and nucleotides in which they describe a fragment ion in the mass spectrum of 5′-AMP(TMS)$_5$ analogous to m and presumably due to the loss of an unusually stable radical formed by the silylated phosphate moiety. Conversely, fragment l is a characteristic fragment in the mass spectra of TMS derivatives of furanose nucleosides but does not occur in the mass spectrum of 5′-AMP-(TMS)$_5$, nor was a fragment ion analogous to l present in our studies of the mass spectrum of nucleocidin (TMS)$_4$.

Table II

Ion	Composition	Obsd mass	Calcd mass
M^+	$C_{22}H_{45}N_6Si_4SO_6F$	652.2158	652.2182
M − CH_3	$C_{21}H_{42}N_6Si_4SO_6F$	637.1950	637.1947
m	$C_{19}H_{35}N_5Si_3O_8F$	484.2035	484.2018
s	$C_{14}H_{33}N_1Si_3SO_6F$	446.1313	446.1291
k	$C_{13}H_{23}N_5Si_2O_2$	337.1394	337.1385
i	$C_{13}H_{24}N_5Si_2O$	322.1513	322.1514
a + H	$C_9H_{14}N_5SiO$	236.0979	236.0966
b + 2H	$C_8H_{14}N_5Si$	208.0998	208.1017
b + 1H	$C_8H_{13}N_5Si$	207.0922	207.0939
b	$C_8H_{12}N_5Si$	206.0845	206.0860

Comparison between the mass spectra and the ^1H nmr spectra of nucleocidin and other nucleosides and nucleotides[9,10] clearly establishes the furanose nucleoside structure **2**. The downfield shift in the ^1H nmr spectrum of the $C_{5'}$-methylene over that of adenosine ($\Delta\delta$ = 0.6 ppm) and the presence of ion m in the mass spectrum, analogous to that found by McCloskey, *et al.*, are strong evidence for assigning the sulfamyloxy group to the $C_{5'}$ position. The assignment of the fluorine to the $C_{4'}$ position is based primarily on its approximately equal coupling to the $C_{5'}$-methylene protons and the H–F vicinal coupling to $C_{3'}H$. Additional work including heteronuclear spin decoupling is planned.

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A New Method for the Synthesis of Protected Ribooligonucleotides with 3′-Phosphate End Groups

Sir:

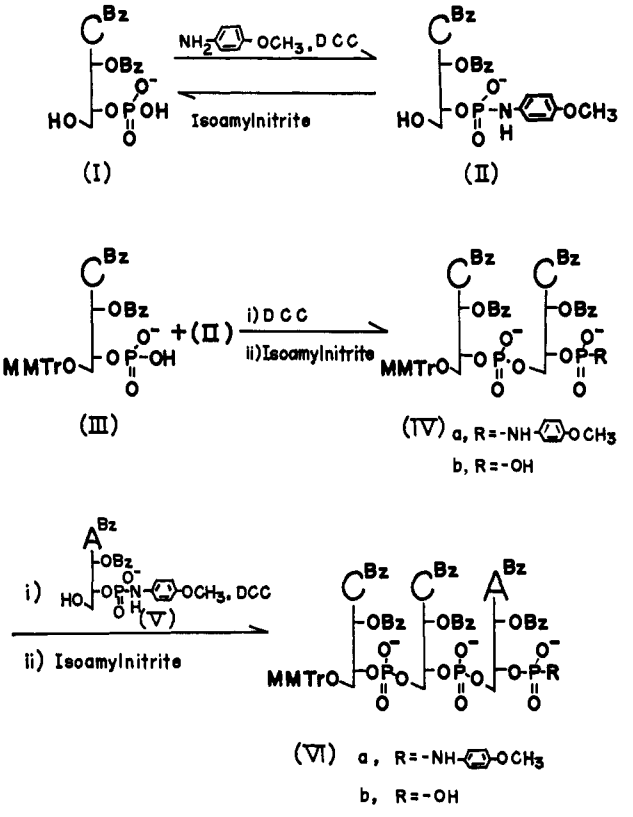
The synthesis of ribonucleotides with specific sequences is of importance for studies of the relationship between the structure and the function of nucleic acids.

In the synthesis of deoxyribopolynucleotides, stepwise condensation of preformed oligonucleotides has proved to be an advantageous method.[1] In the ribonucleotide series, however, a satisfactory procedure for the synthesis of suitably protected oligonucleotides has not been established. It is essential to use specific protection for heterocyclic rings, primary and secondary hydroxyl groups in carbohydrate moieties, and phosphomonoester groups in the synthesis of ribooligonucleotides which can be used for further condensations.

