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SOME DEVELOPMENTS IN THE PHOSPHITE–TRIESTER METHOD FOR SYNTHESIS OF OLIGONUCLEOTIDES[1,2]

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Abstract—1,1-Dimethyl-2,2,2-trichloroethyl has merit as a protecting group for P–O in the phosphite–triester synthesis of oligonucleotides. Use of this group enables one to simplify procedures for preparing active nucleoside phosphorochloridite reagents, to remove oligonucleotide phosphotriester intermediates intact from a solid support, and to conduct block syntheses in solution or on a silica support *via* phosphite chemistry. Deprotection can be achieved by warming the trihaloethyl phosphotriesters with tributylphosphine. The high efficiency of the coupling and deprotection reactions for oligomers bound to an insoluble support make this chemistry especially attractive.

The chemistry of the phosphite–triester scheme for synthesizing oligonucleotides[3] is represented schematically in Chart 1. On sequential treatment of a 5′-O-protected nucleoside (**1**) with (a) $ROPX_2$, (b) a 3′-O-protected nucleoside, (c) iodine–water and (d) an agent to free the 5′-OH, a dinucleoside phosphotriester (**5**) is produced. The chain can be extended by treating **5** with another active phosphorous intermediate, (**2**), and repeating the cycle. After construction of the desired sequence all phosphoryl protecting groups (R′) and the 3′-O-terminal protecting group are removed (e, f) to give the synthetic oligonucleotide. A striking feature of this approach is the speed of all reactions in the building sequence. Since a phosphorodichloridite ($R'OPCl_2$) is more reactive than a monochloridite (**2**), it is important that all dichloridite be consumed or removed before step b. This feature is particularly important in solid support syntheses where a large excess of the nucleoside phosphorochloridite is used. In the initial work with this scheme it was found that $Cl_3CCH_2OPCl_2$ is moderately selective in reacting with nucleosides, affording good yields of **2** at $-78°$.[3] This protecting group has since been used in a number of syntheses conducted in the solution phase.[4]

A Me protecting group for P–O was employed when phosphite chemistry was adapted to solid-phase synthetic methodology.[5] With this group, the phosphotriesters can be converted to phosphodiesters by the action of thiophenol/triethylamine,[6] a reaction that proceeds efficiently with oligomers bound to solid supports[5] as well as with oligomers in homogeneous solution. Selectivity is poor when CH_3OPCl_2 reacts with nucleosides, however; a mixture of CH_3OPCl_2, intermediate **2**, and a dinucleoside 3′-3′ phosphite is obtained even when excess nucleoside is employed.[7] A reagent suitable for solid support syntheses can be obtained by driving off excess dichloridite by coevaporation with pyridine. Alternatively, CH_3OPCl_2 can first be converted to $CH_3OP(NR_2)Cl$, which then reacts selectively with protected nucleosides to give phosphoramidite derivatives **2** (X = NR_2, Chart 1). Relatively unreactive themselves, these phosphoramidites afford highly active phosphitilating agents on treatment with tetrazole in acetonitrile.[8]

Heretofore it has not been practical to utilize trichloroethyl protecting groups for synthesis of oligonucleotides on solid supports. Conventional reducing agents such as Zn metal[9] or aromatic radical anions[3] do not react satisfactorily with trichloroethyl phosphotriesters bound to an insoluble matrix such as silica, and non-selective cleavage reactions make it impractical to remove oligomers from the support prior to deprotection.

We describe in this paper several experiments designed (a) to test the utility of 1,1-dimethyl-2,2,2-trichloroethyl for protecting P–O in the phosphite approach and (b) to find an efficient means to convert trichloroethyl phosphotriester derivatives immobilized on solid supports to phosphodiesters. 1,1-dimethyl-2,2,2-trichloroethyl was selected for study since it is considerably larger than either methyl or 2,2,2-trichloroethyl.[10] As a consequence of steric hindrance, one might therefore expect greater selectivity in reactions of $Cl_3CC(CH_3)_2OPCl_2$ with nucleosides and greater stability of the phosphite (**2, 3**) and phosphotriester (**4, 5**) intermediates. These properties could lead to simplifications in procedures and new options in preparing and handling intermediates in the phosphite scheme.

Dimethyltrichloroethyl derivatives. 1.1-Dimethyl-2,2,2-trichloroethyl phosphorodichloridite is easily prepared and is relatively stable thermally.[11] We found that it reacts readily with nucleosides despite the presence of the bulky alkyl group. Thus successive treatment with 5′-O-dimethoxytritylthymidine and thymidine in THF/pyridine at $-78°$ yielded the 3′-5′ linked dinucleoside phosphite, **3a**, (R = $(CH_3OC_6H_4)_2C(C_6H_5)$-, R′ = $Cl_3CC(CH_3)_2$-, R″ = H, B = thymine), easily isolable by extraction and precipitation from pentane.

