Oligonucleotide Synthesis. II. The Use of Substituted Trityl Groups

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Received April 5, 1971

Several new substituted trityl protecting groups have been prepared and investigated. They are di(*p*-benzyloxyphenyl)phenylmethanol (**2a**), (*p*-hydroxyphenyl)diphenylmethanol (**2b**), (*p*-acetoxyphenyl)diphenylmethanol (**2c**), (*m*-hydroxyphenyl)diphenylmethanol (**2d**), (*m*-acetoxyphenyl)diphenylmethanol (**2e**), and (*p*-bromophenacyloxyphenyl)diphenylmethanol (**2f**). Comparison of the rates of detritylation of the corresponding 5′-trityladenosine derivatives by acetic acid showed that the (*p*-hydroxyphenyl)diphenylmethyl group could be removed under mild conditions in a reasonable length of time. However, this group cannot be used directly in oligonucleotide synthesis, without protection of the phenolic function. Consequently the trityl chloride (*p*-bromophenacyloxyphenyl)diphenylmethyl chloride (BPTrCl) (**3f**) was used as the phenacyl ester is cleaved by mild reduction with zinc and acetic acid to give the *p*-hydroxytrityl group. Two dinucleoside monophosphates TpT (**5a**) and d-UpT (**5b**) have been synthesized using the BPTr group for protection of the 5′-hydroxyl position. The removal of the protecting group was studied both in the presence and absence of other acyl protecting groups. Application to the ribose series was investigated by the preparation of the dinucleoside monophosphate 5′-BPTr-UpU (**12**) and the trinucleoside diphosphate 5′-BPTr-UpUpU (**15**). Removal of the BPTr group from these compounds was achieved with 20% acetic acid and zinc dust. However, it was found that the presence of other acyl protecting groups complicated the detritylation when using zinc and acetic acid, so that for further synthetic work detritylation was achieved with formic acid.

The selective protection of reactive groups in nucleosides and nucleotides is of utmost importance for the successful chemical synthesis of oligo- and polynucleotides of predetermined base sequence.[1] Acid-labile protecting groups such as the trityl group (Tr)[2] and its mono- (MMTr), di- (DMTr), and trimethoxy (TMTr) derivatives are widely used for protection of the 5′ primary hydroxyl function of nucleosides.[3] The introduction of methoxy groups increases the ease of removal of the trityl groups, but also increases the rate of reaction with the secondary hydroxyl groups and the amino functions of the bases.[3] As a result the most widely used trityl group is the MMTr.

As part of a general program on the synthesis of polynucleotides of predetermined base sequence, we have undertaken a study of substituted trityl chlorides to investigate whether improvements could be made of their ease of removal while maintaining selectivity toward the 5′-hydroxyl group. In particular, we have developed the use of the *p*-bromophenacyloxytrityl group and report its use in oligonucleotide synthesis.

Results

The trityl alcohols di(*p*-benzyloxyphenyl)phenylmethanol (DPTrOH, **2a**), (*p*-hydroxyphenyl)diphenylmethanol (*p*-HOTrOH, **2b**), and (*m*-hydroxyphenyl)diphenylmethanol (*m*-HOTrOH, **2d**) were prepared from the corresponding ketones (**1a**, **1b**, and **1d**) *via* a Grignard reaction with phenylmagnesium bromide.[4] The trityl chloride DPTrCl (**3a**) was prepared from **2a** by chlorination with acetyl chloride. Treatment of the methanol **2b** with acetyl chloride gave the corresponding acetoxy derivative (*p*-acetoxyphenyl)diphenylmethyl chloride (**3c**), and, similarly, *m*-acetoxytrityl chloride (**3e**) was prepared from **2d**. Attempts to prepare *p*- and *m*-hydroxytrityl chlorides (**3b** and **3d**) by treatment with hydrogen chloride in ether in the presence of calcium chloride were unsuccessful.

(*p*-Bromophenacyloxyphenyl)diphenylmethyl chloride (BPTrCl, **3f**) was synthesized by reacting the trityl alcohol **2b** with *p*-bromophenacyl bromide and then chlorinating the intermediate trityl alcohol (**2f**) with acetyl chloride.

On treating the trityl alcohol **2f** with zinc dust and 80% acetic acid at room temperature, it was completely reduced to *p*-HOTrOH (**2b**) in under 1 hr. In 20% acetic acid containing zinc the compound was significantly reduced (>25%) in 1 hr and the reaction was complete in 16 hr. No reduction was observed in the absence of the zinc dust or when ethanol was substituted for acetic acid.

The trityl chlorides **3a**, **3c**, **3e**, and **3f** were used to prepare the 5′-protected derivatives 5′-DPTr-A (**4a**), 5′-(*p*-AcOTr)-A (**4c**), 5′-(*m*-AcOTr)-A (**4e**), and 5′-BPTr-A (**4f**). In the deoxyribose series 5′-BPTr-T (**5a**), 5′-BPTr-dU (**5b**), 5′-BPTr-dA (**5c**), and 5′-BPTr-dG (**5d**) were prepared. Deactylation of **4c** and **4e** with ammonia afforded 5′-(*p*-HOTr)-A (**4b**) and 5′-(*m*-HOTr)-A (**4d**), respectively.

The rates of detritylation of the protected ribonucleosides **4a–f** were studied and compared with those of 5′-MMTr-A and 5′-DMTr-A, and the results are summarized in Table I. Detritylation of **4f** was also studied in the presence of zinc dust.

The intermediate *p*-HOTr derivatives **4b** and **6a–d** were isolated by preparative tlc from the corresponding BPTr nucleosides by treatment with 30% acetic acid and zinc dust for 45 min together with unprotected nucleoside.

The use of the BPTr group in oligonucleotide synthesis was demonstrated by the preparation of the deoxyribodinucleoside monophosphates TpT (**7a**) and d-UpT (**7b**) as shown in Scheme I. In these syntheses, the 3′-hydroxyl function of the phosphorylating moiety was protected with the dihydrocinnamoyl group, which is removable by alkaline pH or enzymatically by α-chymotrypsin at neutral pH.[5]

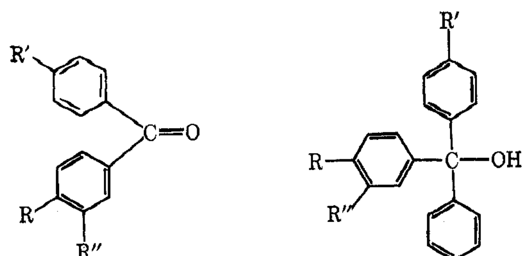
(1) H. Kössel, H. Buchi, T. M. Jacob, A. R. Morgan, S. A. Narang, E. Ohtsuka, R. D. Wells, and H. G. Khorana, *Angew. Chem., Int. Ed. Engl.*, **8**, 387 (1969); H. G. Khorana, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, **19**, 931 (1960).

(2) The system of abbreviations used in this paper is that of H. G. Khorana's group; compare, for example, H. Kössel, H. Buchi, and H. G. Khorana, *J. Amer. Chem. Soc.*, **89**, 2185 (1967).

(3) M. Smith, D. H. Rammler, T. H. Goldberg, and H. G. Khorana, *ibid.*, **84**, 430 (1962).

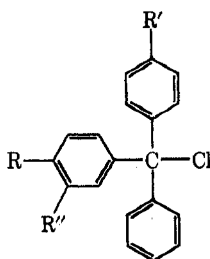
(4) Preparative methods were adapted from M. Gomberg and L. H. Cone, *Justus Liebigs Ann. Chem.*, **370**, 142 (1909).

(5) The rationale for using the dihydrocinnamoyl protecting group is described in H. S. Sachdev and N. A. Starkovsky, *Tetrahedron Lett.*, **9**, 733 (1969).

