and yielded voltammograms of the type shown in Figure 1. While the cyclic voltammograms exhibit some subtle features, the evidence indicates that the charging process involves the formation of $TCNQ^{-}\cdot$ radical anion sites. First, derivative cyclic voltabsorptometry[14] at the 735-nm band, which has been assigned to the $TCNQ^{-}\cdot$ monomer species,[15] produced dA/dE curves of the same form as the conventional cyclic voltammograms obtained simultaneously; see Figure 2. Second, reduction of a $(TCNQ)_x$ film (dip-coated on Pt grid) in an ESR cavity by using the intra muros technique[16,17] yielded a signal ($g = 2.0031$, $\Delta H_{pp} = 0.4$ mT) that increased in intensity in direct proportion to the charge passed. In addition, the two-step EE process (eq 2) is evident in the voltammograms for which the potential is scanned to more negative values, although repeated cycling past the second wave destroys the film electroactivity. However, if the potential excursions are maintained within the limits of Figure 1, the $(TCNQ)_x$ film retains full electrochemical activity over a period of days. Also, the ESR signal intensity and the voltammetric waves did not decrease when the reduced films were exposed to the laboratory atmosphere.

Similar voltammetric behavior was observed in both $NaClO_4$ and $LiClO_4$ electrolytes. In the presence of 0.1 M tetraethylammonium perchlorate, however, the cathodic peak current was significantly attenuated and the corresponding anodic peak was more drawn out than shown in Figure 1. Similar electrolyte effects have been found for donor polymer films.[18,19] Thus the $(TCNQ)_x$ reduction process in aqueous electrolytes can be viewed as "cation controlled" and written as an ionic intercalation of cations into the film phase:

$$(TCNQ)_x + xM^+ + xe^- \rightleftharpoons (TCNQ^-M^+)_x \qquad (3)$$

In this regard, the electrode processes for the $(TCNQ)_x$ films are akin to those observed for TTF–TCNQ "organic metal" electrodes in aqueous media.[20] In the presence of 0.5 M $NaClO_4$ or $LiClO_4$, chronocoulometric experiments indicate rapid diffusion-controlled charging processes for both reduction and oxidation with Cottrell slopes of 1.2×10^{-3} C cm^{-2} s$^{-1/2}$.

The cyclic voltammograms have several features that indicate slow potential-dependent changes in the film structure and/or uptake of electrolyte that are similar to those observed for TTF polymer films.[21] Initial potential scans in the negative direction resulted in a narrow cathodic peak (peak width ca. 50 mV), which was replaced by a broader peak (width 90 mV) on subsequent cycles; see Figure 1. Holding the potential at 0.2–0.3 V for 3–5 min restored the film to the original state. Simultaneous absorption measurements at 735 nm demonstrated that a slow oxidation of the radical anion sites occurred on this time scale. In addition, upon repeated cycles, isopotential points[22] indicative of a two-state system developed in the multicyclic voltammograms as shown in Figure 1. The causes of this behavior are under further study. In spite of these variations, the total charge accepted by a $(TCNQ)_x$ film remained constant, indicating the overall reversibility of the process.

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Registry No. 1·ClC(O)$(CH_2)_4$C(O)Cl copolymer, 83462-96-8; **1**·ClC(O)$(CH_2)_4$C(O)Cl repeating unit, 83462-97-9; Pt, 7440-06-4; $LiClO_4$, 7791-03-9; $(TCNQ)_x$, 26810-79-7.

(14) Bancroft, E. E.; Sidwell, J. S.; Blount, H. N. *Anal. Chem.* **1981**, *53*, 1390.
(15) Boyd, R. H.; Phillips, W. D. *J. Chem. Phys.* **1965**, *43*, 2927.
(16) Geske, D. H.; Maki, A. H. *J. Am. Chem. Soc.* **1960**, *82*, 2671.
(17) Piette, L. H.; Ludwig, P.; Adams, R. N. *Anal. Chem.* **1962**, *34*, 916.
(18) Daum, P.; Murray, R. W. *J. Phys. Chem.* **1981**, *85*, 389.
(19) Chambers, J. Q.; Kaufman, F. B.; Nichols, K. H., to be published in *J. Electroanal. Chem.*
(20) Jaeger, C. D.; Bard, A. J. *J. Am. Chem. Soc.* **1980**, *102*, 5435.
(21) Schroeder, A. H.; Kaufman, F. B. *J. Electroanal. Chem.* **1980**, *113*, 209.
(22) Untereker, D. F.; Bruckenstein, S. *Anal. Chem.* **1972**, *44*, 1009.