The phosphite link in **3**, as well as the trityl ether, is attacked by 80% aqueous acetic acid (extensive cleavage within 30 min at room temp), but compound **3** reacts only very slowly with ammonium hydroxide–dioxane (1:1, v/v) (no decomposition observed after 30 min at room temp; only slight reaction

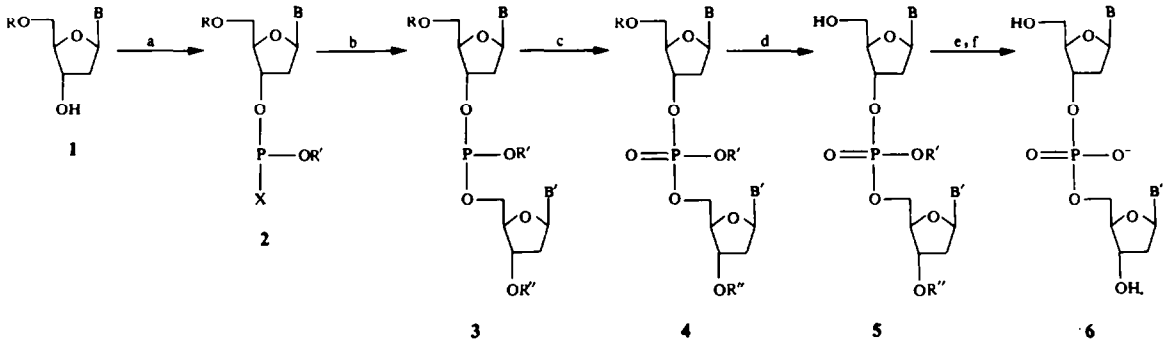


Chart 1. Phosphite–triester synthesis of oligonucleotides.

after 24 hr). Compound **3** also exhibits good stability toward atmospheric oxygen; we have stored samples as dry powders for over a year at 5° without detectable oxidation or hydrolysis (the ^{31}P NMR resonance for the epimers of the phosphite, δ[12] − 151.3, − 151.0,[12] is readily distinguished from that for the corresponding phosphate, δ − 10.36, − 10.23 or the hydrolysis product). On the other hand, oxidation with iodine/water proceeds rapidly and essentially quantitatively. The resulting phosphate, **4**, is more stable to ammonium hydroxide than the corresponding derivatives with methyl or 2,2,2-trichloroethyl protecting groups (no degradation of **4** was observed by TLC test over an 8 hr period at room temperature in ammonium hydroxide–dioxane (1:1, v/v) and only slight reaction was observed after 48 hr.

Tests monitored by ^{31}P NMR spectra indeed showed that $Cl_3CC(CH_3)_2OPCl_2$ is much more selective than $Cl_3CCH_2OPCl_2$ or CH_3OPCl_2 in reacting with the 3′-OH of a nucleoside. Active phosphite derivatives were prepared by adding 1.2 equivalents of d-(DMTr)T to 1.0 equivalent of $ROPCl_2$ in CH_2Cl_2/C_5H_5N (3:2, v/v) at room temperature. The spectrum of the product from $Cl_3CC(CH_3)_2OPCl_2$ showed only a sharp peak (− 177.8 ppm) for the desired active reagent (**2**) and a small peak (− 151.3 ppm) for the 3′-3′ dinucleoside phosphite formed from **2** and excess d-(DMTr)T. All dichloridite (− 213 ppm) had been consumed. On the other hand, each of the other reaction mixtures showed the presence of $ROPCl_2$ (− 198.5 ppm for R = CH_3 and − 195.2 ppm for R = Cl_3CCH_2) in addition to peaks for the active nucleoside phosphoromonochloridite and the 3′-3′ product. Accordingly, use of the hindered protecting group enables one to prepare an active reagent suitable for synthesis directly by mixing the components without subsequent manipulations. To maximize selectivity we routinely prepare the reagents at − 78°, where d-(DMTr)bzA, d-(DMTr)ibG, and d-(DMTr)bzC also exhibit high selectivity with $Cl_3CC(CH_3)_2OPCl_2$.[13] Tetrahydrofuran was used as a solvent in preparing a number of the reagents used in the earlier studies. Methylene chloride works equally well and is easier to dry and to store. For polymer support work it has the added advantage of being a good solvent for pyridine hydrochloride.

Block coupling. As a test of the utility of trichlorodimethylethyl derivatives in joining oligonucleotide blocks, the transformations outlined in Chart 2 were carried out. The 1,1-dimethyl-2,2,2-trichloroethyl ester of 5′-O-dimethoxytrityl-thymidylyl-(3′-5′)-thymidine (**7**) was converted to both phosphoromonochloridite **8** and derivative **9**, which has a 5′-OH and 3′-OAc. Reaction of these two blocks, with **9** as the limiting reagent, afforded the fully protected tetranucleotide, compound **10**, in 93% yield. Similarly, detritylation of **10** followed by condensation with **8** yielded the protected hexamer (81%). d-(DMTr)T*p*T*p*bzA*p*bzA(OAc) (70%) and d-(DMTr)bzA*p*bzA*p*T*p*T(OAc) (73%) were obtained by preparing and coupling appropriate dinucleotide blocks by an analogous sequence. These tetramer derivatives and **10** and **12** were each characterized by

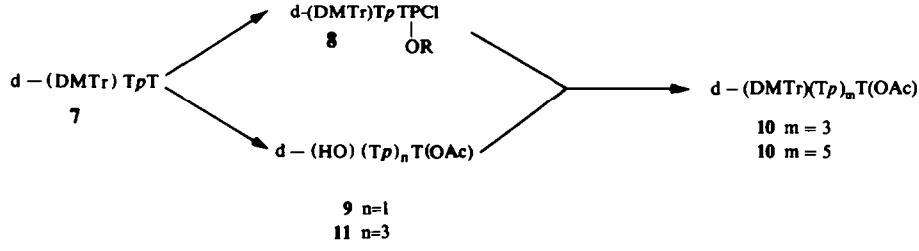


Chart 2. Block condensation scheme; *p* represents a 3′–OPO–5′ internucleoside link. P is trivalent phosphorous at the 3′–O.

conversion to the corresponding oligonucleotides by successive treatment with ammonium hydroxide, Zn, and 80% aqueous acetic acid, and by hydrolysis of the resulting oligomers tothe expected nucleotides and nucleosides with snake venom phosphodiesterase.

