recommendations (6), special attention being directed to sections 3.1 and 3.2. Subscript s preceding phosphate symbol (-) denotes

The several condensations are summarized in Table I: each reaction mixture was subjected to column chromatography (Figs. 1-7), and the peaks characterized by paper chromatography, uv spectroscopy and monomer analysis (see Results). Details are summarized under the figure captions. All chromatographic separations were carried out at 2-4°C. Gel permeation chromatography used the techniques of Narang et al.(11,12).

The blocked dodecamer was subjected to ammonia hydrolysis and treatment with iodine as described earlier (1). Isolation was carried out by preparative paper chromatography in system C (see Table III for definition).

Results and Discussion: The synthetic approach is summarized in Fig. 8. As in previously described chemical syntheses from our laboratory, a nucleoside S-alkyl phosphorothioate served as an anchor terminus carrying the growing oligomer throughout the series of fragment condensations, final deblocking being effected by mild oxidative hydrolysis. Synthesis and, indeed, isolation of trimer 4 were atypical: the incoming (i.e., phosphate donor) dimer, normally used in excess over the acceptor (hydroxyl-bearing) fragment, was made limiting, since the acceptor was the easily accessible monomer. Furthermore, it proved advantageous to isolate the product prior to removal of the 5'-protecting (cyanoethyl) group, to prevent the necessity of separating species of equal charge. It will be noticed that the dimer in the foregoing condensation was used again in the synthesis of 6. Otherwise the synthesis proved comparable to similar ventures of this type: yields are variable, and generally modest, and great effort is expended in separation efforts.

Tables II and III summarize uv and paper chromatographic data of the various oligonucleotides. Table IV summarizes the mono-

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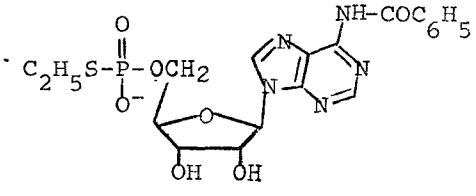
phosphorothioate. Thus, Et_s-bzA is



We wish to express our appreciation to Dr. Waldo E. Cohn, Director of the Office of Biochemical Nomenclature, National Research Council, whose advice in these matters we deem most helpful.

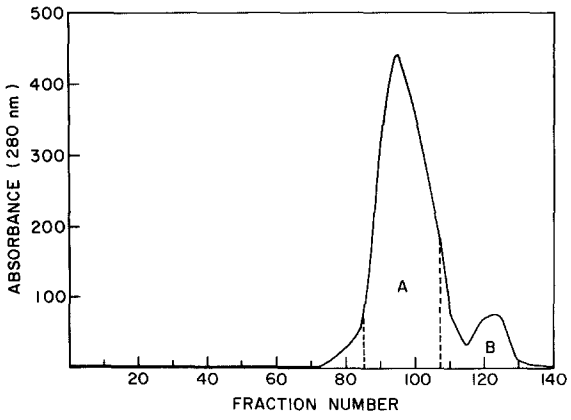


Figure 1: Preparation of 1. - Condensation: 14.2 mmol d(Et$_S$-bzA), 15 mmol d(pibuG(Ibu)) 45 mmol MSC*, 30 ml pyridine. Workup: 90 ml DIEA**, 30 ml water; after overnight storage at 0° adjust volume to 200 ml, treat with 200 ml 2N sodium hydroxide for 10 min at 0°, neutralize with pyridinium Dowex 50, subject to preliminary DEAE-cellulose chromatography (not shown) with convex gradient of 0.3M triethylammonium bicarbonate (pH 7.3) into 6 l. of water. Material emerging with buffer molarity 0.1-0.12 was concentrated, desalted and a one-fifth aliquot subjected to gel permeation chromatography on LH 20 Sephadex. Column size: K50/100, flow rate: 1ml/min, eluent: 70% ethanol, fraction size: 5 ml.