Use of Trichlorodimethylethyl as a Protecting Group and Tributylphosphine as a Deprotecting Agent in Oligonucleotide Synthesis

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Phosphite triester chemistry[1] provides a means for rapid synthesis of oligonucleotides. In this approach 2,2,2-trichloroethyl[1,2] and methyl[3] have been the groups of choice for protecting P–O internucleoside links, the latter proving particularly useful in syntheses conducted on insoluble supports.[4] We describe here two developments that simplify some operations and broaden the scope in syntheses of oligonucleotides by the phosphite approach: (1) use of the bulky 2,2,2-trichloro-1,1-dimethylethyl group for protecting P–O; (2) a new reaction of tributylphosphine useful in cleaving trihaloethyl phosphotriesters.

^{31}P NMR studies show that 2,2,2-trichloro-1,1-dimethylethyl phosphorodichloridite reacts *selectively* with 5′-O-protected deoxyribonucleosides at −78 °C in that the first halogen in the dichlorodite is displaced before the second reacts. Consequently, solutions of active nucleoside reagents (**1**, Chart I) free of dichloridite can be prepared simply by adding a slight excess of the 5′-O-protected nucleoside to $Cl_3CC(CH_3)_2OPCl_2$ in CH_2Cl_2 or THF in the presence of pyridine at −78 °C. In contrast, CH_3OPCl_2 is unselective, and $Cl_3CCH_2OPCl_2$ exhibits only moderate selectivity in reactions carried out under comparable conditions.

The potential of $Cl_3CC(CH_3)_2OPCl_2$ in synthesis was examined by preparing **2** and **3**. Phosphite **2**[7] (^{31}P NMR, δ −151.29, −151.02)[8] was obtained (70%) by reaction of **1** (base = thymine) with excess thymidine in THF–C_5H_5N (−78 → 0 °C). Oxidation with I_2–H_2O converted it quantitatively to the corresponding phosphate, **3**[7] (^{31}P NMR, δ −10.36, −10.23)[8] which was relatively stable to alkaline solutions (no reaction in 4 h with NH_4OH at 25 °C).

The stability of the trichlorodimethylethyl phosphotriesters to alkali permits oligonucleotide triester derivatives to be removed from insoluble supports intact, as demonstrated by synthesis of d-(DMTr)[T_{TCDM}]$_n$T,[9] n = 1–5, on a silica support. The procedure of Matteuci and Caruthers[4a] was adapted to the syringe technique,[4e] and $Cl_3CC(CH_3)_2OPCl_2$ was used in place of CH_3OPCl_2. Aliquots of loaded silica were removed at the end of each cycle and treated with NH_4OH (~23 °C, 3 h) to liberate the oligomers. In each case a dominant spot was observed on reverse-phase TLC[10] corresponding to the nucleoside triester derivative for that stage (R_f 0.45, 0.40, 0.35, 0.29, 0.24, 0.22 for n = 0–5, respectively). The hexamer **4** was identical in properties with d-(DMTr)[T_{TCDM}]$_5$T prepared independently in solution by stepwise condensations of dimer **5** with oligomer blocks, starting with d-T_{TCDM}T(OAc). In confirmation of the structure, successive treatment with zinc and aqueous HOAc converted **4** to d-(Tp)$_5$T,

(1) Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278. Letsinger, R. L.; Lunsford, W. B. *Ibid.* **1974**, *98*, 3655.
(2) Finnan, J. L.; Varshney, A.; Letsinger, R. L. *Nucleic Acids Res., Symp. Ser.* **1980**, *7*, 133. Ogilvie, K. K.; Nemer, M. J. *Can. J. Chem.* **1980**, *58*, 1389.
(3) Van Tamelen, E. E.; Daub, S. U. *J. Am. Chem. Soc.* **1977**, *99*, 3526.
(4) (a) Matteucci, M. D.; Caruthers, M. H. *Tetrahedron Lett.* **1980**, *21*, 719; *J. Am. Chem. Soc.* **1981**, *103*, 3185. (b) Ogilivie, K. K.; Nemers, M. J. *Tetrahedron Lett.* **1980**, *21*, 4159. (c) Alvarado-Urbina, G.; et al. Science (*Washington, D.C.*) **1981**, *270*. (d) Chow, F.; Kempe, T.; Palm, G. *Nucleic Acids Res.* **1981**, *9*, 2807. (e) Tanaka, T.; Letsinger, R. L. *Ibid.* **1982**, *10*, 3249.
(5) For preparation, see: Gerrard, W.; Wyville, P. L. *Research* (*London*) **1949**, *2*, 536.
(6) For a related reagent, see: Eckert, H.; Listl, M.; Ugi, I. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 361.
(7) Satisfactory C, H, N analyses were obtained.
(8) δ is relative to $(C_6H_5O)_3PO$ in THF.
(9) TCDM refers to the internucleoside 2,2,2-trichloro-1,1-dimethylethyl phospho triester link.
(10) 7:3 Me_2CO–H_2O on Whatman MKC_{18} plates.