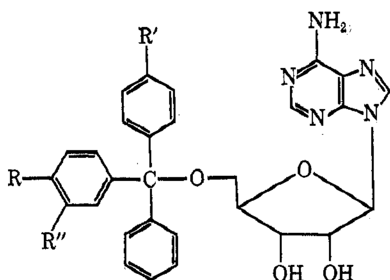


1a, R = R′ = OC_6H_5; R″ = H
b, R = OH; R′ = R″ = H
d, R = OH; R = R′ = H

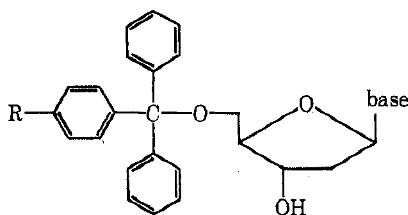
2a, R = R′ = OC_6H_5; R″ = H
b, R = OH; R′ = R″ = H
c, R = OAc; R′ = R″ = H
d, R″ = OH; R = R′ = H
e, R″ = OAc; R = R′ = H
f, R = $OCH_2COC_6H_4Br$; R′ = R″ = H



3a, R = R′ = OC_6H_5; R′ = H
b, R = OH; R′ = R″ = H
c, R = OAc; R′ = R″ = H
3d, R″ = OH; R = R′ = H
e, R″ = OAc; R = R′ = H
f, R = $OCH_2COC_6H_4Br$; R′ = R″ = H



4a, R = R′ = OC_6H_5; R″ = H
b, R = OH; R′ = R″ = H
c, R = OAc; R′ = R″ = H
4d, R″ = OH; R = R′ = H
e, R″ = OAc; R = R′ = H
f, R = $OCH_2COC_6H_4Br$; R′ = R″ = H



5a, R = $OCH_2COC_6H_4Br$; base = Thy
b, R = $OCH_2COC_6H_4Br$; base = Ura
c, R = $OCH_2COC_6H_4Br$; base = Ade
d, R = $OCH_2COC_6H_4Br$; base = Gua
6a, R = OH; base = Thy
b, R = OH; base = Ura
c, R = OH; base = Ade
d, R = OH; base = Gua

Detritylation of **7a** and **7b** was studied in detail and the results are shown in Scheme II. The BPTr group could be removed slowly from **7a** by 80% acetic acid alone (8–24 hr).

The application of this protecting group to ribooligonucleotide synthesis was studied. The mononucleotide 5′-BrTr-U(OAc)-3′-p (**11**) was prepared by reaction of uridine 2′,3′-cyclic phosphate with BPTrCl followed by incubation with pancreatic ribonuclease to open the 2′,3′-cyclic phosphate and protection of the 2′-hydroxyl function by acetylation. The protected monomer was condensed in the usual way[6] with dibenzoyluridine to prepare the ribodinucleoside monophosphate **12** as shown in Scheme III.

TABLE I
TIME REQUIRED FOR FULL DEPROTECTION OF 5′-TRITYLADENOSINE COMPOUNDS WITH ACETIC ACID AT ROOM TEMPERATURE[a]

Compound	Time: 80% HOAc	40% HOAc	20% HOAc
5′-MMTr-A	1 hr	48 hr	1 week
5′-DMTr-A	15 min	3 hr	48 hr
5′-DPTr-A (**4a**)	15 min	3 hr	1 week
5′-(*p*-HOTr)-A (**4b**)	1 hr	1 hr	6 hr
5′-(*p*-AcOTr)-A (**4c**)	1 week		
5′-(*m*-HOTr)-A (**4d**)	48 hr	1 week	
5′-(*m*-AcOTr)-A (**4e**)	1 week		
5′-BPTr-A (**4f**)	5 hr	1 week	
5′-BPTr-A + zinc[b] (**4f**)	1 hr	2 hr	24 hr

[a] 15–20 μmol of 5′-trityladenosine in 0.2 ml of acetic acid. [b] 20 mg of zinc dust.

The acyl protecting groups were removed from **12** by treatment with ammonia for 16 hr to give 5′-BPTr-UpU (**15**) (see Scheme IV). Detailed studies showed that the BrTr group was removed from both **12** and **15** by 20% acetic acid and zinc in under 1 hr, but the detritylation of **15** proved to be cleaner than that of the fully protected dinucleoside monophosphate **12**. In the case of **12** several side products, which were not identified, were also formed. Similar results were obtained with 10, 40, and 50% acetic acid and zinc. However, it was found that a brief treatment with formic acid also removed the BPTr group cleanly from the fully protected dinucleoside monophosphate.

As a result, for further synthetic work the BPTr group was removed from **12** by treatment with 90% formic acid for 10 min, to give **13**. Condensation of **13** with **11** gave the fully protected trinucleoside diphosphate **14** (see Scheme III).

The series of reactions summarized in Scheme V were carried out. Detritylation of the fully protected trinucleoside diphosphate **14** again proved to be more difficult than that of the partially protected trimer **18**, and as in the case of the dinucleoside monophosphates a brief treatment with formic acid gave better results.

We were unable to find conditions under which appreciable amounts of the intermediate *p*-HOTr protected dinucleoside monophosphates and trinucleoside diphosphates could be isolated. The *p*-HOTr group was obviously removed as fast as it was formed with 20% and even with 10% acetic acid.

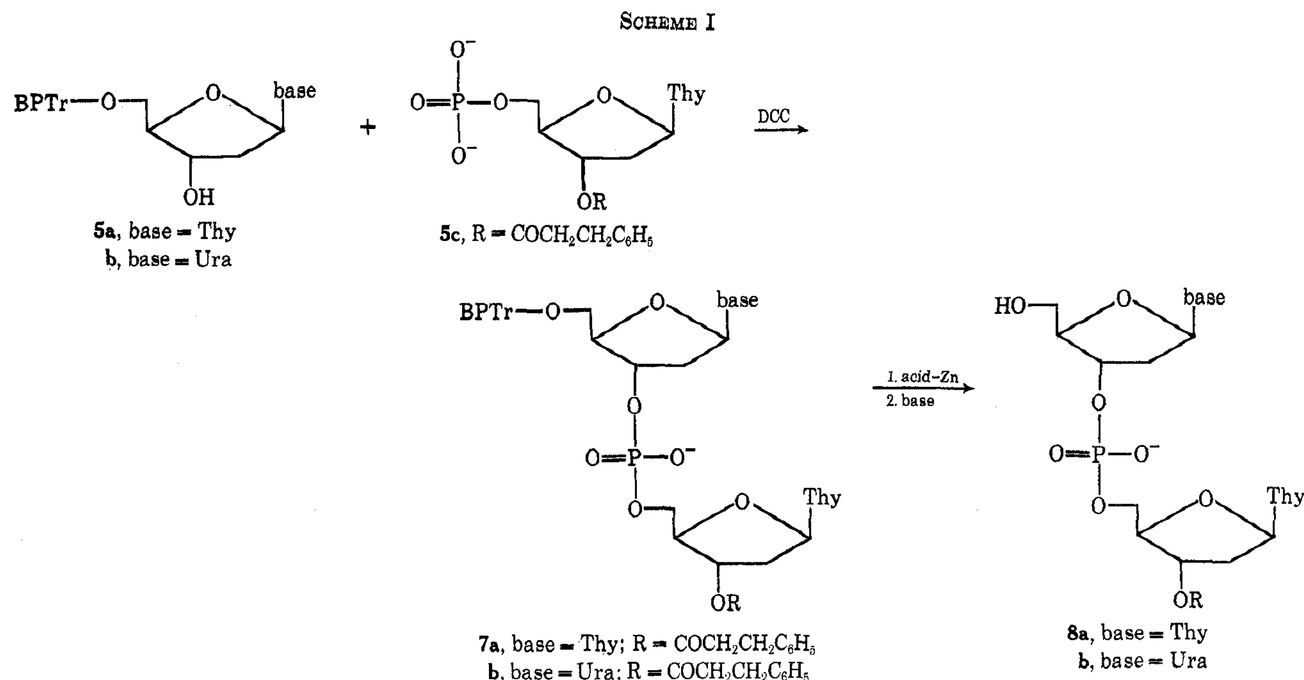
The identity of the dimers TpT, d-UpT, UpU (**16**), and the trimer UpUpU (**19**) was confirmed by degradation with snake venom phosphodiesterase.[7]