The conditions for these condensations have not been optimized. The favorable results obtained, however, indicate that reagents with dimethyltrichloroethyl P–O protecting groups can be used effectively in preparing and joining oligonucleotide blocks *via* phosphite chemistry.

Solid support synthesis of oligothymidylate phosphotriesters. In some cases it could be advantageous to have the capability of removing oligonucleotide derivatives from a solid support with all or some of the potentially labile triester links intact. With current methodology, methyl, chlorophenyl, and trichloroethyl are not suitable protecting groups for P–O for such applications since they are sensitive to the ammoniacal conditions used to sever the acyl functions holding the oligomers to the solid support. The finding that nucleoside dimethyltrichloroethyl phosphotriesters are relatively stable to ammonium hydroxide provides a way around this difficulty. To explore the feasibility of recovering oligonucleotide triesters from a solid support we synthesized a hexathymidylate derivative on silica.

The synthetic cycles were carried out by previously described procedures[7,14] except that compound **2** (R = dimethoxytrityl, R′ = $Cl_3CC(CH_3)_2$–, B = thymine) was used as the active phosphite reagent. At each stage, samples of the loaded silica were removed for recovery of oligomers. Pertinent data on the course of the synthesis are collected in Table 1. Yields based on the trityl cation liberated in the zinc bromide cleavage averaged a little over 80% per step.[15] Samples of oligomers removed by treating the silica with ammonium hydroxide (4 hr, 25°) were analyzed by TLC and HPLC. In each case a major band corresponding to the expected d-(DMTr)(T*p*)nT was obtained. The HPLC bands were broad, reflecting stereoisomers at phosphorus. The hexamer recovered from a cut of the main band corresponded to 15% of the initial thymidine units on the silica gel. On deblocking with zinc, it afforded d-(DMTr) TTTTTT, which appeared as a sharp peak on HPLC.

These experiments demonstrate that nucleoside dimethyltrichloroethyl phosphotriesters are sufficiently stable to be removed from solid supports under mild ammoniacal conditions. Presumably, the triesters would be even more stable in oligomers containing some anionic phosphodiester links.

Deprotection of trihaloethyl phosphotriesters with tributylphosphine. For routine synthesis of oligonucleotides it is desirable to remove phosphoryl protecting groups prior to cleaving the oligomers from the solid support. Since conventional agents for deprotecting trichloroethyl phosphotriesters are unsatisfactory for use with esters bound to a silica support, we looked for other reducing agents to accomplish the desired transformation: Substituted phosphines appeared attractive candidates since triphenylphosphine is known to dehalogenate certain vicinal dibromides.[16]

Triphenylphosphine did not react with the trichloroethyl phosphotriesters, however, tributylphosphine was found to attack dinucleoside trichloroethyl and dimethyltrichloroethyl phosphotriesters slowly at room temperature. The reaction proceeded smoothly at a good rate at 80° in a mixture of tributylphosphine, dimethylformamide and triethylamine (2:4:1, v/v/v), affording 95% or more of the phosphodiester cleavage product (by TLC analysis) within an hour. No side reactions involving the base rings were detected in control experiments with d-bzC, d-bzA, d-ibG and dT. The course of the reaction was confirmed by isolating the nucleotide products from the cleavage of representative compounds. For this purpose dimer or tetramer dimethyltrichloroethyl phosphotriester derivatives were heated (80°) for 2 hr with the tributylphosphine, dimethylformamide, triethylamine mixture, and after cooling, the nucleotide products were precipitated by adding ether–pentane (3:2, v/v). N-protecting groups were removed by NH_4OH and the nucleosides purified by HPLC on a reverse phase column. Products and yields were: d-AA (70%), d-CC (60%), d-GC (73%), d-AATT (60%) and d-TTAA (84%). In our experience the tributylphosphine reaction now provides the most convenient and efficient means avail-

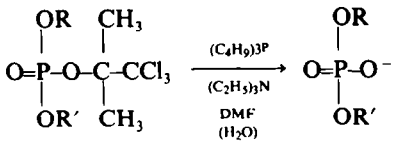
Table 1. Products from synthesis of d-(DMTr)(T*p*)$_n$T on silica

n	% Yield[a]	Rf, TLC[b]	Rf, TLC[c]	Elution time (min.) HPLC[d] MeCN/H_2O, 1:1	3:2
0	—	0.51	0.45	5.44	
1	84	0.32	0.40	8.08	
2	84	0.24	0.35	13.7	
3	80	0.22	0.29	22.8	
4	81	0.20	0.24	40.	8.8
5	—	0.19	0.21	—	11.2

[a]Based on trityl cation. [b]Silica; $CHCl_3/CH_3OH$ 10:1.