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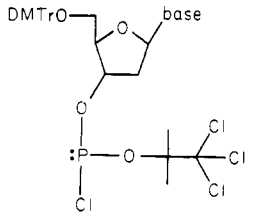
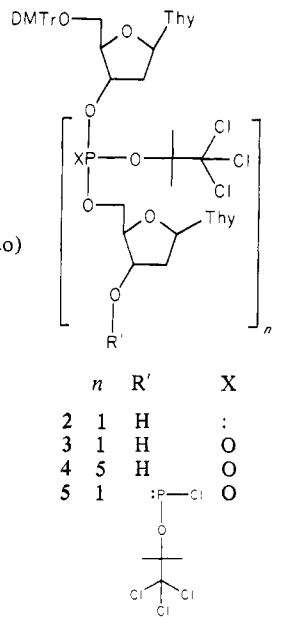
Chart I



1, (base = Thy, bzCyt, bzAde, ibGuo)



	n	R'	X
2	1	H	:
3	1	H	O
4	5	H	O
5	1	:P—Cl	O

which was hydrolyzed completely to d-T and d-pT by snake venom phosphodiesterase.

For routine synthesis of oligonucleotides with phosphodiester backbones it is desirable to cleave the P–O protecting groups before removing the oligomers from the insoluble support. Heretofore this has not been possible with trichloroethyl phosphotriesters since neither zinc[11] nor radical anions[12] are effective on substrates bound to silica supports. A search for a new reagent for deprotection revealed that tributylphosphine[13] in DMF–Et_3N at 80 °C converts nucleoside trichloroethyl and trichlorodimethylethyl phosphotriesters efficiently to the corresponding nucleoside phosphotriesters for reactions conducted both in solution and on insoluble supports.

Use of the phosphorochloridite reagents (**1**) in conjunction with deprotection by Bu_3P is illustrated by the synthesis of d-T_{16} and d-GCAAATATCATTTT. Reactions were carried out on silica (80 mg, 3 μmol of d-(DMTr)T in a column similar to that previously described.[4a] Preliminary experiments showed the following sequence to be effective: treatment with (1) 3% Cl_3CCOOH in CH_3NO_2,[4e] 2.5 min, (2) pyridine, 2 min, (3) reagent **1** in 3:1 CH_2Cl_2–*N*-methylimidazole,[14] 15 min, (4) pyridine, 2 min, (5) I_2 in 40:20:1 THF-pyridine-H_2O or 0.2 M *m*-chloroperbenzoic acid in CH_2Cl_2–pyridine, 2 min, (6) pyridine, 2 min, (7) CH_3NO_2, 2 min. With this procedure yields averaged above 95% per cycle for addition of a nucleotide unit (trityl cation test). On completion, the loaded silica was heated with 2:1:4 Bu_3P–Et_3N–DMF (80 °C, 3 h) and NH_4OH (50 °C, 12 h). The ammoniacal solutions were evaporated and the products analyzed by HPLC. The chromatographic profiles (Figure 1) show that the efficiency through the synthetic cycles and deprotection steps is good. Samples purified by HPLC and chromatography on silica gel[4c] were characterized by hydrolysis to the component nucleotides and nucleosides (d-T or d-G) by snake venom phosphodiesterase and, after labeling with ^{32}P (polynucleotide kinase), by sizing by electrophoresis on a polyacrylamide gel (single spots were obtained corresponding to a hexadecamer for the thymidine derivative and to a tetradecamer for the mixed oligomer).