Discussion

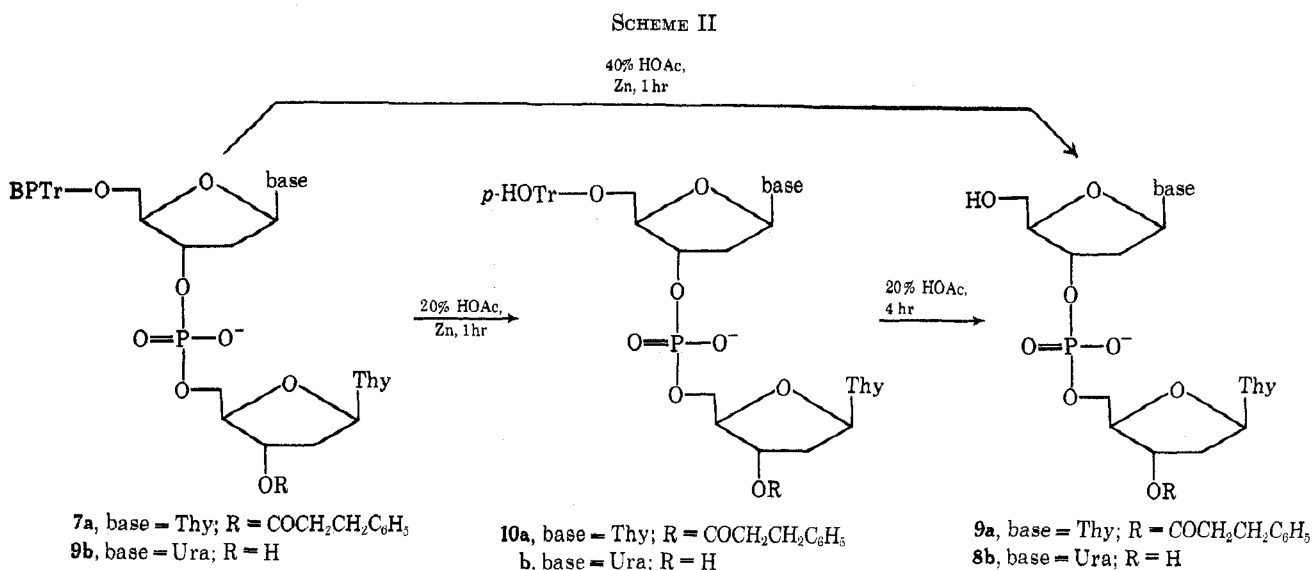
At present the MMTr and DMTr groups are the most frequently used acid-labile protecting groups for the 5′-hydroxyl functions of nucleosides. The DMTr group can be removed under milder conditions than the MMTr group, but it is also less specific for the primary hydroxyl function. In addition, the greater lability of

(6) S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Amer. Chem. Soc.*, **87**, 2988 (1965).
(7) H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *ibid.*, **83**, 686 (1961); H. G. Khorana and J. P. Vizsolyi, *ibid.*, **83**, 675 (1961).

SCHEME I



SCHEME II



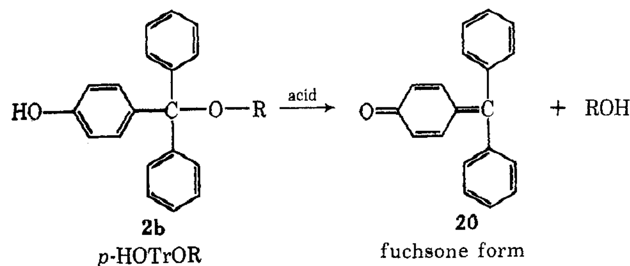
this trityl group can lead to unwanted removal of the protecting group during a synthetic sequence.

We have used these two groups for comparison with the substituted trityl groups described in this paper. Particular attention has been given to the selectivity of the trityl chlorides for primary and secondary hydroxyl functions and to the ease of removal from nucleosides.

The DPTr group was synthesized in order to determine the effect of size on the selectivity of a trityl group. This bulky trityl group showed excellent selectivity for the primary hydroxy function, no other isomer being observed. Similarly, BPTrCl reacted selectively with the 5′-hydroxyl group of nucleosides to give excellent yields of the protected compounds.

The p-HOTr group was synthesized as detritylation of a nucleoside protected by this group should be particularly easy because of the formation of the fuchsone form (**20**) of the trityl alcohol in the presence of acid.

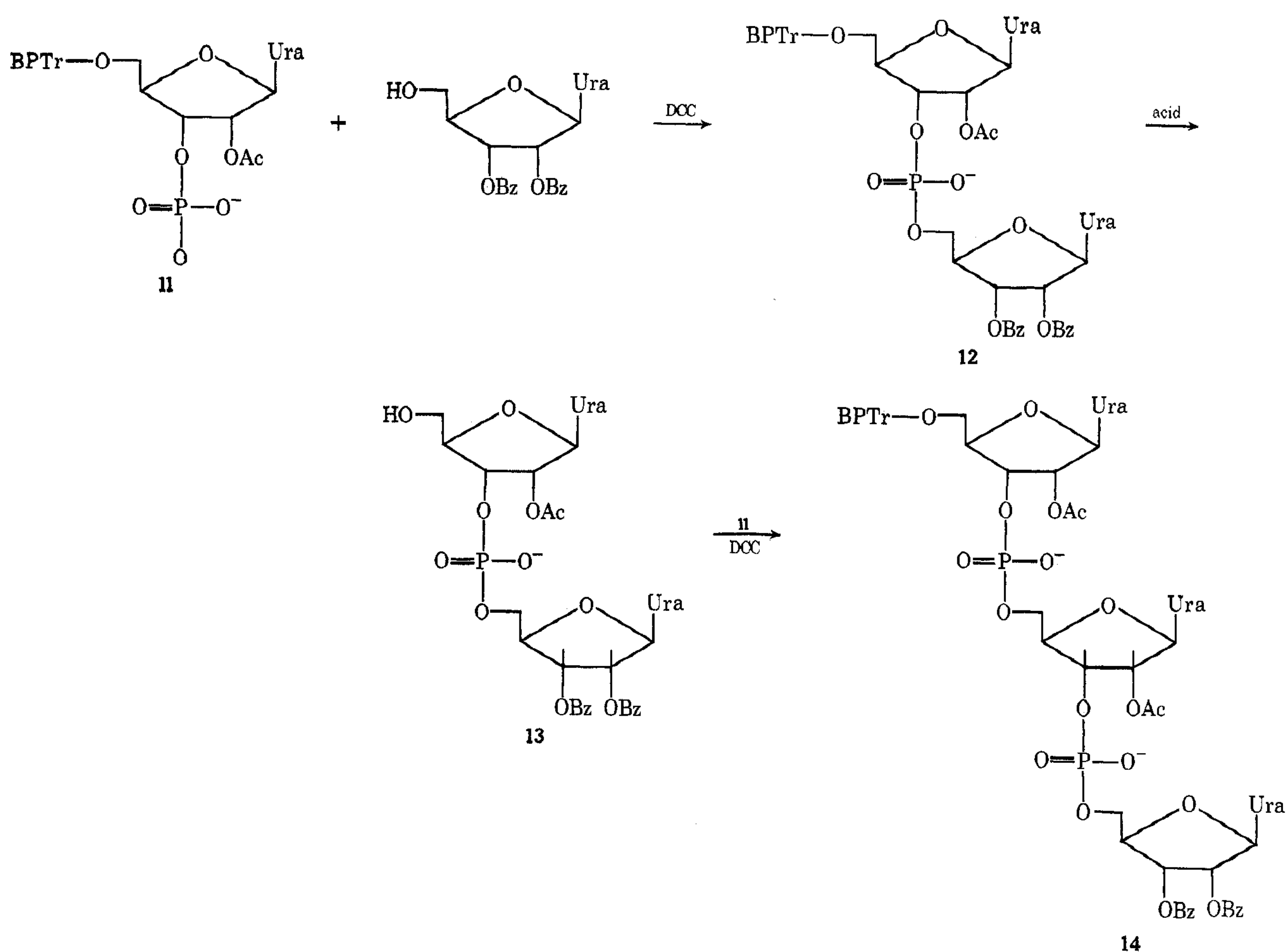
(p-Hydroxyphenyl)diphenylmethanol (**2b**) is peculiar in that when crystallized from ammoniacal alcohol the crystals are colorless, whereas those obtained from 50% acetic acid are yellow. The yellow color is thought to be due to the presence of the fuchsone.[8]



