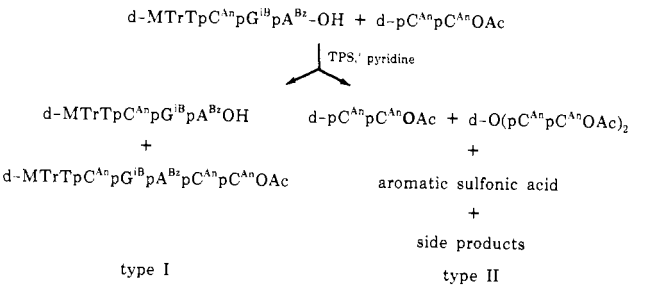
Use of Trityl- and α-Naphthylcarbamoylcellulose Derivatives in Oligonucleotide Synthesis†

P. J. Cashion,‡ M. Fridkin, K. L. Agarwal, E. Jay, and H. G. Khorana*

ABSTRACT: The reaction mixtures in the standard deoxyribopolynucleotide synthesis contain two types of products, those that contain trityl groups and those that do not. To facilitate purification of the required trityl-containing products, a method has now been devised for the rapid separation of the reaction mixtures into the above two types of compounds. Two lipophilic derivatives (trityl and naphthylcarbamoyl) of cellulose have been prepared. On passage of the reaction mixtures through columns of these derivatives in low ethanolic solvent, the trityl-containing compounds are selectively absorbed. These are subsequently eluted quantitatively by increasing ethanol and, in some cases, salt concentration. The general applicability of the method has been demonstrated by separating a large variety of oligonucleotides of different base composition and size.

The current methodology for the synthesis of double-stranded DNA involves the chemical synthesis of short deoxyribopolynucleotide chains corresponding to the entire two strands and their subsequent enzymatic joining when they are appropriately aligned to form bihelical complexes (Agarwal *et al.*, 1972; Miller *et al.*, 1971; Agarwal *et al.*, 1970). The chemical synthesis is easily the more demanding and time-consuming part of the DNA synthesis and efforts continue to be made to introduce new and more convenient procedures in order to increase efficiency and speed in this work (Jay *et al.*, 1972; Agarwal *et al.*, 1971, 1973). In the present methods, the bulk of the time is expended in achieving separation of the reaction products by anion-exchange chromatography. In the present paper, a new procedure is described which shortens the overall time taken at the separation step and is also capable of giving improved resolution of the required products. The chemical synthesis involves the condensation of a protected oligonucleotide containing a 5′-phosphate group with the 3′-hydroxyl end group of a growing oligonucleotide chain, the 5′-hydroxyl end group of which usually carries a methoxytrityl group. An example of a typical reaction mixture and products is given in Scheme I. Thus the products can be grouped into the two types shown, one of which contains a trityl group while the second type does not. Since the interest resides primarily in compounds of the type I, attempts were made to achieve their rapid separation from compounds of the type II by taking advantage of the lipophilic trityl group present in them. In the present work, it has been shown that the above separation can be accomplished by the use of highly lypophilic cellulose derivatives. A number of these were investigated (Agarwal *et al.*, 1973) but only the two (trityl and naphthylcarbamoyl) shown in Figure 1 proved

SCHEME I



satisfactory and the present paper describes the preparation of these derivatives and the design of conditions for their successful use in the separation of a large number of synthetic reaction mixtures. The above concept in nucleotide separation was first introduced by Gillam *et al.* (1967) who devised ingenious methods for tRNA separation. The use of benzoyldiethylaminoethylcellulose in oligonucleotide separation has also been recorded by von Tigerstrom and Smith (1970) and by Narang *et al.* (1970).

Experimental Section

Materials

Cellulose powder, CF-11, was a product of Whatman Chemical Co. Trityl chloride was from Fisher Chemical Co.; it was recrystallized before use from anhydrous *n*-pentane in the presence of $SOCl_2$. Reagent grade pyridine (Mallinckrodt) was purified and dried as described previously (Agarwal *et al.*, 1971). Naphthyl isocvanate was purified by fractional distillation. All of the deoxyribonucleotide reaction mixtures used in these studies were intermediates in the synthesis of oligonucleotides required for the synthesis of bihelical DNA. The chemical synthesis of these oligonucleotides will be described elsewhere.

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[1] Abbreviations used are: TEAB, triethylammonium bicarbonate, pH 7.8–8.0; DEAE-cellulose, diethylaminoethylcellulose; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride; Bz, benzoyl; An, anisoyl; iB, isobutyryl; mB, 2-methylbutyryl; MTr, monomethoxytrityl; d-$pA^{Bz}pA^{Bz}$, the dinucleotide of 5′-deoxyadenylic acid with its amino groups blocked by benzoyl functions; TPM, *p*-(triphenylmethyl)aniline.