[c]Reverse phase (C-18); acetone/H_2O 7:3. [d]Whatman Partisil ODS3 column, 2mL/min flow rate.

able for obtaining oligonucleotides from soluble trichloroethyl phosphotriester derivatives.



Importantly, the tributylphosphine reaction is equally effective with trihaloester derivatives bound to a solid support, though the reaction is somewhat slower. Following three rounds of synthesis, the silica support bearing the dimethyltrichloroethyl phosphotriesters was treated with the tributylphosphine reagent (80°, 3 hr) and then ammonium hydroxide (5 hr, 50°). The alkaline solution was collected, concentrated, taken up in water, and chromatographed. Absence of sizeable peaks other than the major one for d-TTTT attests to the efficiency of the coupling reactions and the deprotection scheme (the purity indicated by the HPLC profile was similar to that indicated in Fig. 2).

Other phosphines and trihalo esters can also be employed in these transformations. Tris(dimethylamino)phosphine is an effective reagent, but has no apparent advantage over tributylphosphine. Tribromoethyl phosphotriesters (e.g. 2,2,2-tribromethyl *bis*-(5'-O-dimethoxytritylthymidyl)phosphate) react rapidly with tributylphosphine even at room temperature. Tribromoethyl therefore has some potential as a P–O protecting group; however, we have not yet been able to achieve as high yields in synthesizing oligomers with the tribromoethyl protecting group as with trichlorodimethylethyl.

Synthesis of oligonucleotides. Investigation of variables in the coupling reactions involving nucleoside dimethyltrichloroethyl phosphorochloridites led to a protocol which gave good coupling yields on silica supports for derivatives of all four common nucleosides. Several experiments were carried out in a small column equipped with a pump to cycle solvents. The operations for addition of one nucleotide unit were: (1) Detritylate with 3% trichloroacetic acid in nitromethane, 2.5 min, (2) wash with pyridine, 2 min, (3) react with the nucleoside chloridite reagent, which is injected as a solution in dichloromethane (0.4 mL) with N-methylimidazole (0.1 mL) and recycled for 15 min, (4) wash with pyridine, 2 min, (5) oxidize with 0.1 M iodine in THF–pyridine–H_2O or with 0.2 M *m*-chloroperbenzoic acid in dichloromethane–pyridine (0.5 mL, (9:1, v/v) 2 min, (6) wash with pyridine, 2 min, (7) wash with nitromethane, 2 min. Coupling yields (trityl cation test) averaged better than 95% per cycle for the preparation of d-CCCCT, d-AAAAT, d-GGGGT, d-TTTTTT, d-T_{16} and d-GCAAATATCATTTT (for the separation profile of the reaction mixture see Fig. 1). The longer two oligomers were characterized by labeling with ^{31}P by polynucleotide kinase and sizing by polyacrylamide gel electrophoresis. d-GCAAATATCATTTT was also hydrolyzed to the component nucleotides by snake venom phosphodiesterase.

A simple and equally effective method for manipulating the silica support utilizes a syringe as the reaction vessel. In this case the same protocol was used but the washing steps involved three or four transfers of solvents into and out of the syringe. With reagents prepared from the protected nucleosides and dimethyltrichloroethyl phosphorodichloridite, d-AGGGACCT was prepared on a silica support in yields averaging 94% per cycle.[17] The oligonucleotide was obtained by treating the silica loaded sample successively with trichloroacetic acid, tributylphosphine and ammonium hydroxide in the standard manner. The HPLC profile for the product obtained directly from the silica is shown in Fig. 1. Rechromatography of the major peak yielded high quality octamer (shown by ^{31}P labeling and gel electrophoresis).