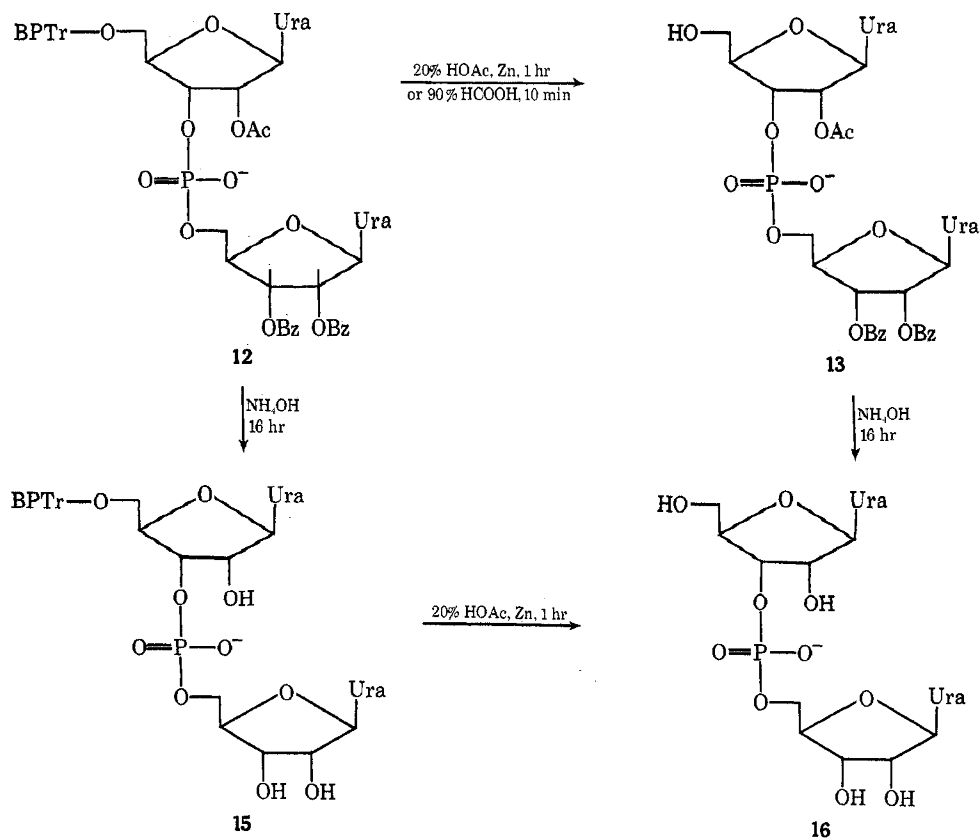
However, the p-HOTr group cannot be used directly in oligonucleotide synthesis for two reasons. Firstly, during the condensation step the phenolic function must be protected as sulfonyl chlorides, used as condensing agents, will react with phenols to form sulfonic esters, and, secondly, the corresponding trityl chloride

(8) L. C. Anderson and M. Gomberg, *J. Amer. Chem. Soc.*, **35**, 203 (1913); K. I. Beynon and S. T. Bowden, *J. Chem. Soc.*, 4247 (1957).

SCHEME III



SCHEME IV



SCHEME V

BPTr—O—[Ura, OAc]—O—P(=O)(O⁻)—O—[Ura, OAc]—O—P(=O)(O⁻)—O—[Ura, OBz OBz] **14** —(20% HOAc, Zn, 1 hr or 90% HCOOH, 10 min)→ HO—[Ura, OAc]—O—P(=O)(O⁻)—O—[Ura, OAc]—O—P(=O)(O⁻)—O—[Ura, OBz OBz] **17**

14 —(NH_4OH, 16 hr)→ **18**; **17** —(NH_4OH, 16 hr)→ **19**

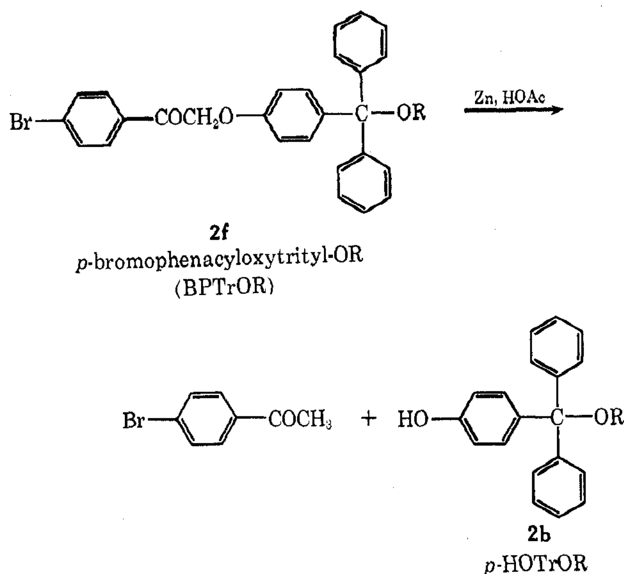
BPTr—O—[Ura, OH]—O—P(=O)(O⁻)—O—[Ura, OH]—O—P(=O)(O⁻)—O—[Ura, OH OH] **18** —(20% HOAc, Zn, 1 hr)→ HO—[Ura, OH]—O—P(=O)(O⁻)—O—[Ura, OH]—O—P(=O)(O⁻)—O—[Ura, OH OH] **19**

p-HOTrCl is unstable and loses HCl spontaneously to give **20**.[9] The synthesis of *m*-HOTrCl by chlorination of the methanol *m*-HOTrOH with acetyl chloride has been reported.[9] However, we were unable to repeat this work, as we obtained only the acetoxy derivative *m*-AcOTrCl in high yield from this reaction. Similarly *p*-AcOTrCl[10] was the only product from the reaction of *p*-HOTrOH with acetyl chloride. Treatment of either trityl alcohol with dry hydrogen chloride in ether in the presence of calcium chloride was also unsuccessful.

The phenolic function of the *p*-HOTr group could be protected by the acetyl group as 5′-(*p*-AcOTr)-A (**4c**) could be deacetylated readily with ammonia to give 5′-(*p*-HOTr)-A (**4b**). However, the acetyl group was not thought to be the ideal protecting group during oligonucleotide synthesis, as other alkali-labile protecting groups are used both in the ribose and deoxyribose series. Consequently a phenacyl ether, which can be removed by reductive cleavage with zinc and dilute acid,[11] was utilized to protect the phenolic function of the *p*-HOTr group.

Comparison of the ease of detritylation of all these substituted trityl compounds was carried out using the corresponding 5′-trityladenosine derivatives, 5′-MMTr-A and 5′-DMTr-A, and the results are shown in Table I. The DPTr group was hydrolyzed by 80 and 60% acetic acid at a rate comparable to the DMTr group but much slower with 20% acid. It should be noted that complete and rapid removal of the trityl group in the presence of alkali-labile base-protecting groups frequently leads to depurination, so that it is advisable when complete deprotection is sought to submit the protected compound first to treatment with alkali and then with acid.[12-14] However, in the

(9) S. T. Bowden and K. I. Beynon, *J. Chem. Soc.*, 4253 (1957).
(10) M. Gomberg, *J. Amer. Chem. Soc.*, **35**, 209 (1913).
(11) J. B. Hendrickson and C. Kandall, *Tetrahedron Lett.*, 343 (1970).
(12) P. T. Gilham and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 6212 (1958).
(13) H. Schaller, G. Weiman, B. Lerch, and H. G. Khorana, *ibid.*, **85**, 3821 (1963).
(14) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3828 (1963).



sequential synthesis of oligonucleotides, it is essential to be able to remove the trityl group efficiently while keeping the alkali-labile base protecting groups intact. As a check on the use of removal of the DPTr group under these conditions $N,N',O^{2'},O^{3'}$-tetrabenzoyladenosine was synthesized by successive tritylation, benzoylation, and detritylation of adenosine using the DPTr group. The yield and purity of the product was slightly better than that obtained using the DMTr group.

Both acetoxytrityl groups, as expected, proved to be very resistant to hydrolysis. The *m*-HOTr group was similar. However, the *p*-HOTr group could be removed rapidly be acetic acid and, in fact, more easily with 20% acetic acid than either the MMTr or DMTr groups. The BPTr group on treatment with acetic acid alone was relatively resistant, but on addition of zinc dust detritylation took place at a rate comparable to the DMTr group. Detritylation occurred in two stages, the first, and rate-determining step, being removal of the phenacyl ether to give the *p*-HOTr derivative. Attempts to find conditions under which the *p*-HOTr derivative could be isolated quantitatively failed as under all conditions investigated the compound was isolated together with the free nucleoside.