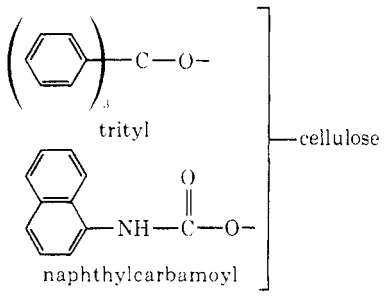


FIGURE 1: Lypophilic cellulose derivitives prepared.

Methods

Preparation of Tritylcellulose. A typical medium-scale preparation of tritylcellulose is as follows. Cellulose powder (15 g, 93 mmol of glucosyl residues) was dried overnight at 100° *in vacuo* and was mixed with 38 g (136 mmol) of recrystallized trityl chloride in the presence of 180 ml of anhydrous pyridine in a round-bottom flask fitted with a reflux condenser. The suspension was stirred slowly and brought to reflux. After 4 hr the reaction was terminated by the addition of an excess of 10% aqueous ethanol. The reaction suspension was then cooled and poured over a cheesecloth, and most of the excess liquid was removed by squeezing. The resinous product was then packed into a column and the residual pyridine and trityl alcohol were completely removed by washing with many bed volumes of ethanol (or in some cases with benzene) until the effluent was completely free from pyridine or trityl alcohol. After air-drying, 19 g of a white powder was obtained.

For determination of the trityl content, a sample was dried *in vacuo* at 80° for 3–4 hr. The dry sample (about 10 mg) was suspended in an appropriate volume of 60% perchloric acid. The absorbance of the yellow solution was read at 430 nm. The ϵ_{430} of the trityl cation was determined to be 35,200. In the above preparation, it was found to be 0.23 trityl group/glucosyl residue of cellulose. A sample removed after 2-hr refluxing of the reaction mixture contained 0.19 trityl group/glucose residue.

Preparation of Naphthylcarbamoylcellulose. Cellulose powder (60 g) was dried overnight *in vacuo* at 80° and suspended in anhydrous pyridine (300 ml). The suspension was stirred mechanically and 1-naphthyl isocyanate (29.5 g, 0.17 mol) was added. The reaction mixture was then heated at 70° for 16 hr with exclusion of moisture. To the brown reaction mixture, a mixture of $CHCl_3$–MeOH (600 ml; 1:2 v/v) was added. The insoluble product was collected by filtration and washed with the same solvent until the washings were free from the ultraviolet (uv) absorption at 280 nm. The product was finally washed with ethanol and dried *in vacuo;* the yield of naphthylcarbamoylcellulose was 84 g. The extent of derivatization was calculated to be 15% of the total hydroxyl groups on the basis of the increase in weight of the cellulose.

By following the same procedure, naphthylcarbamoylcelluloses of varying degree of derivatization (30 and 100%, respectively) were prepared by using correspondingly larger amounts of naphthyl isocyanate.

Chromatographic Methods. Columns were packed and elutions were carried out usually at 4°. A slurry of the trityl- or naphthylcarbamoylcellulose in 20–40% ethanol containing 0.05 M triethylammonium bicarbonate (pH, 7.5) was poured into a column of desired size and allowed to pack at a fast flow rate (about 10 ml/min). In most cases, a rather short fat column (*e.g.*, 6 × 20 cm) affording a fast flow rate proved to be satisfactory. A solution of the oligonucleotide mixture (concentration, 1 mM) in the above-mentioned solvent was applied to the column and washing with the same solvent was continued to remove the non-trityl compounds. The trityl-containing compounds were then eluted by increasing the ethanol (to 75%) and salt concentration. A marked increase in the flow rate was observed with the increase of ethanol concentration in the eluent.

The column effluents were monitored for their absorbance in the uv region with a Zeiss PMQ spectrophotometer. The monomethoxytrityl content of the oligonucleotide fractions was checked by mixing one volume of 60% $HClO_4$ with one volume of an appropriately diluted sample and reading the absorbance at 472 nm within 5–30 min. When necessary, fractions were pooled and concentrated protecting groups were removed, and the products were checked by paper chromatography: system A, isopropyl alcohol–NH_3–H_2O (7:1:2); system B, *n*-propyl alcohol–NH_3–H_2O (55:10:35); system C, ethanol–1 M ammonium acetate (7:3, pH 7.5). The recovery of both trityl and non-trityl oligonucleotides was quantitative from both the trityl- and naphthylcarbamoylcelluloses. Both cellulose derivatives are stable under the conditions of chromatography after many months of use.