For construction of extended oligonucleotide

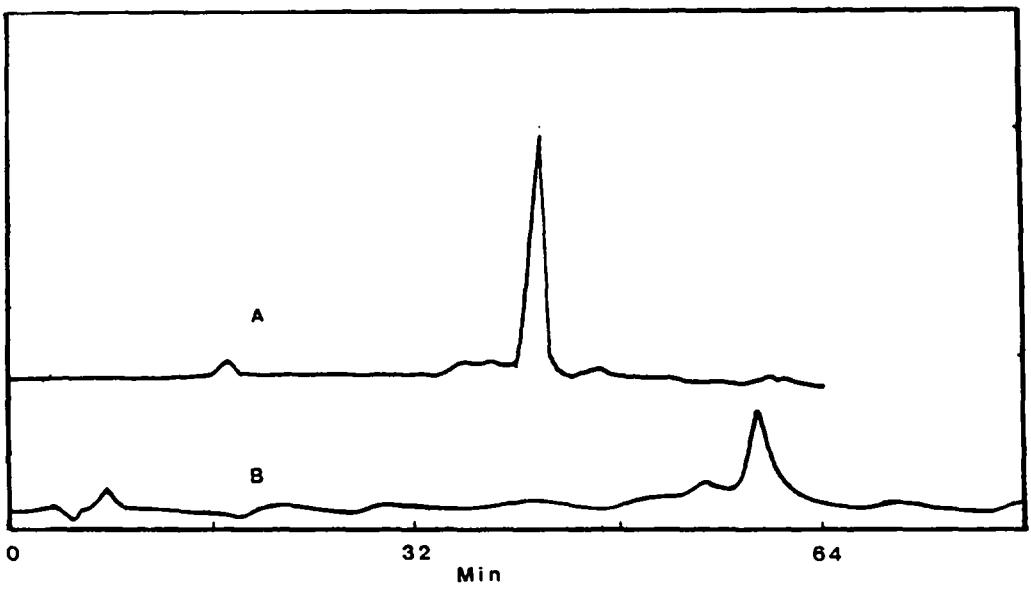


Fig. 1. HPLC analysis of crude reaction mixtures from preparation of (A) d-AGGGACCT and (B) d-GCAAATATGATTTT.

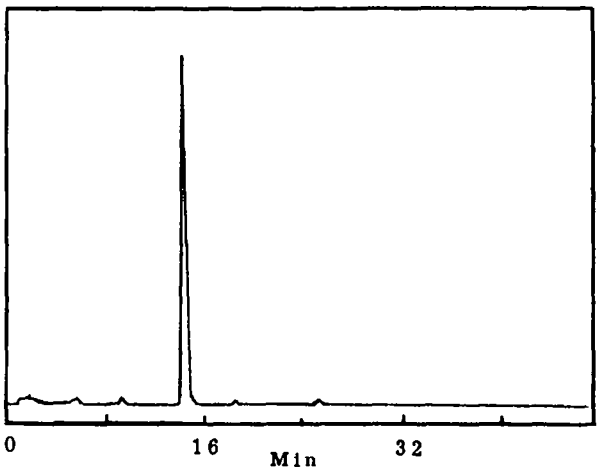


Fig. 2. HPLC analysis of crude reaction mixture from preparation of d-T_5 by dimer block synthesis on a silica support.

chains (e.g. in the 25–50 nucleotide range) on insoluble supports, synthesis with dimer blocks would be advantageous. Block synthesis has been employed in the conventional triester approach, but has not been exploited with phosphite chemistry. Use of the bulky, relatively stable dimethyltrichloroethyl group for protecting phosphorus enables one to prepare dimer blocks conveniently in solution and to convert them to active phosphite derivatives for syntheses on solid supports. An important question, however, concerns the efficiency of coupling dimer blocks to oligonucleotide fragments on an insoluble support. As a preliminary test we have prepared d-T_5 by two cycles of reaction of dimer **8** with d-T bound to a silica support. Yields by the cation test were 97 and 94%, in the range of those observed in monomer couplings (a longer coupling time, 20 min, was used to compensate for the use of a sterically larger reagent). In addition, HPLC of the crude mixture obtained from the silica without purification of the nucleotides indicated high conversion to the pentamer with very little side product from the synthesis or deprotection steps (Fig. 2). In view of this excellent result the studies are being extended to use of other dimer blocks and the synthesis of longer chains.

CONCLUSIONS

Use of dimethyltrichloroethyl phosphorodichlorodite facilitates the preparation of active nucleoside reagents for use in synthesis of oligonucleotides, and the tributylphosphine reaction provides a convenient means for converting trihalophosphotriesters to phosphodiesters, both in solution and in solid phase work. Good yields and the relative simplicity of operation make the phosphite approach utilizing these reagents an attractive alternative to other synthetic procedures. The nucleoside reagents are easier to prepare than those derived from methyl phosphorodichloridite. Because of moisture sensitivity they are more difficult to handle than nucleoside phosphoramidites, but at prevailing prices they are cheaper to make than phosphoramidites. The chemistry is applicable to syntheses on polystyrene[18] as well as silica supports; however, silica offers some advantage in ease of changing solvents and reagents. In addition, these studies indicate that the hindered monochloridite reagents have high potential for use in block syntheses conducted on solid supports as well as in solution. The relatively good stability of dimethyltrichloroethyl phosphotriester derivatives of nucleosides toward ammonium hydroxide also permits one to cleave synthetic oligomers from a solid support with the phosphotriester function intact.

EXPERIMENTAL

The preparation of solvents, reagents and silica supports followed previously described procedures.[14] Acetonitrile was dried by standing over CaH_2. Davison 62 silica (140–200 mesh, 14 nm average pore diam, 1.1 mL/g pore volume, 300 m^2/g surface area) was employed as the solid phase. Syringe reactions were carried out in Glenco 2.5 mL syringes equipped with a Teflon plunger and a polyethylene filter at the base as previously described.[14,19] The pumping system for semiautomated syntheses was similar to that of Matteucci and Caruthers.[5] ^{31}P NMR spectra were obtained with a JEOL FX90 Q instrument. Chemical shifts are reported relative to triphenyl phosphate. HPLC was carried out with a Whatman Partisil PXS ODS-3 column (Fig. 1) or an IBM-C18 column (Fig. 2) with aqueous 0.1 M $Et_3NH^+OAc^-$ and CH_3CN (increased at rate of 0.1%/min) starting at 10% CH_3CN (11% for B in Fig. 2).