In the deoxyribose series the nucleosides thymidine, deoxyuridine, deoxyadenosine, and deoxyguanosine were protected with the BPTr group. Detritylation of these compounds was studied giving particular attention, in the case of deoxyadenosine and deoxyguanosine, to depurination.[12-14] As can be seen from Table II, detritylation could be achieved without depurination with 20 and 40% acetic acid and zinc, although some depurination was detected with 80% acid. Depurination was more noticeable with deoxyguanosine than with deoxyadensoine.

From these results it can be seen that the BPTr group combined the best properties of all the substituted trityl groups investigated. It showed excellent selectivity for the 5′-hydroxyl function of nucleosides because of its large size, and could be removed easily with acetic acid containing zinc dust. In the absence of zinc dust the group was resistant to hydrolysis. In oligonucleotide synthesis it is obviously advantageous to use a hydrolysis resistant group such as the BPTr

TABLE II

TIME REQUIRED FOR FULL DEPROTECTION OF 5′-BPTr NUCLEOSIDES BY ACETIC ACID AND ZINC AT ROOM TEMPERATURE[a]

Compound	Time, hr		
	80% HOAc	40% HOAc	20% HOAc
5′-BPTr-A (**4f**)	1	2	24
5′-BPTr-T (**5a**)	1	2	8
5′-BPTr-dU (**5b**)	1	2	8
5′-BPTr-dA (**5c**)	1 (2%)[b]	2 (<1%)	8 (<1%)
5′-BPTr-dG (**5d**)	1 (5%)[b]	2 (1%)	8 (<1%)

[a] 15–20 μmol of 5′-trityl nucleoside in 0.2 ml of acetic acid containing 20 mg of zinc dust. [b] % depurination as measured by elution of spots from paper chromatograms and measurement of absorbance at λ_{max}.

group, which at the correct time can be converted into a more labile group.

The use of this protecting group in synthesis was demonstrated by the preparation of the deoxyribodinucleoside monophosphates TpT (**8a**) and d-UpT (**8b**) as shown in Scheme I. Removal of the BPTr group was studied in one case (**8a**) before and in the other (**8b**) after removal of the dihydrocinnamoyl group from the 3′-hydroxyl position. The presence of other protecting groups appeared to have no effect on the ease of removal of the BPTr group.

The results on the detritylation of the two deoxyribodinucleoside monophosphates paralleled the results at the monomer level. The BPTr group can be removed rapidly and completely by mild acid and zinc. In the absence of zinc the BPTr group was stable.

In the ribose series the dinucleoside monophosphate UpU (**16**) and the trinucleoside diphosphate UpUpU (**19**) were synthesized utilizing the BPTr group. It was found that the BPTr group could be removed from ribose dinucleoside monophosphates and trinucleoside diphosphates under very mild conditions, but when other acyl protecting groups were present the removal was complicated by the formation of side products which we were unable to identify. There was no evidence of hydrolysis of the glycosidic bonds.

However, it was noted that a brief treatment with formic acid removed the BPTr group without any complications at both the dinucleoside monophosphate and trinucleoside diphosphate levels. This was, therefore, the method of choice for these particular compounds for further synthetic work.

It appears from these results that the *p*-bromophenacyloxytrityl group, while eminently suitable for use in synthesis in the deoxyribose series, is not the ideal diprotecting group when used in the ribose series. The *p*-HOTr group itself is entirely satisfactory being removed rapidly by mildly acidic conditions (less than 20% acetic acid), but a better protecting group for the phenolic function, than the *p*-bromophenacyl ether, is mandatory.

Experimental Section

General Methods.—Paper chromatography was carried out by the descending technique using Whatman No. 1 or Whatman No. 3MM paper. The solvent systems used were (A) ethyl alcohol–1 *M* ammonium acetate (pH 7.5) (7:3, v/v); (B) ethyl acetate–ethanol (9:1); (C) *n*-PrOH–concentrated NH_4OH–H_2O (55:10:35); (D) *i*-PrOH–concentrated NH_4OH–H_2O (7:1:2). Thin layer chromatography was carried out on silica gel plates (F-254 E Merck).

The trityl groups or substituted trityl groups in compounds

were detected by spraying the chromatograms with 10% aqueous perchloric acid and drying in warm air. The trityl-containing compounds appeared yellow or orange. The presence of phenolic functions was detected by lightly spraying the chromatograms with a saturated solution of *p*-nitrobenzenediazonium fluoroborate followed by spraying with 20% sodium bicarbonate solution. Compounds containing phenolic functions appeared as pink spots.

Reagent grade pyridine was purified by distillation over chlorosulfonic acid and potassium hydroxide and stored over 4A molecular sieve beads (Linde Co.). All evaporations were carried under reduced pressure below 25°. Whenever necessary, reagents and reaction mixtures were rendered anhydrous by repeated evaporation of added dry pyridine *in vacuo*.

Enzymatic degradations were carried out by standard methods.[7]

Melting points are uncorrected. Elemental analyses were carried out by Dr. C. Fitz, Needham Heights, Mass.

The amounts of nucleotides in solution were estimated by their absorption at neutral pH at 260 mμ.

Adaptations of published procedures were used to prepare 5′-MMTr-A,[15] 5′-DMTr-A,[15] 2′,3′-dibenzoyluridine,[13] *p*-HOTrOH,[8,9] *m*-HOTrOH,[8,9] DPTrOH,[8,9] DPTrCl,[9] *p*-AcOTrCl,[10] and *m*-AcOTrCl.[10]

(*p*-Bromophenacyloxyphenyl)diphenylmethanol (2f).—(*p*-Hydroxyphenyl)diphenylmethanol (**2b**) (5.52 g, 20 mmol), *p*-bromophenacyl bromide (5.56 g, 20 mmol), and powdered potassium carbonate (20 g) were stirred overnight in dry acetone (200 ml) at 30°. The reaction mixture was filtered and evaporated to dryness. The residue was crystallized from methanol (20 ml) to give 8.0 g (88%) of **2f**: mp 118–120°; λ_{max} (EtOH) 260 mμ (ε 21,800); ir (Nujol) 2.80, 2.92, 5.84, 6.20, 6.30, 8.15, 8.44, 9.10 μ.

Anal. Calcd for $C_{27}H_{21}O_3Br$: C, 68.5; H, 4.5; Br, 16.9. Found: C, 68.2; H, 4.7; Br, 16.5.

(*p*-Bromophenacyloxyphenyl)diphenylmethyl Chloride (3f).—The above methanol (2f) (1 g) was dissolved in acetyl chloride (15 ml) with warming. The deep yellow solution was kept at room temperature 15 min, diluted to 125 ml with petroleum ether (bp 30–60°), and kept at 0° overnight. Colorless crystals of **3f** separated and were filtered, washed with dry petroleum ether, and dried *in vacuo* to give 0.91 g (88%) of product which decomposed without melting.

Anal. Calcd for $C_{27}H_{20}O_2BrCl$: C, 65.8; H, 4.1; Br, 16.3; Cl, 7.2. Found: C, 65.6; H, 4.3; Br, 14.3; Cl, 6.8.

Action of Zinc Dust and Acetic Acid on BPTrOH.—When a 0.5% solution of the trityl alcohol in acetic acid (clarified, if necessary, by the addition of a few drops of acetone) was kept at room temperature overnight and then examined by tlc (silica gel, solvent B), no degradation of the compound was observed. In the presence of zinc dust (100 mg), however, the compound was significantly hydrolyzed (>25%) to *p*-HOTrOH in 20% acetic acid in 1 hr. Hydrolysis was complete with 80% acetic acid containing zinc dust in 1 hr. No hydrolysis was observed when ethanol was substituted for acetic acid.

Preparation of 5′-Trityladenosine Derivatives.—Compounds **4a**, **4c**, **4e**, and **4f** were prepared as follows. A solution of adenosine (1.5 g, 5.22 mmol), dried by repeated evaporation from anhydrous pyridine, in a mixture of dry dimethylformamide (35 ml) and pyridine (65 ml) was treated with a solution of the trityl chloride (5.2 mmol) in dry dimethylformamide (10 ml). After standing at room temperature for 5 days, the reaction mixture was poured into ice-cold water (800 ml). The precipitate thus obtained was washed with water, dried over P_2O_5 *in vacuo*, and recrystallized from ethyl acetate–benzene. Information concerning the properties of substituted trityladenosines is given in Table III.