Results

Mononucleotides with or without their amino groups acylated showed essentially no adsorption to tritylcellulose columns when they were applied in 10% ethanol + 0.05 M TEAB. Similarly dinucleotides containing thymidylic and/or guanylic acids such as d-pTpT, d-pG^{iB}pG^{iB}, or d-pG^{iB}pT showed negligible adsorption to tritylcellulose when applied under the above conditions. On the other hand, dinucleotides such as d-pC^{An}pC^{An}, d-pA^{Bz}pA^{Bz}, or d-pC^{An}pA^{Bz} and, especially, their symmetrical pyrophosphate derivatives, showed significant adsorption in 10% ethanol + 0.05 M TEAB. These dinucleotides could be removed by increasing the ethanol concentration to about 35%. The stronger adsorption of these latter dinucleotides and their pyrophosphates to tritylcellulose is probably due to the additive effects of the aryl, anisoyl, and benzoyl protecting groups.

Oligonucleotides containing the trityl or monomethoxytrityl group at their 5′ end were adsorbed to tritylcellulose quantitatively even in the presence of 20–40% ethanol. However, if the trityl-containing oligonucleotides were especially rich in thymidylic or guanylic acid residues, their affinity for tritylcellulose decreased slightly. The above results were consistent with the expectation that there were lipophilic interactions between tritylcellulose and trityloligonucleotides containing a varying number of aromatic blocking groups. In view of the foregoing observations it was clear that oligonucleotide mixtures should be applied to a tritylcellulose column with the appropriate amount of ethanol so that only the trityl-containing oligonucleotides would adsorb to the column, the non-trityl-containing oligonucleotides being eluted off. The amount of ethanol required for elution of the non-trityl oligonucleotides was determined by studying the degree of binding of the strongly aromatic dinucleotide d-pA^{Bz}pA^{Bz} to tritylcellulose as a function of the ethanol concentration. As may be seen from Table I the ethanol concentration required to elute quantitatively the dinucleotide was in the range of 35–40% ethanol. At this ethanol concentration the corresponding trityl-terminated dinucleotide, d-MTrA^{Bz}pA^{Bz}, was retained completely by the tritylcellulose column. Thus, the separation of a mixture of d-MTrA^{Bz}pA^{Bz}

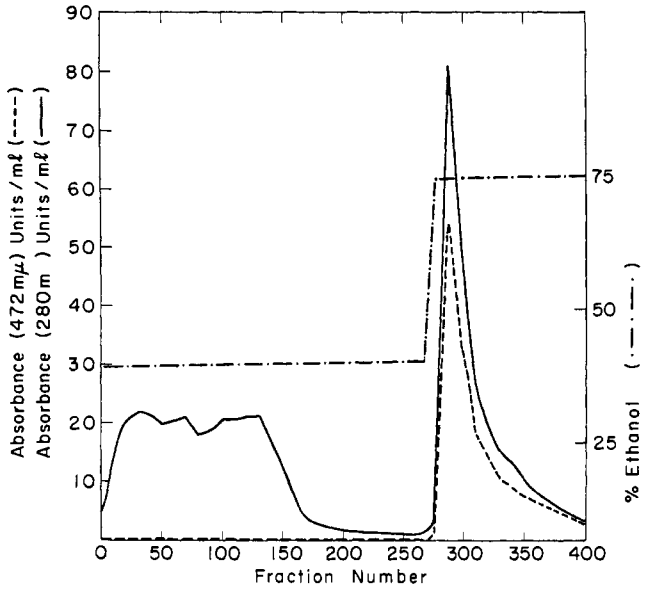


FIGURE 2: Purification of a reaction mixture on tritylcellulose involving the condensation of d-MTrTpC^{An}pG^{iB}pA^{Bz} with d-pC^{An}pC^{An}OAc. Details of the synthesis will be given in a later report (P. J. Cashion, unpublished data). The compounds were dissolved in 3.5 l. of 45% ethanol–0.10 M TEAB and applied to a tritylcellulose column (6 × 32 cm, equilibrated with 45% ethanol–0.10 M TEAB). The column was washed with 2 l. of 45% ethanol–0.10 M TEAB followed by 2.5 l. of 75% ethanol–0.50 M TEAB. Fractions of 20 ml were collected every 2–3 min.

and d-pA^{Bz}pA^{Bz} was effected by applying them to a tritylcellulose column in a 35% ethanol–0.05 M TEAB solution. After removal of d-pA^{Bz}pA^{Bz}, the ethanol concentration was increased to 90% and the trityl-terminated oligonucleotide was eluted as a sharp peak.