1,1-*Dimethyl*-2,2,2-*trichloroethyl* 5′-O-*mono*-p-*methoxytritylthymidyl*-3′ *thymidyl*-5 *phosphite* (**3**, R = MTr, R′ = $Cl_3CC(CH_3)_2$–, R″ H, B = B′ = thymine). 5′O-Mono-*p*-methoxytritylthymidine (d-(MMTr)T) (145 mg, 0.28 mmol) in THF–pyridine (0.9 mL, 2:1, v/v) was added dropwise (10 min) to a stirred soln of $Cl_3CC(CH_3)_2OPCl_2$[11] (80 μL, 0.44 mmol) at −78°. The empty flask was rinsed with THF–pyridine (0.6 mL), and the rinsings added to the mixture. After 15 min, thymidine (174 mg, 0.72 mmol)[20] in THF–pyridine (2.9 mL, 5:3, v/v)[21] was added. The mixture was stirred 15 min at − 78° and warmed to 0° over a 15 min period. Dilution with CH_2Cl_2 (100 mL), extraction with NH_4OH (50 mL), concentration of the CH_2Cl_2 layer and chromatography (silica plate $CHCl_3$–CH_3OH (10:1) gave **3**, isolated by elution with $CHCl_3$ and precipitated by dropwise addition of the $CHCl_3$ soln (2 mL) into pentane (25 mL); 189 mg (70%) ^{31}P NMR (THF) – 151.3, 151.0 ppm. R_f ($CHCl_3$/CH_3OH 10:1) 0.48. (Found: C, 54.92; H, 4.82; N, 5.72. Calc for $C_{44}H_{48}Cl_3N_4O_{12}P$: C, 54.92; H, 5.04; N, 5.82%.)

Oxidation of **3** (2 mg, R_f 0.23 on reverse phase TLC, acetone–H_2O, 7:3) with I_2–THF–H_2O[1], showed complete

conversion to the phosphate (7), R_f 0.43 on reverse phase TLC, acetone–H_2O, 7:3.

Phosphotriester **4a**. The preparation of **3** was repeated. At the stage the mixture had warmed to 0°, the phosphite was oxidized by addition of excess I_2 in THF–H_2O (3 mL, 2:1). After 40 min, excess I_2 was reduced with $Na_2S_2O_3$; then the product was worked up as in the case of the phosphite to give 149 mg of **7** (54%); ^{31}P NMR (THF) −10.36, −10.23 ppm. R_f silica ($CHCl_3$/CH_3OH, 10:1) 0.42; R_f reverse phase (acetone–H_2O, 7:3) 0.43. (Found: C, 53.69; H, 5.06; N, 5.62. Calc for $C_{44}H_{48}Cl_3N_4O_{13}P$. C, 54.02; H, 4.95; N, 5.73%.)

Detritylation of **7**; [*1,1-Dimethyl-2,2,2-trichloroethyl thymidyl-3′ thymidyl-5′ phosphate*]. On standing in 80% aq. AcOH for 12 hr, **4a** (52 mg) was converted to the 5′-OH derivative, which was recovered by coevaporation with pyridine and precipitation by dropping the pyridine soln (1 mL) into ether: yield, 31.4 mg (85%); ^{31}P NMR (THF–pyridine, 8:2) − 10.30 ppm; R_f reverse phase (acetone–H_2O, 7:3) 0.85. (Found: C, 39.85; H, 4.85; N, 8.11. Calc. for $C_{24}H_{32}Cl_3N_4O_{12}P{\cdot}H_2O$: C, 39.81; H, 4.85; N, 7.78%.)

Deprotection with Zn and acetylacetone,[9] filtration, and precipitation of residual Zn ion by H_2S afforded d-TT, which hydrolyzed to dpT and dT (1.0:1.04) on digestion with snake venom phosphodiesterase.

Block syntheses in solution. Data on yields and properties are summarized in Table 2. Dimer units d-(DMTr)T*p*T (**7**; 70%) and d-(DMTr)bzA*p*bzA (**13**; 45%) were prepared by the procedure used for **4a**, except that the ratio of d-(DMTr) nucleoside–$Cl_3CC(CH_3)_2OPCl_2$–nucleoside was 1.0:1.3:2.0. Compound **9** was prepared by acetylation of **7** (101 mg; 0.2 mL acetic anhydride and 0.1 g *p*-dimethylaminopyridine in 1.33 mL THF, 0.36 mL pyridine, 10 min) followed by detritylation with 80% aqueous AcOH (1 hr, r.t.). d-bzA*p*bzA(OAc), **14**, was similarly prepared from **13**, except that detritylation was effected with $C_6H_5SO_3H$ (30 mg) in $CHCl_3$–CH_3OH (6 mL; 7:3) at 0° for 10 min. All products were purified by chromatography on silica plates. The product band was eluted with CH_3OH, and after evaporation the residue was taken up in $CHCl_3$ (1 mL) and precipitated in pentane (20 mL).