The ease of preparing the active reagents, high selectivity in reactions,[15] stability of the intermediate triesters, and efficiency in deprotection make this approach promising for routine synthesis of oligonucleotides for studies in molecular biology. In addition,

(11) Eckstein, F.; Rizk, I. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 695.
(12) Letsinger, R. L.; Finnan, J. L. *J. Am. Chem. Soc.* **1975**, *97*, 7197.
(13) Other phosphine derivatives, e.g., $[(CH_3)_2N]_3P$, are also active.
(14) *N*-Methylimidazole accelerates the condensation reaction.
(15) For another means of achieving selectivity see: Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859.

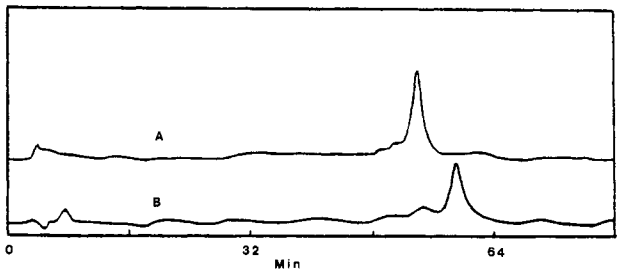


Figure 1. HPLC analysis of reaction mixtures from preparation of (A) d-T_{16} and (B) d-GCAAATATCATTTT; Whatman Partisil PXS ODS-3 column, starting with 11% CH_3CN, 89% 0.1 M aqueous $Et_3NH^+OAc^-$ and increasing CH_3CN at rate of 0.1%/min.

in combination with procedures utilizing methyl protecting groups for P–O, this chemistry provides flexibility in synthesizing and subsequently modifying oligonucleotides possessing enzyme-resistant sites (i.e., stable triester links) at specified points in phosphodiester chains.

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Rhenium(VII) Neopentylidene and Neopentylidyne Complexes[1]

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In the past several years a variety of d^0 alkylidene[2] and alkylidyne[3] complexes of Nb, Ta, Mo, and W have been prepared, most of them by a variation of the α-hydrogen abstraction reaction.[4] An interesting question is whether as yet unknown Re(VII) alkylidene and alkylidyne complexes can be prepared by using related methods. We report here that they can be, but so far at least one other π-bonding ligand (dianion or trianion) must be present in order for the metal to sustain its relatively high oxidation state.

The starting point in this chemistry is $Re(N\text{-}t\text{-}Bu)_3(OSiMe_3)$, a compound that can be prepared in high yield from Re_2O_7 and $NH(SiMe_3)\text{-}t\text{-}Bu$.[5] Addition of 4 equiv of gaseous HCl to $Re(N\text{-}t\text{-}Bu)_3(OSiMe_3)$ in dichloromethane produces 1 equiv of $t\text{-}BuNH_3Cl$ and orange $Re(N\text{-}t\text{-}Bu)_2Cl_3$ in >85% yield.[6] Since $Re(N\text{-}t\text{-}Bu)_2Cl_3$ is a monomer in dichloromethane and the *tert*-butyl groups are equivalent by ^1H and ^{13}C NMR, we propose that it is a trigonal bipyramidal species with equatorial imido ligands (cf. other five-coordinate d^0 complexes containing two π-bonding ligands isoelectronic with imido ligands such as $Ta(CHCMe_3)_2(mesityl)(PMe_3)_2$[7] and $W(O)(CHCMe_3)(PEt_3)Cl_2$[8]).

(1) Multiple Metal-Carbon Bonds. 30. For part 29 see ref 15.
(2) Schrock, R. R. In "Reactions of Coordinated Ligands"; Braterman, P. S., Ed.; Plenum Press: New York, in press.
(3) (a) Clark, D. N.; Schrock, R. R. *J. Am. Chem. Soc.* **1978**, *100*, 6774. (b) Wengrovius, J. H.; Sancho, J.; Schrock, R. R. *Ibid.* **1981**, *103*, 3932.
(4) Wood, C. D.; McLain, S. J.; Schrock, R. R. *J. Am. Chem. Soc.* **1979**, *101*, 3210.
(5) (a) Nugent, W. A.; Harlow, R. L. *J. Chem. Soc., Chem. Commun.* **1979**, *1105*. (b) Nugent, W. A.; Haymore, B. L. *Coord. Chem. Rev.* **1980**, *31*, 123.
(6) Anal. Calcd for $ReC_8H_{18}N_2Cl_3$: C, 22.09; H, 4.17. Found: C, 22.15; H, 4.11.
(7) Churchill, M. R.; Youngs, W. J. *Inorg. Chem.* **1979**, *18*, 1930.

0002-7863/82/1504-6806$01.25/0