Separation of Polynucleotide Mixtures. As an example of the separation of longer oligonucleotides, the reaction mixture shown in Scheme I was applied to a suitable tritylcellulose column in 45% ethanol containing 0.05 M TEAB. Non-tri-

TABLE I: Elution of the Oligonucleotide d-pA^{Bz}pA^{Bz} from Tritylcellulose as a Function of the Ethanol Concentration.[a]

% Ethanol	% d-pA^{Bz}pA^{Bz} Removed
5	32
10	38
25	80
40	100

[a] The dinucleotide, pA^{Bz}pA^{Bz} (400 OD_{280}), was dissolved in 250 ml of 5, 10, 25, and 40% aqueous ethanol containing 50 mM triethylammonium bicarbonate, respectively. The samples were applied to a preequilibrated tritylcellulose column (3.8 × 6 cm). Aliquots of the effluent were checked at 280 nm for their d-pA^{Bz}pA^{Bz} content. The percentage of the applied OD_{280} units collected in the first 400 ml of effluent was the percentage of d-pA^{Bz}pA^{Bz} removed from the column. Beyond this point a negligible amount of the dinucleotide was eluted.

TABLE II: Separation of Oligonucleotide Mixtures on Tritylcellulose.

No.	Oligonucleotide Mixture: Reactants	Oligonucleotide Mixture: Product	% Ethanol–TEAB (M) for Elution of Oligonucleotides: Type II	Type I
1	d-MTrT + d-pC^{An}-OAc	d-MTr-TpC^{An}	40–0.10	90–0.10
2	d-MTrA^{Bz} + d-pG^{iB}OiB	d-MTr-A^{Bz}pG^{iB}	30–0.05	90–0.05
3	d-MTrTpC^{An} + d-pG^{iB}pA^{Bz}OAc	d-MTr-TpC^{An}pG^{iB}pA^{Bz}	30–0.05	90–0.05
4	d-MTrA^{Bz}pG^{iB} + d-pG^{iB}pTOAc	d-MTr-A^{Bz}pG^{iB}pG^{iB}pT	30–0.05	90–0.05
5	d-MTrTpC^{An}pG^{iB}pA^{Bz} + d-pC^{An}pC^{An}OAc	d-MTr-TpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An}	45–0.10	75–0.50
6	d-MTrA^{Bz}pG^{iB}pG^{iB}pT + d-pC^{An}pG^{iB}OAc	d-MTr-A^{Bz}pG^{iB}pG^{iB}pTpC^{An}pG^{iB}	30–0.10	75–0.50
7	d-MTrG^{mB}pTpA^{Bz}pA^{Bz} + d-pTpG^{iB}OAc	d-MTrG^{mB}pTpA^{Bz}pA^{Bz}pTpG^{iB}	30–0.05	75–0.50
8	d-MTrTpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An} + d-pTpG^{iB}OAc	d-MTrTpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An}pTpG^{iB}	45–0.10	75–0.50
9	d-MTrA^{Bz}pG^{iB}pG^{iB}pTpC^{An}pG^{iB} + d-pA^{Bz}pA^{Bz}OAc	d-MTrA^{Bz}pG^{iB}pG^{iB}pTpC^{An}pG^{iB}pA^{Bz}pA^{Bz}	45–0.10	75–0.50
10	d-MTrA^{Bz}pG^{mB}pTpC^{An}pC^{An}pA^{Bz} + d-pC^{An}pTpTOAc	d-MTrA^{Bz}pG^{mB}pTpC^{An}pC^{An}pA^{Bz}pTpC^{An}pA^{Bz}pC^{An}pTpT	40–0.05	75–0.50
11	d-MTrTpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An}pTpG^{iB} + d-pC^{An}pA^{Bz}pG^{iB}pA^{Bz}OAc	d-MTrTpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An}pTpG^{iB}pC^{An}pA^{Bz}pG^{iB}pA^{Bz}	45–0.10	75–0.50
12	d-MTrG^{mB}pTpA^{Bz}pA^{Bz}pTpG^{iB} + d-pC^{An}pTpTOAc	d-MTrG^{mB}TpA^{Bz}pA^{Bz}pTpG^{iB}pC^{An}pTpTpT	30–0.05	75–0.50
13	d-MTrTpG^{iB}pA^{Bz}pC^{An}pG^{iB} + d-pC^{An}pC^{An}OAc	d-MTrTpG^{iB}pA^{Bz}pC^{An}pG^{iB}pC^{An}pC^{An}-OAc	30–0.10	70–0.10
14	TPMpT + pTOAc	TPMpTpT-OAc	25–0.10	70–0.10