Trityl derivatives for the protection of primary hydroxyl groups and the acyl protection for heterocyclic amino groups and 2′-hydroxyl groups have been used successfully in the synthesis of triribonucleotides without phosphomonoester end groups[2] and other ribooligonucleotides.[3] With these protecting groups, acidic or alkaline treatment cannot be used for the specific removal of the protecting group on the phosphomonoester.

In this communication it has been found that aromatic amidate can be used for the protection of phosphomonoester. This group may be removed with isoamyl nitrite in a buffered solution without damaging other protecting groups on the base and the sugar moiety. Using this method a large-scale synthesis of ribooligonucleotides was performed.

Since thymidine 5′-phosphoramidate was converted by the treatment with amyl nitrite to the 5′-phosphate,[4] we have tested this reaction with 3′-phosphoranisidate of a protected nucleoside. When N,2′-O-dibenzoylcytidine 3′-phosphoro-*p*-anisidate (II), which was pre-



(1) H. Kössel, M. W. Moon, and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2148 (1967); H. Kössel, H. Büchi, and H. G. Khorana, *ibid.*, **89**, 2185 (1967); E Ohtsuka and H. G. Khorana, *ibid.*, **89**, 2195 (1967).

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(3) D. H. Rammler, Y. Lapidot, and H. G. Khorana, *ibid.*, **85**, 1989 (1963); Y. Lapidot and H. G. Khorana, *ibid.*, **85**, 3852 (1963); R. Lohrmann and H. G. Khorana, *ibid*, **86**, 4188 (1964)

(4) M. Ikehara, S. Uesugi, and T. Fukui, *Chem. Pharm. Bull.*, (Tokyo), **15**, 440 (1967).

pared from the corresponding 3′-phosphate (I) by a method similar to the case of adenosine 5′-phosphoro-*p*-anisidate,[5] was allowed to react with excess isoamyl nitrite in an equal volume mixture of pyridine and acetic acid at room temperature, the phosphate I was obtained exclusively. In DMF solution the isoamyl nitrite treatment of II showed small amounts of N-debenzoylation and deamination. Therefore, this procedure was applied to the synthesis of a protected ribotrinucleotide with a 3′-phosphomonoester end group (IVb) which could be further condensed with a protected oligonucleotide block with a free 5′-hydroxyl group. Compound II (1 mmole) was condensed with 5′-O-monomethoxytrityl-N,2′-O-dibenzoylcytidine 3′-phosphate (III) (0.7 mmole) in pyridine using DCC as a condensing agent. After 4 days, the aqueous pyridine treatment was given overnight and the azeotropically dried pyridine solution of IVa was precipitated with ether. The precipitate was treated with isoamyl nitrite (10 mmoles) in a mixture of pyridine (5 ml) and acetic acid (5 ml) for 4 hr at room temperature. The nucleotides were precipitated in ether to remove pyridinium acetate. The precipitate was dissolved in pyridine and 95% ethyl alcohol and applied to a column of TEAE-cellulose (acetate form). The elution conditions and the pattern are shown in Figure 1. Peak VIII contained the pure dinucleotide (IVb). The yield was 3500 OD_{304} units (0.2 mmole, 28%). The spectral properties in ethyl alcohol were λ_{max} 262 and 304 mμ, λ_{min} 250 and 290 mμ, and $\epsilon_{304}/\epsilon_{280} = 0.81$; $\epsilon_{(p)}$ at 304 mμ was 8800. The purity was checked by paper chromatography and electrophoresis after partial removal of the protecting groups. Methanolic ammonia treatment gave MMTr-CpCp[6] (R_f 0.27 in solvent A,[7] R_{Cp} 0.80 in paper electrophoresis at pH 7.5). The benzoylated dinucleotide obtained by 80% acetic acid treatment showed R_f 0.71 in solvent B and R_{Cp} 0.81 in paper electrophoresis at pH 7.5. The acidic treatment and subsequent removal with ammonia gave the unprotected dinucleotide, CpCp (R_{Cp} 0.43 in solvent A and R_{Cp} 1.03 in paper electrophoresis at pH 7.5), which was completely degraded by pancreatic RNase.