5′-(*p*-Hydroxyphenyl)diphenylmethyladenosine (4b).—A solution of **4c** (0.6 g, 0.83 mmol) in dimethylformamide (5 ml) was treated with aqueous 58% ammonium hydroxide (5 ml) and the mixture stirred for 6 hr at room temperature. After evaporation under reduced pressure to a dry residue, the product was crystallized twice from ethanol to give 0.50 g (82%) of **4b**, mp 192–193°.

5′-(*m*-Hydroxyphenyl)diphenylmethyladenosine (4d).—This compound was prepared in the same way as the above compound from **4e** as crystals (78% yield), mp 182–184°.

(15) R. Lohrmann and H. G. Khorana, *J. Amer. Chem. Soc.*, **86**, 4188 (1964).

Table III

Properties and Yields of 5′-Trityl Nucleosides and Nucleotides

Compound	Yield, %	R_f Tlc, silica, solvent B	R_f Whatman No. 1, solvent A
5′-DPTr-A (**4a**)	53	0.57	0.85
5′-(*p*-HOTr)-A (**4b**)	82[a]	0.37	0.84
5′-(*p*-AcOTr)-A (**4c**)	60	0.41	0.84
5′-(*m*-HOTr)-A (**4d**)	78[a]	0.36	0.84
5′-(*m*-AcOTr)-A (**4e**)	65	0.39	0.84
5′-BPTr-A (**4f**)	88	0.52	0.82
5′-BPTr-T (**5a**)	90	0.80	0.97
5′-BPTr-dU (**5b**)	73	0.75	0.86
5′-BPTr-dA (**5c**)	91	0.65	0.83
5′-BPTr-dG (**5d**)	89	0.65	0.80
5′-(*p*-HOTr)-T (**6a**)	49	0.72	0.90
5′-(*p*-HOTr)-dU (**6b**)	40	0.65	0.85
5′-(*p*-HOTr)-dA (**6c**)	39	0.50	0.75
5′-(*p*-HOTr)-dG (**6d**)	33	0.51	0.72
5′-BPTr-TpT-DHC (**7a**)	42		0.83
5′-BPTr-dUpT-DHC (**7b**)	54		0.85
5′-BPTr-dUpT (**9d**)	94		0.80
5′-(*p*-HOTr)-TpT-DHC (**10a**)	31		0.78
5′-(*p*-HOTr)-dUpT (**10b**)	50		0.69
5′-BPTr-U(OAc)-3′-p (**11**)	97		0.79
5′-BPTr-U(OAc)pU(OBz)$_2$ (**12**)	47		0.83
5′-BPTr-U(OAc)pU(OAc)pU(OBz)$_2$ (**14**)	34		0.84
5′-BPTr-UpU (**15**)	83		0.75
5′-BPTr-UpUpU (**18**)	83		0.63

[a] Obtained by alkaline hydrolysis of the corresponding acetoxy derivatives.

Preparation of 5′-BPTr Deoxyribonucleosides.—Compounds **5a–d** were prepared as follows. A pyridine solution (2 ml) of the deoxyribonucleoside (1.0 mmol) was treated at 0° with BPTrCl (0.54 g, 1.1 mmol) for 4 hr and then overnight at room temperature. Water (100 ml) was added and the mixture extracted with methylene chloride (three 100-ml portions). The organic extracts were dried ($MgSO_4$), and the solvent was removed *in vacuo* and the residue recrystallized from benzene. The yields and properties of these compounds are summarized in Table III.

Detritylation Experiments.—Samples of the 5′-trityladenosine compounds (**4a–f**, **5a–d**, and MMTr-A and DMTr-A) (1.5–2 μmol) were treated with 20, 40, and 89% acetic acid (0.2 ml) at room temperature. The reactions were followed by tlc using silica plates (solvent B) and on Whatman No. 1 paper (solvent A). The results are summarized in Tables I and II.

The detritylation of **4f** and **5a–d** was also studied under the same conditions in the presence of zinc dust (20 mg).

Preparation of 5′-(*p*-HOTr) Derivatives from the Corresponding 5′-BPTr Nucleosides (4b from 4f, 6a from 5a, 6b from 5b, 6c from 5c, and 6d from 5d).—Samples of **4b** and **5a–d** (0.1 mmol) were treated with 30% acetic acid (2 ml) and zinc dust (200 mg) for 45 min at room temperature. The solutions were filtered, neutralized to stop the reactions, and chromatographed on preparative tlc (silica, solvent B). Bands of product were eluted and crystallized from benzene. Yields were: **4b**, 41% (adenosine 32%); **6a**, 49% (thymidine 33%); **6b**, 40% (deoxyuridine 37%); **6c**, 39% (deoxyadenosine 29%); and **6d**, 33% (deoxyguanosine 28%).

5′-(*p*-Bromophenacyloxytrityl)thymidylyl-(3′–5′)-3′-dihydrocinnamoylthymidine (5′-BPTr-TpT-DHC, 7a).—A mixture of 5′-(*p*-bromophenacyloxytrityl)thymidine (**5a**, 353 mg, 0.5 mmol) and 3′-dihydrocinnamoylthymidine 5′-monophosphate[5] (414 mg, 0.91 mmol) together with dry Dowex 50W-X8 (pyridinium) resin (1.0 g) were dried by azeotroping with pyridine. The mixture was dissolved in dry pyridine (7 ml) and a solution of dicyclohexylcarbodiimide (840 mg, 4.08 mmol) in pyridine (1 ml) was added; the mixture was stirred at room temperature for 5 days. The solution was cooled and treated with an equal volume of water and after standing 2 hr extracted with three portions of cyclohexane (40 ml). The aqueous layer was stored overnight

at 0° and then filtered and concentrated *in vacuo*. The product was isolated by paper chromatography on Whatman No. 3MM, solvent A, to give the protected dinucleoside monophosphate, 245 mg (42%), R_f 0.83 (solvent A).

Thymidylyl-(3′–5′)-thymidine (8a).—A sample of 5′-BPTr-TpT-DHC (**7a**, 20 mg) was treated with acetic acid (40%) (1 ml) and zinc dust (25 mg) for 1 hr and the solution chromatographed on Whatman No. 3MM (solvent D). The band at R_f 0.67 was eluted and the solution lyophilized to give TpT-DHC (**9a**, 11 mg (91%). The dihydrocinnamoyl group was removed from this dinucleoside monophosphate by the enzyme α-chymotrypsin (see ref 5) to give TpT (**8a**).

5′-(*p*-Hydroxytrityl)thymidylyl-(3′–5′)-3′-dihydrocinnamoylthymidine (5′-(*p*-HOTr)-TpT-DHC, 10a).—The hydrolysis of 5′-BPTr-TpT-DHC (**7a**) (150 OD's) was studied using 20, 40, and 60% acetic acid (0.1 ml) and zinc dust (2 mg) and followed by tlc (cellulose, solvent A). In 1 hr using 60 or 40% acetic acid there was complete detritylation to give TpT-DHC (**9a**). However, on using 20% acid and zinc for 1 hr the intermediate 5′-(*p*-HOTr)-TpT-DHC (**10a**) could be isolated by paper chromatography (solvent A) together with TpT-DHC (**9a**). The yields follow: **10a**, 15 OD_{260} units (31%), and **9a**, 18 OD_{260} units (39%). After standing for 4 hr the only product was **9a**.