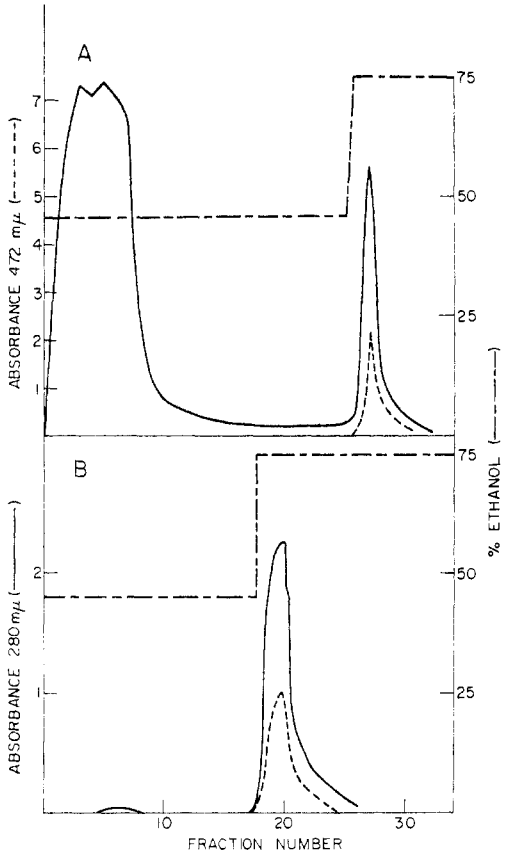


FIGURE 3: (A) Purification of a reaction mixture on tritylcellulose involving the condensation of d-MTrTpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An}pTpG^{iB} with d-pC^{An}pA^{Bz}pG^{iB}pA^{Bz}OAc. Details of the synthesis will be given in a later report (P. J. Cashion, unpublished data). The reaction mixture was diluted with 1 l. of 45% ethanol–0.10 M TEAB and was applied to a tritylcellulose column (3 × 5 cm, equilibrated with 45% ethanol–0.10 M TEAB). The column was then washed with 2 l. of 45% ethanol–0.10 M TEAB followed by 1.5 l. of 75% ethanol–0.50 M TEAB. Fractions of 20 ml were collected every 2–3 min. (B) Rechromatography on tritylcellulose of trityl-positive fractions in part A. Representative aliquots from the trityl-positive peak in part A were pooled and applied in a concentration of 45% ethanol–0.10 M TEAB solution (100 ml) to a small tritylcellulose column (1 × 4 cm, equilibrated with 45% ethanol–0.10 M TEAB). The column was washed with 200 ml of 45% ethanol–0.10 M TEAB followed by 300 ml of 75% ethanol–0.50 M TEAB.

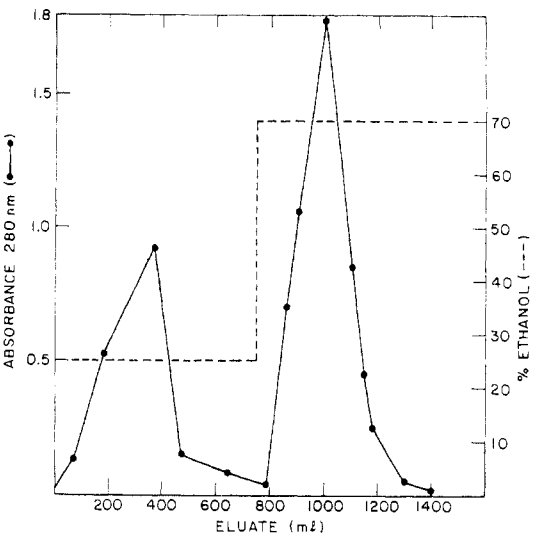


FIGURE 4: Purification of a reaction mixture on naphthylcarbamoylcellulose involving the condensation of TPMpT with pTOAc. The reaction mixture was diluted to 200 ml with 25% ethanol–0.10 M TEAB and applied to a naphthylcarbamoylcellulose column (3.8 × 7 cm, equilibrated with 25% ethanol–0.10 M TEAB). The column was washed with 25% ethanol–0.10 M TEAB (700 ml) followed by 400 ml of 70% ethanol–0.10 M TEAB. Fractions of 10 ml were collected.

tyl-containing compounds were all eluted on continued elution with this solvent. The ethanol concentration was then raised to 75% and the buffer concentration to 0.50 M. As seen in Figure 2, the trityl-containing compounds were now eluted as a sharp peak. It has been a general experience that trityl-containing oligonucleotides of chain length 6 or greater require a higher salt concentration (*e.g.*, 0.50 M TEAB), while oligonucleotides of shorter chain length can be eluted with 0.05 M TEAB concentration.