For the synthesis of trinucleotide VIb, the protected dinucleotide IVb (3250 OD_{304} units, 0.185 mmole) was allowed to react with N,O$^{2'}$-dibenzoyladenosine 3′-phosphoro-*p*-anisidate (V) (3110 OD_{280} units) and DCC in pyridine. Using essentially the same procedure as above, the protecting group in VIa was removed and the trinucleotide VIb, which was eluted at about 0.3 *M* salt concentration in TEAE-cellulose (acetate) column, was isolated in a yield of *ca.* 30% (1050 OD_{304} units). The spectral properties in ethanol were λ_{max} 264 mμ and shoulder at 280 and 300 mμ, λ_{min} 250 mμ, and $\epsilon_{304}/\epsilon_{280} = 0.50$. Ammonia treatment gave MMTr-CpCpAp (R_f 0.14 in solvent A, R_{Cp} 0.86 in paper electrophoresis at pH 7.5) and a trace of a trityl-negative side product. The benzoylated trinucleotide showed R_f 0.79 in solvent B, R_{Cp} 0.81 in paper electrophoresis at pH 7.5. The completely deprotected trinucleotide CpCpAp (R_{Cp} 0.14 in solvent A and R_{Cp} 1.03 in electrophoresis at pH 7.5) was degraded with pancreatic RNase to give cytidine phosphate (R_f 0.73) and adenosine 3′-phosphate (R_f 0.17) in the ratio of 2.03 : 1.00 in solvent C.

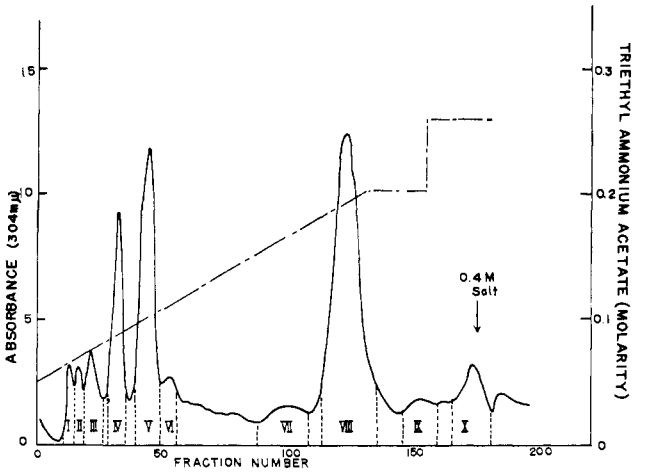

Figure 1. Chromatography of the products obtained in the synthesis of the dinucleotide IVb on a TEAE-cellulose (acetate) column (2.7 × 50 cm) preequibrated with 95% ethanol. Elution was carried out using a linear salt gradient of triethylammonium acetate (pH 6.5) in 95% ethanol (2 l. of 0.05 *M* salt in the mixing vessel and an equal volume of 0.25 *M* salt in the reservoir). Fractions of 21 ml were collected every 15 min. Peak VIII contained the dinucleotide IVb.

Thus the properly protected ribotrinucleotide with 3′-phosphate was obtained in quantity using the anisidate protection. Further condensation of the trinucleotide with oligonucleotide blocks having free 5′-hydroxyl groups and polymerization of the detritylated trinucleotide are in progress.

Application of this method for the protection of 5′-phosphomonoester in the synthesis of deoxyribopolynucleotides and the use of phosphoramidates with different stabilities are under investigation.

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(6) The system of abbreviations is essentially the same as used in the *Journal of Biological Chemistry*. The letter p at the right indicates 3′-phosphate and MMTr at the left means 5′-monomethoxytrityl group.

(7) Paper chromatography was performed using the descending technique. The solvent systems used were: solvent A, isopropyl alcohol–concentrated ammonia–water (7 : 1 : 2, v/v); solvent B, ethyl alcohol–1 *M* ammonium acetate, pH 7.5 (7.3, v/v); solvent C, saturated ammonium sulfate–water–isopropyl alcohol (79 : 19 : 2, v/v).

Catalytic Formation of Hydrocarbons (C_1–C_5) from Hydrogen and Carbon Monoxide over the Electron Donor–Acceptor Complex Films of Alkali Metals with Transition Metal Phthalocyanines or Graphite

Sir:

Various phthalocyanine films and graphite exhibit marked catalytic activities for hydrogen exchange, hydrogenation of unsaturated hydrocarbons,[1] and isomerization of butenes[2] at room temperature when they are

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