Similar studies were made using 40, 60, and 80% acetic acid in the absence of zinc dust. Only in the case of 80% acetic acid was any hydrolysis observable after 24 hr.

d-5′-(*p*-Bromophenacyloxytrityl)uridylyl-(3′–5′)-3′-dihydrocinnamoylthymidine (5′-BrTr-dUpT-DHC, 7b).—This was prepared in the same way as **7a** using 5′-BrTr-dU (**5b**, 300 mg, 0.44 mmol) and pT-DHC (350 mg, 0.77 mmol). The product was isolated by chromatography on Whatman No. 3MM (solvent A), followed by lyophilization after removal of the salts, to give the dinucleoside monophosphate **7b** as a white solid, 270 mg (54%), R_f 0.85 (solvent A).

d-Uridylyl-(3′–5′)-thymidine (8b).—The preceding fully protected dinucleoside monosphosphate (**7b**, 20 mg) was dissolved in 50% ethanol (2.5 ml), diluted with an equal volume of pyridine, cooled to 0°, and treated with cold (0°) 2 *N* sodium hydroxide solution (5 ml). After standing at 0° for 5 min, the solution was neutralized with Dowex 50W-X8 resin (pyridinium form). The solution was filtered, concentrated, and chromatographed on Whatman No. 3MM (solvent C). Elution of the zone R_f 0.85 gave the dinucleoside monophosphate 5′-BPTr-d-UpT (**9b**), 16.7 mg (94%).

This dinucleoside monophosphate (4 mg) was dissolved in 40% acetic acid (10 ml) containing zinc dust. After standing at room temperature overnight the solution was filtered, evaporated, and chromatographed on paper (solvent A). The main zone had R_f 0.50 and on elution gave d-UpT (**8b**), λ_{max} 263 mμ, 50 OD_{260} units (74%).

d-5′-(*p*-Hydroxytrityl)uridylyl-(3′–5′)-thymidine (5′-(*p*-HOTr)-dUpT, 10b).—5′-BPTr-d-UpT (**9b**, 4 mg) was dissolved in 20% acetic acid (10 ml), the solution treated with zinc dust (500 mg), and the mixture shaken for 1 hr at room temperature. The mixture was filtered, concentrated, and chromatographed. Work-up of the zone at R_f 0.69 (solvent A) gave 5′-(*p*-HOTr)-dUpT, **10b**, 35 OD_{260} units (50%).

When 10 OD_{260} units of 5′-(*p*-HOTr)-dUpT were dissolved in 90% formic acid (1 ml) for 10 min or 20% acetic acid (1 ml) for 4 hr, and the solutions were chromatographed on Whatman No. 1 (solvent A), the unprotected dinucleoside monophosphate d-UpT (**8b**) was formed in both cases, R_f 0.48, 8.1 OD_{260} units (85%) and 7.8 OD_{260} units (82%), respectively.

5′-(*p*-Bromophenacyloxytrityl)uridine 2′,3′-Cyclic Phosphate.—Uridine 2′,3′-cyclic phosphate (350 mg, 0.9 mmol) was dissolved in a mixture of dimethylformamide (10 ml) and pyridine (1 ml) and treated with *p*-bromophenacyloxytrityl chloride (490 mg, 1.0 mmol). The mixture was stirred at room temperature for 2 days and then treated with water (2 ml); the solution was evaporated to dryness and azeotroped with small portions of dry pyridine. The gummy residue was dissolved in pyridine (5 ml) and precipitated with dry ether (200 ml) at 0°. The white precipitate was filtered and dried *in vacuo* to give 5′-BPTr-U>p, 650 mg (86%), R_f 0.77 (solvent A).

5′-(*p*-Bromophenacyloxytrityl)uridine 3′-Phosphate.—The above compound (650 mg, 0.78 mmol) was taken up in dimethylformamide (8.0 ml) and 2.5 *M* ammonium acetate buffer (3.5 ml) and incubated at 37° for 24 hr with pancreatic ribonuclease (Bovine) (11 mg). The pH of the solution was maintained between 7.5 and 7.6 by addition of 1.0 *M* ammonium hydroxide from a microsyringe. The solution was diluted with 1% aqueous ammonia until turbidity developed and then extracted with ethyl acetate (two 65-ml portions). The aqueous phase was saturated with sodium sulfate and extracted with *n*-butyl alcohol (four 65-ml portions). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* in the presence of added pyridine, the residue taken up in 5% pyridine, and the solution passed through a column of Dowex 50W-X8 (pyridinium, 4 × 19 cm). The eluate was evaporated and rendered anhydrous by repeated evaporations of dry pyridine. The residue was taken up in dry pyridine (5 ml), precipitated with cold dry ether (200 ml), collected, and dried *in vacuo* to give 5′-BPTr-U-3′-p, 490 mg (74%), R_f 0.71 (solvent A).

2′-Acetyl-5′-(*p*-bromophenacyloxytrityl)uridine 3′-Phosphate (11).—The above compound (440 mg, 0.57 mmol) was acetylated by dissolving it in acetic anhydride (0.6 ml) in the presence of tetraethylammonium acetate (6.0 mmol). The mixture was stirred for 16 hr at room temperature and then treated with a mixture of methanol–pyridine, 4:1, for 10 min. The solution was evaporated and the residue taken up in a mixture of methanol–pyridine–water, 3:1:1 (50 ml), and the solution passed through a column of Dowex 50W-X8 (pyridinium) resin (2 × 18 cm). The eluate was concentrated, dried, and precipitated with pyridine–ether in the usual way to give **11** as a white powder, 500 mg (97%), R_f 0.79 (solvent A).

5′-(*p*-Bromophenacyloxytrityl)-2′-acetyluridylyl-(3′–5′)-2′,3′-dibenzoyluridine (5′-BPTr-U(OAc)pU(OBz)$_2$, 12).—Dibenzoyluridine (113 mg, 0.25 mmol), 5′-BPTr-U(OAc)-3′-p (**11**, 324 mg, 0.36 mmol), and anhydrous Dowex (pyridinium) resin (1.0 g) were azeotroped and then dissolved in dry pyridine (4 ml). A solution of dicyclohexylcarbodiimide (750 mg, 3.7 mmol) in dry pyridine (6 ml) was added and the mixture stirred at room temperature for 3.5 days. It was then treated with water (10 ml) for 2 hr, extracted with cyclohexane (three 20-ml portions), and stored overnight at room temperature. The solution was filtered, concentrated, and chromatographed on Whatman No. 3 MM (solvent A). The fully protected dimer was eluted as a zone R_f 0.83 and after drying precipitated from pyridine–ether as an off-white powder, 150 mg (47%).

5′-(*p*-Bromophenacyloxytrityl)uridylyl-(3′–5′)-uridine (5′-BPTr-UpU, 15).—The above fully protected dimer (27 mg) was treated with methanol saturated with ammonia at 0° for 16 hr. The solvent was removed *in vacuo* and the residue dissolved in pyridine and precipitated with ether to give **15**, 18 mg (83%).

Detritylation of the Dimers 5′-BPTr-U(OAc)pU(OBz)$_2$ (12) and 5′-BPTr-UpU (15).—Portions (2 mg) of the dinucleoside monophosphates **12** and **15** were treated with 10, 20, 40, 50, 60, and 80% acetic acid (0.4 ml) and zinc dust (4 mg) at room temperature. The progress of the reactions was followed by tlc (cellulose, solvent A) and by paper chromatography (Whatman No. 1, solvent A). In the case of **15** (5′-BPTr-UpU), R_f 0.73, detritylation was complete in under 1 hr with 20–80% acetic acid and in 4 hr with 10% acid, to give UpU (**16**), R_f 0.45. The detritylation of **12** (5′-BPTr-U(OAc)pU(OBz)$_2$), R_f 0.90, was also complete in under 1 hr with 20–80% acetic acid, to give U(OAc)pU(OBz)$_2$ (**13**), R_f 0.82 (36%), but in addition other side products were formed with R_f 0.76 (38%) and 0.86 (26%). Both products were trityl negative and showed the presence of uracil in their uv spectra.

A second sample of 5′-BPTr-U(OAc)pU(OBz)$_2$, prepared from U(OBz)$_2$ (75 mg, 0.17 mmol) and 5′-BPTr-U(OAc)-3′-p (100 mg, 0.11 mmol), was not isolated but treated directly with 90% formic acid for 10 min at room temperature. After rapid evaporation of the formic acid *in vacuo* the residue was chromatographed on Whatman No. 3 MM paper (solvent A) and the band at R_f 0.82 eluted to give **13**, U(OAc)pU(OBz)$_2$, 32 mg (32%). An aliquot of this dinucleoside monosphosphate was treated with methanol saturated with ammonia for 16 hr to give UpU which was identical with that prepared from **15**.