A large number of separations carried out by using this method are shown in Table II. In particular, it should be noted that the tri- as well as tetranucleotide blocks together with their pyrophosphates can also be separated from the trityl-containing reaction components. An example is given in Figure 3A which shows in the synthesis of the dodecanucleotide d-MTrTpC^{An}pG^{iB}pA^{Bz}pC^{An}pC^{An}pTpG^{iB}pC^{An}pA^{Bz}pG^{iB}pA^{Bz} the excess of the strongly aromatic tetranucleotide d-pC^{An}pA^{Bz}pG^{iB}pA^{Bz}, as well as its pyrophosphate derivative, could be removed from the trityl-containing components of the reaction mixture by passage through tritylcellulose in 45% ethanol–0.05 M TEAB. Increasing the ethanol–salt concentrations to 75%–0.50 M removed the trityl-terminated dodecamer together with the unreacted octamer. When a representative fraction from the trityl-containing peak shown in Figure 3A was reapplied to a small tritylcellulose column and eluted under the conditions described above, a negligible amount of uv-absorbing and trityl-negative component was detected as shown in Figure 3B.

Separation of Oligonucleotides with Unprotected Amino Groups. Even when the aromatic amino protecting groups are not present in the oligonucleotides, the monomethoxytrityl groups are sufficient by themselves to bind to tritylcellulose. A mixture of d-MTrTpGpApCpG and d-MTrTpGpApCpGpCpC and d-pCpC and O(pCpC)$_2$ was applied to tritylcellulose in 10% ethanol–0.05 M TEAB and washed until the OD_{260} was 0.15; finally, the ethanol–TEAB concentrations were raised to 50%–0.50 M to remove the trityl-terminated oligomers. Paper chromatography and thin-layer chromatography (tlc) demonstrated the completeness of the separation and lack of cross-contamination.

Naphthylcarbamoylcellulose. The cellulose used in the following experiments contained 15% of its hydroxyl groups derivatized with naphthylcarbamoyl groups. A number of separations carried out by using this method are shown in Table III. Generally speaking naphthylcarbamoylcellulose columns required lower ethanol concentrations to minimize lipophilic interactions with aromatic-type diblocks such as d-pA^{Bz}pA^{Bz} than do tritylcellulose columns. Thus, d-pA^{Bz}pA^{Bz} was quantitatively recovered from a naphthylcarbamoylcellulose column by application and elution with a 20% ethanol–0.10 M TEAB solution. Similarly when a mixture of d-MTrA^{Bz}pA^{Bz} and the pyrophosphate O(pA^{Bz}pA^{Bz})$_2$ was applied to a naphthylcarbamoyl column in a 40% ethanol–0.1 M TEAB solution, the pyrophosphate component was recovered quantitatively from the eluate; the trityl-containing

TABLE III: Separation of Oligonucleotide Mixtures on Naphthylcarbamoylcellulose.

Oligonucleotide Mixture		% Ethanol–TEAB (M) Used to Elute Oligonucleotides	
Reactants	Product	Type II	Type I
d-MTrA^{Bz}pA^{Bz} + O(pA^{Bz}pA^{Bz})$_2$		40–0.10	70–0.10
TPMpT + d-pTOAc	TPMpTpT	25–0.10	70–0.10
d-MTrG^{mB}pG^{mB}pT + d-pG^{mB}pG^{mB}OAc	d-MTrG^{mB}pG^{mB}pTpG^{mB}pG^{mB}	25–0.05	40–0.5
d-MTrTpG^{iB}pA^{Bz}pC^{An}pG^{iB} + d-pC^{An}pC^{An}OAc	dpMTrTpG^{iB}pA^{Bz}pC^{An}pG^{iB}pC^{An}pC^{An}	30–0.10	70–0.10

component of the mixture was eluted quantitatively from the column with 70% ethanol–0.10 M TEAB.

Figure 4 shows that the TPM-containing nucleotides TPMpT and TPMpTpTOAc may be separated from the simple mononucleotide d-pT-OAc by application to a naphthylcarbamoylcellulose column in a 25% ethanol–0.10 M TEAB solution, followed by a 70% ethanol–0.10 M TEAB elution. As may be seen from Table II, a similar separation of these trityl-terminated phosphoramidates may be obtained with tritylcellulose.