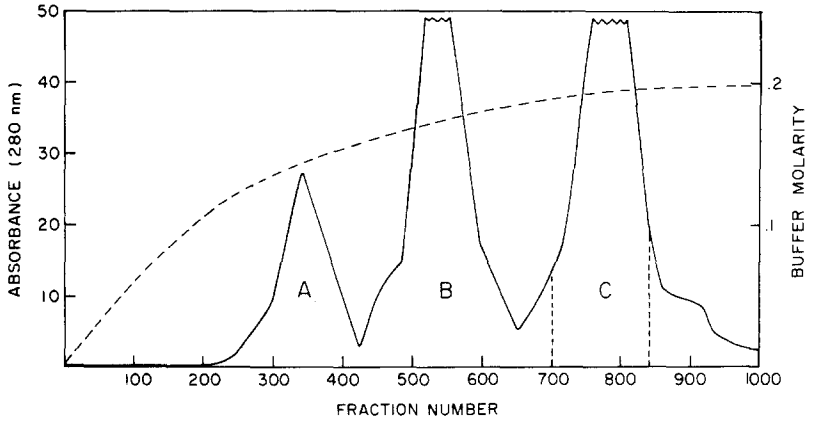


Figure 2: Preparation of 2. - Condensation: 4.2 mmol 1, 12.6 mmol d(pibuG(Ibu)), 38 mmol MSC, 40 ml pyridine. Workup: 80 ml DIEA, 40 ml water. Removal of 3'-block as in fig. 1. Chromatography on DEAE-cellulose in bicarbonate phase; column size: 6 x 90 cm. Convex gradient, 0.2M (pH 7.5) TEABIC*** into 5 l. of water in mixing chamber. Fraction size: 20 ml, rate: 2.5 ml/min.

* mesitylene sulfonyl chloride; ** diisopropylethylamine, 1N in pyridine.
*** triethylammonium bicarbonate.

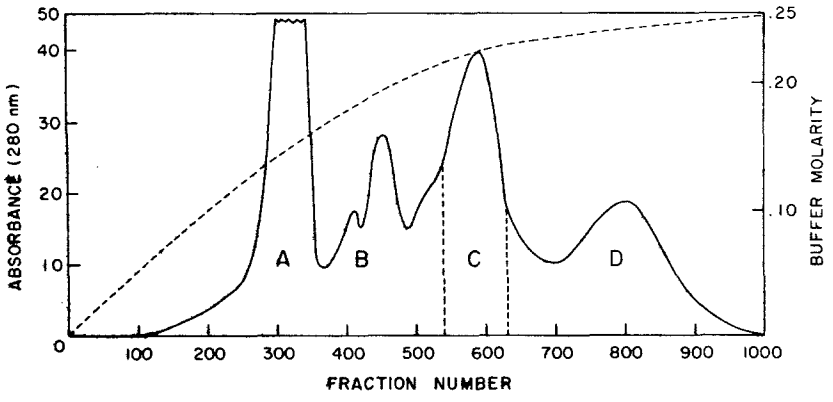


Figure 3: Preparation of 4. - Condensation 25 mmol d(CE-ibuG), 5mmol d(panC-anC(Ac)), 15 mmol dicyclohexylcarbodiimide, 2g of dry Dowex 50-pyridinium resin, 45 ml pyridine. Shaken in the dark at room temperature for 63 hr, then 100 ml of cold water added. After 4 hr, filtered, extracted with ether, made up aqueous portion to 1.6 l. with water. Chromatography on DEAE-cellulose in bicarbonate phase; column size: 7 x 92 cm. Convex gradient of 0.25M TEABIC into 6.5 l. of water. Flow rate: 2.7 ml/min, fraction size 20 ml. Product in peak C was deblocked with 1N sodium hydroxide as in fig. 1 and reacetylated at 3'-terminus.

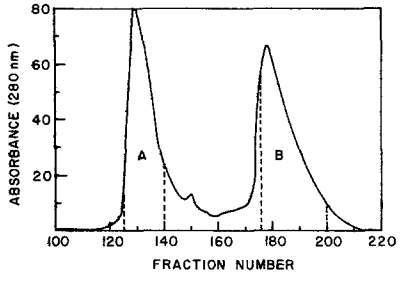


Figure 4: Preparation of 6. Condensation: 0.9 mmol d(CE-ibuG-anC), 1.9 mmol d(panCanC), 5.8 mmol MSC in 5.5 ml pyridine. Workup: 12 ml DIEA, 12 ml. water. Unblocking as usual in total volume of 100 ml 1N in sodium hydroxide. Preliminary fractionation on DEAE-cellulose (not shown): Column size 4 x 60 cm, convex gradient 0.35M TEABIC into 2.5 l. of water. Flow rate 2.5 ml/min. Right behind MSA, dimer d(panCanC) and product 6 emerged in admixture. One half of this was separated by gel permeation chromatography on superfine Sephadex G-25. Column size K50/100, eluent 0.2M TEABIC (pH 7.5), fraction size: 5ml; flow rate: 0.5 ml/min.