5′-(*p*-Bromophenacyloxytrityl)-2′-acetyluridylyl-(3′–5′)-2′-acetyluridylyl-(3′–5′)-2′,3′-dibenzoyluridine (5′-BPTr-U(OAc)-pU(OAc)pU(OBz)$_2$, 14).—The dinucleoside monophosphate **13** (U(OAc)pU(OBz)$_2$) (32 mg, 35 μmol), **11** (5′-BPTr-U(OAc)-3′-p) (74 mg, 82 μmol), and anhydrous Dowex resin (pyridinium) (10 mg) were dried by coevaporation of pyridine and treated with a solution in dry pyridine (5 ml) of dicyclohexylcarbodiimide (100 mg, 485 μmol). The mixture was stored at room temperature for 6 days and then treated with water (5 ml). After extraction with cyclohexane (three 10-ml portions) the aqueous phase was stored at 0° overnight, filtered, and evaporated to

dryness. Chromatography (Whatman No. 3MM, solvent A) gave the product **14**, R_f 0.84, 21 mg (34%).

5′-(*p*-Bromophenacyloxytrityl)uridylyl-(3′-5′)-uridylyl-(3′-5′)-uridine (5′-BPTr-UpUpU, 18).—The preceding trinucleoside diphosphate **14** (7 mg) was treated with concentrated ammonia (5 ml) for 15 hr. Preparative paper chromatography on Whatman No. 3MM paper (solvent A) gave **18** (5′-BPTr-UpUpU), R_f 0.63, 4.5 mg (83%).

Detritylation of 14, 5′-BPTr-U(OAc)pU(OAc)pU(OBz)$_2$, and 18, 5′-BPTr-UpUpU.—Portions (0.5 mg) of the trinucleoside diphosphates **14** and **18** were treated with 10, 20, and 40% acetic acid (0.2 ml) and zinc dust (2 mg) and the reactions followed by tlc (cellulose, solvent A).

The detritylation of **18**, R_f 0.63, was complete in 1 hr with 20 and 40% acetic acid and zinc to give UpUpU, R_f 0.27. In the case of **14**, R_f 0.84, the detritylation was also complete in under 1 hr with 20 and 40% acid to give **17**, U(OAc)pU(OAc)pU(OBz)$_2$, R_f 0.43, but again side products were formed, R_f 0.49 and 0.53.

Treatment of **14**, 5′-BPTr-U(OAc)pU(OAc)pU(OBz)$_2$ (30 OD_{260} units), with 90% formic acid (1 ml) at room temperature for 10 min, followed by evaporation and chromatography (Whatman No. 3MM, solvent A), gave **17**, U(OAc)pU(OAc)pU(OBz)$_2$, 21 OD_{260} units, R_f 0.43. Treatment with concentrated ammonia for 16 hr gave UpUpU.

Registry No.—**2f**, 33608-41-2; **3f**, 33608-42-3; **4a**, 33531-85-0; **4b**, 33608-43-4; **4c**, 33531-86-1; **4d**, 33531-87-2; **4e**, 33531-88-3; **4f**, 33531-89-4; **5a**, 33531-90-7; **5b**, 33531-91-8; **5c**, 33531-92-9; **5d**, 33531-93-0; **6a**, 33531-94-1; **6b**, 33531-95-2; **6c**, 33531-96-3; **6d**, 33531-97-4; **7a**, 33531-98-5; **7b**, 33531-99-6; **8a**, 1969-54-6; **8b**, 10300-41-1; **9b**, 33532-02-4; **10a**, 33532-03-5; **10b**, 33532-04-6; **11**, 33532-05-7; **12**, 33532-06-8; **14**, 33545-29-8; **15**, 33608-44-5; **18**, 33608-45-6; 5′-(*p*-bromophenacyloxytrityl)uridine 2′,-3′-cyclic phosphate, 33532-07-9; 5′-(*p*-bromophenacyloxytrityl)uridine 3′-phospate, 33532-08-0.

Acknowledgment.—This work was supported by National Institute of Medical Sciences, National Institutes of Health, Bethesda, Md. (Contract PH-67-1157). Valuable discussions with Dr. O. M. Friedman, Dr. J. B. Hendrickson, and Dr. D. Söll are gratefully acknowledged. We also thank Mr. P. B. Szabady for technical assistance.

Partial Asymmetric Induction in the Ene Reaction

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Received July 19, 1971

Asymmetric induction in the ene reaction of (−)-menthyl glyoxylate with pent-1-ene has been studied. Optical yields were found to depend on temperature, solvent, and catalyst. The configuration of the new dissymmetric center in the obtained adducts changed with catalyst. In the presence of $SnCl_4$, BF_3, and $TiCl_4$ configuration *S* was induced, whereas with $AlCl_3$ center with configuration *R* was obtained. Postulation of an equilibrium between transition states derived from single- (s-) cisoid and transoid conformations of carbonyl groups of (−)-menthyl glyoxylate accounts for the results of asymmetric induction in the examined ene reaction.

Studies of partial asymmetric synthesis are of theoretical and preparative interest. On one hand they may be used as a tool to establish or relate configuration,[1] or, when configuration of the substrate and product is known, asymmetric induction may serve as a criterion of the assumed geometry of a transition state. On the other hand, high (70–100%) optical yields achieved for several reactions[2] open the possibility of applying asymmetric synthesis as a method for the preparation of optically active compounds with the desired absolute configuration. Though the area has been studied extensively with respect to both of these possibilities, little is known about asymmetric induction in the ene[3] reaction, for which so far only two examples have been examined.[4] In this paper we describe the results of the asymmetric induction in the ene condensation of pent-1-ene with (−)-menthyl glyoxylate in the presence of Lewis acid type catalyst.

Results

Data reported by Klimova, *et al.*,[5] indicate that butyl glyoxylate is an enophile of low reactivity. The thermal reaction (150°) with olefins gives poor yields; however, when catalyzed by Lewis acids it takes place readily at room temperature. Accordingly, we found that (−)-menthyl glyoxylate in the presence of 1 equiv of tin tetrachloride at room temperature reacted with pent-1-ene to afford in 87% yield the expected adduct, (−)-menthyl 2-hydroxy-4-heptenoate (**1**). Likewise high yields of adduct **1** were obtained with other Lewis acids ($AlCl_3$, BF_3, $TiCl_4$). The structure of **1** was confirmed by analysis, spectral data (ir, nmr), and chemical transformations shown in Scheme I.

Adduct **1** was comprised of two components[6] (vpc) which we assumed to be cis and trans isomers, since catalytic hydrogenation of the double bond of adduct **1** yielded dihydro derivative **2**, giving only one peak in vpc, whereas methanolysis of **1** gave methyl ester **3** as a two-component mixture (vpc).

The optical yield of the ene reaction and the absolute configuration of the new dissymmetric center predominantly formed in adduct **1** were established by correlation of the latter with a compound of known specific rotation and absolute configuration, *i.e.*, methyl (−)-malate. To this end adduct **1** was subjected to ozonolysis, oxidative decomposition of the ozonide, and subsequent hydrolysis and methylation of malic acid with diazomethane (Scheme I). The methyl malate

(1) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 72.

(2) T. D. Inch, *Synthesis*, 466 (1970), and references cited therein.

(3) For the review, see H. M. R. Hoffman, *Angew. Chem., Int. Ed. Engl.*, **8**, 556 (1969).

(4) R. K. Hill and M. Rabinowitz, *J. Amer. Chem. Soc.*, **86**, 965 (1964).

(5) (a) E. I. Klimova and Y. A. Arbuzow, *Dokl. Akad. Nauk SSSR*, **167**, 1060 (1966); *Chem. Abstr.*, **65**, 3736h (1966); (b) E. I. Klimova, E. G. Treshchova, and Y. A. Arbuzow, *Dokl. Akad. Nauk SSSR*, **180**, 865 (1968); *Chem. Abstr.*, **69**, 67173b (1968); (c) E. I. Klimova and Y. A. Arbuzow, *Dokl. Akad. Nauk SSSR*, **173**, 1332 (1967); *Chem. Abstr.*, **67**, 108156c (1967).

(6) In principle, adduct **1** is a four-component mixture: geometric isomers of two diastereoisomers. However, separation by vpc of isomers other than cis and trans in this case is rather unlikely, as follows from the vpc examination of the hydrogenation and methanolysis products.