Figure 5 shows that a reaction mixture dealing with the synthesis of a pentanucleotide d-MTrG^{mB}pG^{mB}pTpG^{mB}pG^{mB} may be separated into its respective trityl-containing and non-trityl-containing components by application in a 25% ethanol–0.05 M TEAB solution to a naphthylcarbamoylcellulose column. After removal of the dinucleotide d-pG^{mB}pG^{mB} and its pyrophosphate at the lower ethanol concentration, the trityl components were eluted with 40% ethanol–0.05 M TEAB.

Figure 6 compares the abilities of tritylcellulose and naphthylcarbamoylcellulose to effect a separation of the trityl and non-trityl components of a reaction mixture dealing with the synthesis of a heptanucleotide d-MTrTpG^{mB}pA^{Bz}pC^{An}pG^{mB}pC^{An}pC^{An}. The strongly aromatic diblock d-pC^{An}pC^{An}, its pyrophosphate, and the arylsulfonic acid were removed quantitatively from both columns with a 40% ethanol–0.10 M TEAB solution; increasing the ethanol concentration to 75% brought out the trityl-terminated pentamer and heptamer.

Discussion

That 5′-*O*-trityl-substituted oligonucleotides are held more strongly on cellulose anion-exchanger columns than their non-trityl counterparts has been evident throughout the separation work in polynucleotide synthesis. The retardation of the trityl compound could only be ascribed to nonionic interactions. As expected, elutions were always facilitated by increasing ethanol concentration in the eluent. Tener's group (Gillam *et al.*, 1967) clearly recognized and elegantly exploited nonionic interactions by using BD-cellulose for tRNA separations. The present work has described another application of the same concept for the rapid grouping of the reaction mixtures encountered in synthetic work.

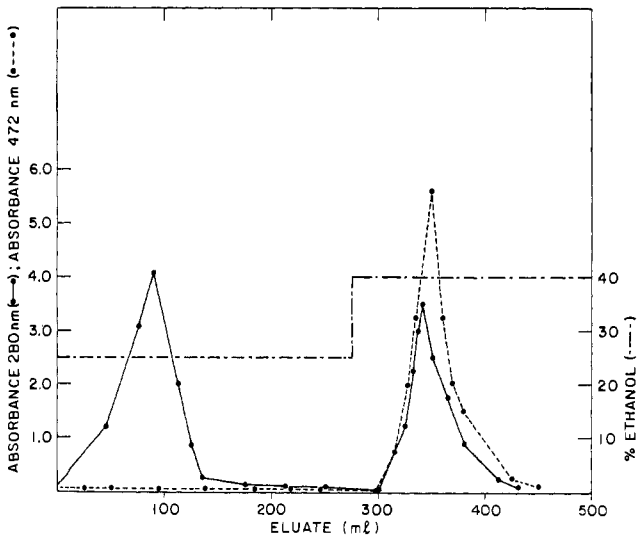


FIGURE 5: Purification of a reaction mixture on naphthylcarbamoylcellulose involving the condensation of d-MTrG^{mB}pG^{mB}pT with d-pG^{mB}pG^{mB}OAc. The reaction mixture was diluted to 50 ml with 25% ethanol–0.05 M TEAB and was applied to a naphthylcarbamoylcellulose column (1.4 × 11 cm, equilibrated with 25% ethanol–0.05 M TEAB). The column was washed with 300 ml of 25% ethanol–0.05 M TEAB followed by 40% ethanol–0.05 M TEAB. Fractions of 10 ml were collected.

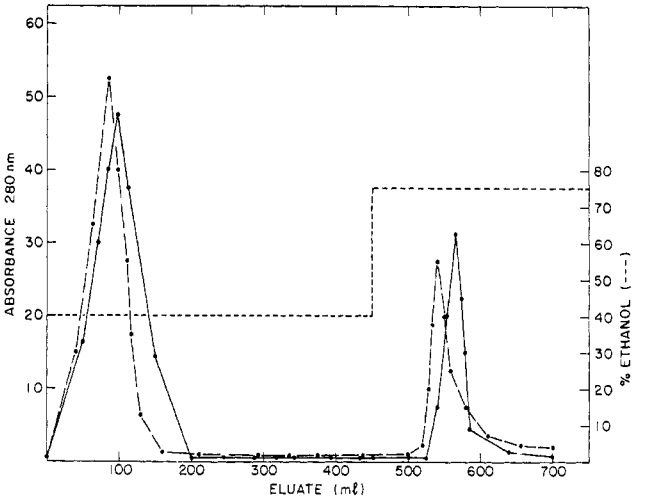
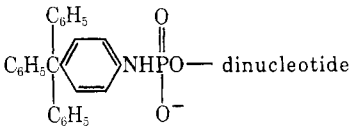