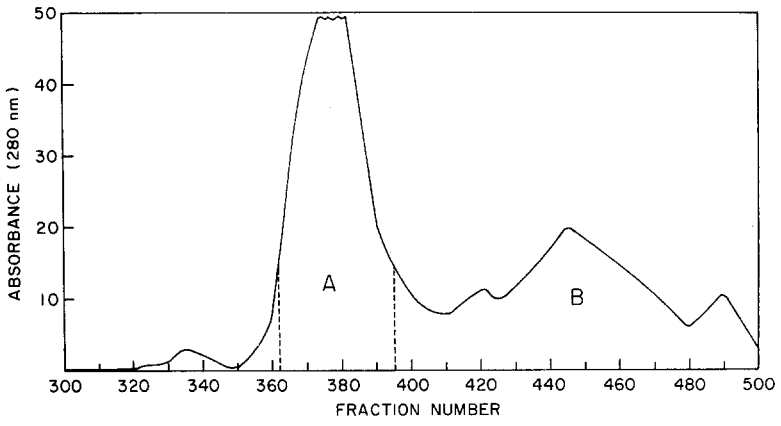


Figure 5: Preparation of 3. - Condensation: 0.5 mmol d(Et$_s$-bzA-ibuG-ibuG), 1.0 mmol d(pT-anC(Ac)), 3 mmol MSC in 10 ml pyridine. Workup: 3 ml DIEA, 3 ml water. Unblocking in 60 ml, 1N in sodium hydroxide. Preliminary fractionation (not shown) on DEAE-cellulose bicarbonate cycle, 5 x 100 cm; convex gradient, 0.32 M TEABIC (pH 7.5) into 4 l. of water; flow rate 2.5 ml/min; fraction size 17.5 ml. From fractions 390-435, obtained impure pentamer by concentration, dissolution in dry pyridine and precipitation from ether. One third of the 600 mg thus obtained were rechromatographed on Sephadex G-50 (superfine) on a K50/100 column; eluent 0.2M TEABIC (pH 7.5); flow rate: 0.5ml/hr; fraction size: 4 ml.

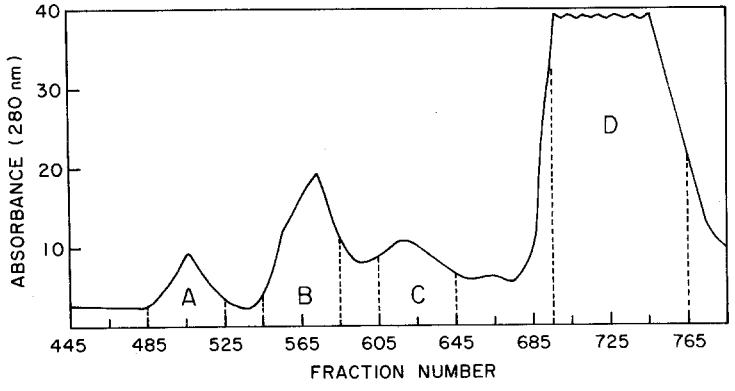


Figure 6: Preparation of 5.- Condensation: 25 µmol d(Et$_s$-bzA-ibuG-T-anC), 150 µmol d(pibuG-anC-anC(Ac)), 1mMol MSC in 2 ml pyridine. Workup: 2 ml DIEA, 2 ml water. Unblocking as usual in 20 ml total, 1N in sodium hydroxide. Chromatography on Sephadex G-50 (superfine), K50/100 column. Eluent: 0.2M TEABIC (pH 7.5); flow rate: 0.5 ml; fraction size: 4 ml.