Reagent **8**, used in dimer coupling, was prepared by adding **7** (504 mg, 0.5 mmol) in THF–pyridine (1 mL, 1:1) to $Cl_3CC(CH_3)_2OPCl_2$ (137 mL, 0.75 mmol) in THF–pyridine (1.5 mL, 2:1) at − 78°. After 20 min at − 78° the mixture was centrifuged and the supernatant dropped into pentane (40 mL) with stirring. After settling, pentane was removed from the ppt by syringe. The residue was washed with pentane and taken up in THF (1.5 mL) and pyridine (0.5 mL). Care was taken to keep moisture away from the active reagent at all times. Activity was tested by adding a small portion of the soln to CH_3OH on one hand and H_2O on the other. Conversion to the methyl ester (~ 80%; R_f 0.8 in EtOAc on silica) with only a minor amount of hydrolysis product (R_f 0.5) indicated high conversion of **7** to the active phosphite reagent (**8**).

The corresponding deoxyadenosine derivative, d-(DMTr)bzA*p*bzAP(Cl)OC$(CH_3)_2CCl_3$, was similarly prepared in ~ 60% yield from **13** (164 mg) and $Cl_3CC(CH_3)_2OPCl_2$. The R_f of the methyl ester obtained on reaction with methanol was 0.71 (silica, EtOAc).

Block couplings were accomplished by treating the appropriate 5′-OH, 3′-OAc derivative with 2 equivs of the 5′DMTr, 3′O-phosphorochloridite reagent in THF–pyridine (2:1) at room temp for 10 min, then with excess I_2 in THF–H_2O (2:1) for 40 min to oxidize the phosphite. Products were worked up in the usual manner, and were isolated by chromatography on silica plates.

Oligomers **10**, **12a**, **15** and **16** were deprotected by reductive cleavage with Zn,[9] ammonium hydroxide and AcOH as usual. Purification by chromatography on Avicel, in n-propyl alcohol–NH_4OH–H_2O, 55:10:35, yielded the corresponding oligonucleotides (d-T_4, d-T_6, d-AATT and d-TTAA) in yields of 30–35%. Each hydrolyzed in presence of snake venom phosphodiesterase to the correct ratios of nucleotides and nucleosides.

Solid support syntheses. Reactions in columns were carried out with 100 mg of silica support loaded with 3–5 μmole of thymidine. For syringe reactions 30–50 mg of the loaded silica was used. Approximately 15 fold excess of active nucleoside chloridite was used in the coupling reactions per initial thymidine on the silica. Nucleoside phosphoromonochloridite reagents were prepared by dropwise addition (10 min) of a soln of nucleoside (0.2 mmol) in CH_2Cl_2–pyridine (1 ml, 4:1) into a stirred soln of $Cl_3CC(CH_3)_2OPCl_2$ (0.18 mmol) in CH_2Cl_2–pyridine (0.7 mL, 6:1) at − 78. After 5 min the mixture was allowed to warm to room temp. It is essential that the nucleoside be in slight molar excess relative to the dichloridite; otherwise low yields in the subsequent coupling steps will be encountered. Dimer reagents were prepared in the same manner. For syringe syntheses we now prefer CH_3CN to pyridine to dry the loaded silica prior to a coupling reaction. A con-

Table 2. Block synthesis in solution

Product	Nucleotide Precursors	% Yield	Rf[a]	Rf[b]
d-(DMTr)TpT, 7	d-(DMTr)T, T	70	.36	.54
d-(HO)TpT(OAc), 9	7	76	.31	.75
d-(DMTr)(Tp)3T(OAc), 10	7, 9	93	.39	.34
d-(DMTr)(Tp)3T, 10a	10	81	.20	.43
d-(HO)(Tp)3T(OAc), 11	10	89	.24	.59
d-(DMTr)(Tp)5T(OAc), 12	7, 11	81	.31	.28
d-(DMTr)(Tp)5T, 12a	12	89	.20	.33
d-(DMTr)bzApbzA, 13	d-(DMTr)bzA d-bzA	62	.40	.34
d-bzApA)(OAc), 14	13	73	.48	—
d-(DMTr)(bzAp)2TpT(OAc), 15	13, 9	71	.49	.26
d-(DMTr)(Tp)2bzApbzA(OAc), 16	7, 14	70	.48	.23

[a]TLC, silica, $CHCl_3$/CH_3OH 10/1. [b]TLC, RP (C-18), Acetone/H_2O 7/3.

venient protocol is: (1) wash with CH_2Cl_2 3 ×; (2) detritylate with 5% dichloroacetic acid in CH_2Cl_2 or 3% trichloroacetic acid in nitromethane, (3) wash with CH_3CN–pyridine (4:1) 3 ×; (4) dry with dry CH_3CN 5 ×; (5) couple with active chloridite; 15 min with monomer reagent or 20 min with dimer reagent, (6) wash with CH_3CN–pyridine (4:1) 3 ×, (7) oxidize with 0.1 M iodine in THF, pyridine, H_2O (10:5:1), wash with CH_3CN 3 ×. At the end of the synthetic cycles oligomers are deprotected with dichloroacetic acid in CH_2Cl_2 followed by treatment with $(C_4H_9)_3P$ and by concd NH_4OH as indicated in the discussion. The aqueous ammonia soln is lyophilized, and the oligomer is taken up in water or buffer and purified by HPLC on a reverse phase column. As an example of recovery, a preparation of d-T_3 by monomer additions on 50 mg of silica loaded with thymidine (1.48 μmol afforded after HPLC on a C-18 column 1.13 μmol of purified d-T_3. Some losses of the oligonucleotides occur in the various manipulations in isolation. In this preparation the couplings by the trityl cation test were quantitative and the HPLC profile indicated efficient reactions (similar to that in Fig. 2).