FIGURE 6: Comparison of the purification of a reaction mixture on both trityl- and naphthylcarbamoylcellulose involving the condensation of d-MTrTpG^{mB}pA^{Bz}pC^{An}pG^{mB} with d-pC^{An}pC^{An}OAc. The reaction mixture was diluted with 200 ml of 40% ethanol–0.10 M TEAB and applied in equal portions to both tritylcellulose and naphthylcarbamoylcellulose columns (each 1.4 × 10 cm, equilibrated with 40% ethanol–0.10 M TEAB). After application the columns were washed with 1 l. of 40% ethanol–0.10 M TEAB followed by 1 l. of 75% ethanol–0.50 M TEAB in the case of tritylcellulose (—·—·—·—), and by 1 l. of 75% ethanol–0.10 M TEAB in the case of naphthylcarbamoylcellulose (————).

Both trityl- and naphthylcarbamoylcelluloses were, in general, satisfactory for the separation of the trityl from the non-trityl products. However, one may be preferred over the other depending upon the nature of the reaction mixtures. Trityl-containing compounds are absorbed to tritylcellulose more strongly than to naphthylcarbamoylcellulose and, consequently, with larger oligonucleotides, higher salt concentration was required for eluting from tritylcellulose. Elutions from naphthylcarbamoylcellulose require low salt and a further practical point is that the latter derivative is prepared more easily and inexpensively.

While the trityl group was the major source of the nonionic interaction, the groups used to protect the amino groups, such as benzoyl and anisoyl, also contributed in a supplementary way to the overall afinity of the trityl oligonucleotides for the tritylcellulose. Guanine nucleotides when protected with an aliphatic group, such as the isobutyryl group, showed less affinity for both cellulose derivatives.

Both tritylcellulose and naphthylcarbamoylcellulose columns show considerable affinity for other aromatic-type groups present within oligonucleotides such as TPM-phosphoramidates of the general structure



Oligonucleotides containing only the naphthylcarbamoyl grouping at their 3′ terminus such as d-TpTpTpA-CONHC$_{10}$H$_7$ were likewise absorbed strongly to tritylcellulose but showed weak binding to naphthylcarbamoylcellulose (Agarwal and Khorana, 1972). Recently these observations have been extended to a 3′-naphthylcarbamoyl-terminated octanucleotide (K. L. Agarwal, unpublished data). Finally the method developed in this paper may find applications in other areas such as in polypeptide or carbohydrate chemistry.

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Nuclear Double-Stranded Ribonucleic Acid of Mammalian Cells. Characteristics and Biosynthesis†

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ABSTRACT: Ribonuclease-resistant RNA synthesis was examined in Sarcoma-180 cells, Ehrlich ascites tumor cells, and L cells in the presence of high or low concentrations of actinomycin D. The RNase-resistant species were characterized by sucrose gradient analysis, Sepharose 2B column chromatography, and thermal denaturation studies. These species are predominantly of nuclear origin and their synthesis is sensitive to 5 μg/ml of actinomycin D. In all the cell lines, this RNA species is polydisperse on sucrose gradient and of low molecular weight. By thermal denaturation profile, it appears to be heterogeneous and not completely base paired. Sarcoma-180 cells not only contain the low molecular weight species, but also a high molecular weight double-stranded RNA that has characteristics of a completely base-paired molecule, when compared with the replicative form of a picornavirus.

Ribonuclease-resistant "presumptive" double-stranded species of RNAs have been detected recently in low amounts in uninfected animal cells (Montagnier, 1968; Stollar and Stollar, 1970; Kimball and Duesberg, 1971). A portion of this double-stranded RNA (ds-RNA)[1] has been reported to be synthesized in the presence of actinomycin D in Burkitt lymphoma cells (Stern and Friedman, 1971).

ds-RNA is also known to occur in the replicative cycle of

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[1] Abbreviations used are: ds-RNA, double-stranded ribonucleic acid; Hn-RNA, heterogeneous nuclear ribonucleic acid; PFU, plaque-forming units; MEM, minimum essential medium; RF, replicative form; SSC, standard saline citrate; ss-RNA, single-stranded ribonucleic acid.