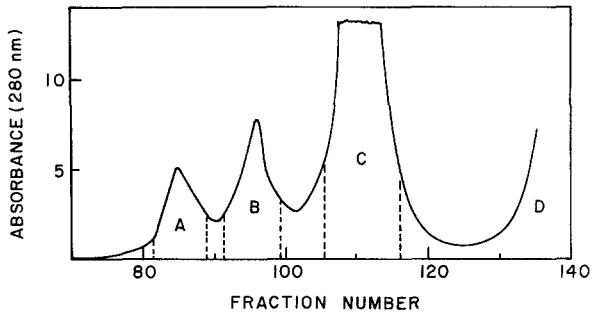


Figure 7: Preparation of 7. -Condensation: 2 µmol d(Et$_S$-bzA-ibuG-ibuG-T-anC-ibuG-anC-anC), 20 µmol d(pibuG-anC-anC-anC), 116 µmol MSC, in 0.5 ml pyridine. Workup: 250 λ DIEA, 250 λ water; unblocking in total volume of 2 ml, 1N in sodium hydroxide. Chromatography on Sephadex G-75 (superfine), K25/100 column. Eluent: 0.2M TEABIC (pH 7.5); flow rate: 0.5 ml/min; fraction size: 3.7 ml.

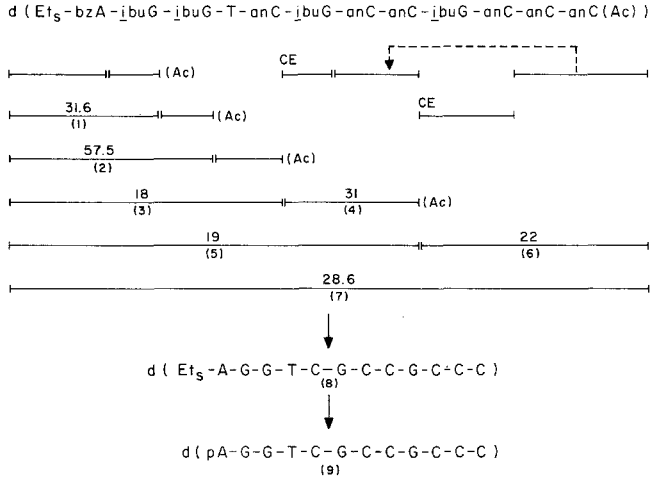


Figure 8: Scheme of Dodecamer Synthesis.

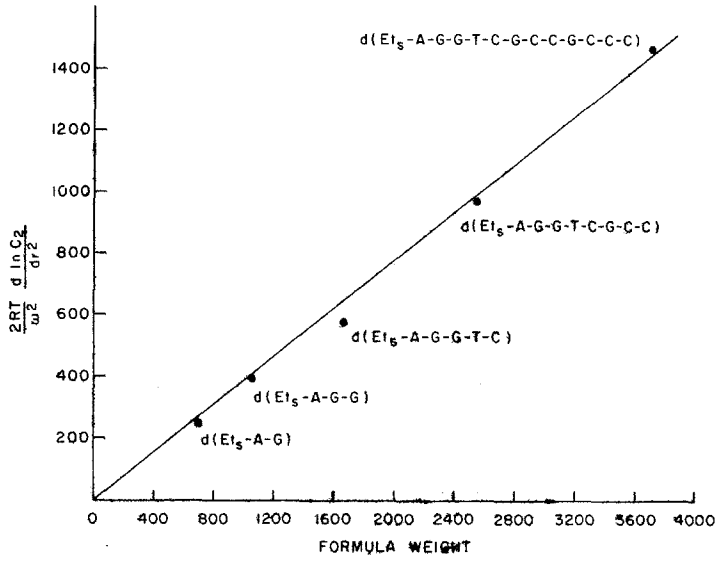


Figure 9: Molecular Weights by Equilibrium Sedimentation.

Table I: Identity of Chromatographic Peaks from the Several Condensations.- For details, see figures and ref. 5, Experimental Section.