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REFERENCES

[1]For a preliminary communication of a portion of this material see: R. L. Letsinger, E. P. Groody and T. J. Tanaka, *J. Am. Chem. Soc.* **104**, 6805 (1982).

[2]Abbreviations: The symbolism for nucleosides is represented by d-(DMTr)bzA, which refers to 5′O-p,p-dimethoxytrityl-N-benzoyldeoxyadensine. Internucleoside phosphotriester links are indicated by *p*, as in d-T*p*T(OAc), which is a derivative of thymidylyl-[3,5′]-thymidine with a 1,1-dimethyl-2,2,2-trichloroethyl group at O of P–O, and an acetyl group at the 3′-terminal O.

[3]R. L. Letsinger J. L. Finnan, G. A. Heavner and W. B. Lunsford, *J. Am. Chem. Soc.* **97**, 3278 (1975); R. L. Letsinger and W. B. Lunsford, *Ibid.* **98**, 3655 (1976).

[4]J. L. Finnan, A. Varshney and R. L. Letsinger, *Nucleic Acids Res. Sym.* Ser. **7**, 133 (1980); K. K. Ogilvie, N. Theriault and K. L. Sadana, *J. Am. Chem. Soc.* **99**, 7741 (1977); K. K. Ogilvie and N. Y. Theriault, *Tetrahedron Letters* 2111 (1979); K. K. Ogilvie, A. L. Schifman and C. L. Penney, *Can. J. Chem.* **57**, 2230 (1979); K. K. Ogilvie and N. Y. Theriault, *Ibid.* **57**, 3140 (1979); K. K. Ogilvie and R. T. Pon, *Nucleic Acids Res.* 2105 (1980); K. K. Ogilvie and M. J. Nemer, *Can. J. Chem.* **58**, 1389 (1980); K. K. Ogilvie, M. J. Nemer, N. Theriault, R. Pon and J. Seifert, *Nucleic Acids Res.*, Sym. Ser. **7**, 147 (1980).

[5]M. D. Matteucci and M. H. Caruthers, *Tetrahedron Letters* **21**, 719 (1980); K. K. Ogilvie and M. J. Memer, *Can. J. Chem.* **14**, 1389 (1980).

[6]G. W. Daub and E. E. van Tamelen, *J. Am. Chem. Soc.* **99**, 3526 (1977).

[7]M. D. Matteucci and M. H. Caruthers, *Ibid.* **103**, 3185 (1981).

[8]S. L. Beaucage and M. H. Caruthers, *Tetrahedron Letters* 1859 (1981); L. J. McBride and M. H. Caruthers, *Ibid.* 245 (1983).

[9]F. Eckstein and I. Rizk, *Angew. Chem.* Int. Ed. **6**, 695, 949 (1967); R. W. Adamiak, E. Biata, K. Grzeskowiak, R. Kierzek, A. Kraszewski, W. T. Markiewicz, J. Stawinski and M. Wiewiorowski, *Nucleic Acids Res.* **4**, 2321 (1977).

[10]The attractive features of the dimethyltrichloroethyl group were indicated in the use of $Cl_3CC(CH_3)_2OCOCl$ in protecting amino groups in amino acids and nucleosides: H. Eckert, M. Listl and I. Ugi, *Angew Chem.* Int. Ed. **17**, 361 (1978); R. G. K. Schneiderwind and I. Ugi, *Z. Naturforsch.* **36b**, 1173 (1981).

[11]W. Gerrard and P. L. Wyville, *Research London* **2**, 536 (1949); H. A. Kellner and I. Ugi, *Z. Naturforsch* **34b**, 1159 (1979). This compound is now available from the Aldrich Chem. Co.

[12]δ is relative to $(C_6H_5O)_3PO$ in THF.

[13]When d-(DMTr)ibG was treated with excess $Cl_3CC(CH_3)_2OPCl_2$, a new resonance peak was observed at −114.1 ppm in addition to that at −178.4 ppm for the monochloridite, indicative of a side reaction of dichlorodite with the guanine base ring. This peak was absent when the active reagent was prepared with excess nucleoside at −78° and then warmed to room temp.

[14]T. Tanaka and R. L. Letsinger, *Nucleic Acids Res.* **10**, 3249 (1982).

[15]As described later, with optimization of conditions yields in the range of 95% per cycle can be obtained.

[16]See, e.g. C. C. Tung and A. J. Speziale, *J. Org. Chem.* **28**, 1521 (1963); K. Pilgram and H. Ohse, *Angew. Chem.* Int. Ed. **5**, 837 (1966). For elimination by a trialkyl phosphite see S. Denshowitz and S. Proskauer, *J. Org. Chem.* **26**, 3595 (1961).

[17]This synthesis was carried out by Margaret E. Schott.

[18]These experiments were carried out by Lenore Martin.

[19]R. L. Letsinger, in press, *Genetic Engineering* (Edited by J. K. Seltow and A. Hollaender), Vol. 5, p. 191. Plenum Press, New York (1983).

[20]Later work showed that a mole ratio of d-MTrT/$ROPCl_2$/dT of 1.0/1.3/2 was preferable.

[21]Thymidine is first dissolved in pyridine, then THF is added.