Fig \ Peak	A	B	C	D
1	d(Et$_s$-bzA-ibuG) Product 1	d(pibuG)	-	-
2	MSA*	Mixture of d(Et$_s$-bzA-ibuG) and d(pibuG(ibu))	d(Et$_s$-bzA-ibuG-ibuG) Product 2	-
3	d(CE-ibuG)	d(pibuG) impure	CE-ibuG-anC-anC Product 4	d(panC-anC(pAc))
4	d(pibuG-anC-anC-anC) Product 6	d(panC-anC)	-	-
5	d(Et$_s$-bzA-ibuG-ibuG-T-anC) Product 3	d(Et$_s$-bzA-ibuG-ibuG)	-	-
6	Unknown**	d(Et$_s$-bzA-ibuG-ibuG-T-anC-ibuG-anC-anC) Product 5	d(Et$_s$-bzA-ibuG-ibuG-T-anC)	d(pibuG-anC-anC)
7	d(Et$_s$-bzA-ibuG-ibuG-T-anC-ibuG-anC-anC-ibuG-anC-anC-anC) Product 7	d(Et$_s$-bzA-ibuG-ibuG-T-anC-ibuG-anC-anC)	d(pibuG-anC-anC-anC)	MSA*

* mesitylene sulfonic acid

** upon hydrolysis, appears identical with unblocked octomer from B. Possibly due to paradoxical peak multiplicity (see ref. 12).

Table II: Ultraviolet Characteristics of Intermediates. - Measurements taken in unbuffered distilled water.

TABLE II

	λ max	λ min	$\frac{260}{280}$	$\frac{280}{300}$
d(Et$_s$-bzA-ibuG)	278,259	267,228	0.97	2.66
d(Et$_s$-bzA-ibuG-ibuG	277,257	268,226	1.07	2.60
d(pibuG-anC-anC)	295,261	266,234	0.88	0.92
d(pibuG-anC-anC-anC)	294-6,260 (shoulder)	236	0.84	0.88
d(Et$_s$-bzA-ibuG-ibuG-T-anC)	274-6,262-3	270,233	1.05	1.62
d(Et$_s$-bzA-(ibuG)$_2$-T-anC-ibuG-anC-anC)	280,263	268,236	0.97	1.25
d(Et$_s$-bzA-(ibuG)$_2$-T-anC-ibuG-anC-(anC)$_3$)	282,258-9	265,235	0.95	1.15
d(pA-G-G-T-C-G-C-C-G-C-C-C)	258,260	232,233	1.57	-

Table III: Paper Chromatography.-R_f values relative to thymidylate. Whatman #1, systems as in ref. (7).

TABLE III

COMPOUND \ SYSTEM	A	B	C
d(Et$_s$-A-G)	0.96	0.95	1.2
d(Et$_s$-A-G-G)	0.31	0.60	0.77
d(pG-C-C)	0.07	0.36	0.37
d(Et$_s$-A-G-G-T-C)	0.06	0.36	0.52
d(Et$_s$-A-G-G-T-C-G-C-C)		0.05	0.20
d(pG-C-C-C)	0.03	0.25	0.28
D(Et$_s$-A-G-G-T-C-G-C$_2$-G-C$_3$			0.02

Table IV: Monomer Composition of Oligodeoxyribonucleotides.-For procedure, see earlier papers (1,7).

	d(pT)	d(pA)	d(pC)	d(pG)
d(Et_s-A-G)		47.7(50)		52.3(50)
d(Et_s-A-G-G)		31.9(33.3)		68.1(66.7)
d(pG-C-C)			68.6(66.7)	31.4(33.3)
d(pG-C-C-C)			76.9(75)	23.1(25)
d(Et_s-A-G-G-T-C)	22.9(20)	17.1(20)	21.4(20)	38.6(40)
d(Et_s-A-G-G-T-C-G-C-C)	12.0(12.5)	11.4(12.5)	39.1(37.5)	37.5(37.5)
d(Et_s-A-G-G-T-C-G-C-C-G-C-C-C)	8.7(8.3)	6.9(8.3)	53.5(50)	30.9(33.3)

nucleotide composition of the unblocked species, as obtained by snake venom diesterase hydrolysis and high pressure liquid chromatography (13), and Fig. 9 shows molecular weight determinations by a sedimentation equilibrium method (14).

We are currently studying the interaction of dodecamer 9 with λ-phage DNA. Work is in progress to learn if it can be joined to the macromolecule upon annealing and treatment with polynucleotide ligase and ATP. As suggested by Agarwal, et al,(15), the transfer of synthetic genetic information into a cell might be accomplished by covalent attachment to a viral genome in this manner.

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