Tetrahedron Letters,Vol.23,No.3, pp 275-278,1982 0040-4039/82/030275-04$03.00/0
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METAL IONS AS SELECTIVE TRIGGERS FOR REMOVING OLIGODEOXYNUCLEOTIDE PHOSPHOTRIESTER PROTECTING GROUPS

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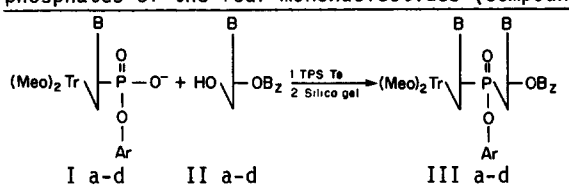
The o-bromophenyl group was successfully tested as a phosphotriester protecting group for internucleotide bonds. This protecting group can be readily and selectively removed using various transition metal salts in aqueous pyridine

The phosphotriester approach (1) in various modified forms (2-6) has been used extensively for synthesizing oligonucleotides Yields and purity of the final fully protected products are usually satisfactory and biochemically active oligonucleotides can be generated A continuing problem has been the choice of a protecting group for the internucleotide phosphotriester (1, 3, 7) A major advancement in this area was achieved by introducing aryl protecting groups derived from phenols with pKa's close to 8 5 (*p*-chlorophenyl or *o*-chlorophenyl) and N^1, N^1, N^3, N^3-tetramethyl guanidinium *syn*-p-nitrobenzaldoxime as a deblocking agent (8) In this communication, we report preliminary investigations involving an alternative solution to this problem. Specifically, we report that various transition metal salts will catalyze removal of an *o*-bromophenyl protecting group from an internucleotide triester.

The synthesis of deoxyoligonucleotides containing *o*-bromophenyl phosphotriesters was completed as described for other aryl protecting groups The synthetic procedure is outlined schematically in Figure 1 The 5'-O-di-*p*-anisylphenylmethyldeoxyribonucleoside -3'-*o*-bromophenyl phosphates of the four mononucleotides (compounds I a-d) were synthesized basically as described



I a-d II a-d III a-d

Ia, IIa, IIIa, B = 1-Thyminyl
Ib, IIb, IIIb, B = 1-(N-4-Benzoylcytosinyl)
Ic, IIc, IIIc, B = 9-(N-6-Benzoyladeninyl)
Id, IId, IIId, B = 9-(N-2-Isobutyrylguaninyl)

Figure 1 Outline for Deoxyoligonucleotide Synthesis. Bz, benzoyl, Ar, *o*-bromophenyl, TPS Te, triisopropylbenzenesulphonyltetrazolide, $(MeO)_2Tr$, di-*p*-anisylphenylmethyl

previously (9) The first step was condensation of a 5'-O-di-*p*-anisylphenylmethyl and base protected deoxynucleoside with *o*-bromophenylphosphorodichloridate in the presence of 1, 2, 4-triazole. The intermediate triesters were formed by addition of 2-cyanoethanol and isolated in 70% to 80% yield by chromatography on silica gel. These phosphotriesters were converted essentially quantitatively to the phosphodiesters using triethylamine in aqueous pyridine and were isolated as the triethylammonium salts by precipitation into ether Compounds I a-d were tested as intermediates in deoxyoligonucleotide synthesis by preparing d(C-C-G-A-T-A-A-C-A-A-T-T) Thus compound Ia was condensed with IIa using TPS Te and the dinucleotide (compound IIIa) was isolated by silica gel column chromatography The next step was removal of the di-*p*-anisylphenylmethyl group using a 4% solution of *p*-toluenesulfonic acid in methanol.chloroform (3·7, v/v)followed by condensation with compound Ic. Thus the synthesis proceeds in a 3' to 5' direction

from the 3'-protected nucleoside (10). A summation of our isolated yields (comparable to results using other substituted phenols) is presented in Table 1.

Table 1 Yields Obtained During the Synthesis of d(C-C-G-A-T-A-A-C-A-A-T-T).[a]

5'-Protected Component (mmol)		5' Hydroxyl Component	(mmol)	Product[b] (% Isolated Yield)
$(MeO)_2$Trd[T*p*]	(4 5)	d[T-Bz]	(3 0)	(78%)
$(MeO)_2$Trd[*Ap*]	(3.0)	d[T*p*T-Bz]	(2 0)	(78%)
$(MeO)_2$Trd[*Ap*]	(2 25)	d[*Ap*T*p*T-Bz]	(1 5)	(75%)
$(MeO)_2$Trd[*Cp*]	(1 5)	d[*ApAp*T*p*T-Bz]	(1 1)	(71%)
$(MeO)_2$Trd[*Ap*]	(1 0)	d[*CpApAp*T*p*T-Bz]	(0.64)	(66%)
$(MeO)_2$Trd[*Ap*]	(0.5)	d[*ApCpApAp*T*p*T-Bz]	(0 3)	(52%)
$(MeO)_2$Trd[*GpApTp*]	(0.16)	d[*ApApCpApAp*T*p*T-Bz]	(0.11)	(38%)
$(MeO)_2$Trd[*CpCp*]	(0.025)	d[*GpApTpApApCpApAp*T*p*T-Bz]	(0.015)	(35%)

[a]The abbreviated nomenclature suggested previously has been used (10). N-benzoyldeoxycytidine, N-benzoyldeoxyadenosine and N-isobutyrldeoxyguanosine are represented by *C*, *A*, and *G* respectively. The *o*-bromophenyl group on phosphate is represented by *p*.

[b]Yield after purification on silica gel

Metal ions were observed to catalyze removal of the *o*-bromophenyl group from phosphotriesters The results using $(MeO)_2$Trd(T*p*T-Bz) as a model compound are summarized in Table 2 Cupric acetate, Cupric chloride, zinc acetate, zinc chloride, cobalt chloride and nickel chloride were tested. Analyses using thin layer chromatography indicated that these metal salts did remove the *o*-bromophenyl group. Our results also indicated that the deprotection rate was variable and was determined by the choice of cation and anion Among the cations tested, Cu^{+2} was superior (compare $CuCl_2$, $ZnCl_2$, $NiCl_2$ and $CoCl_2$). Furthermore a comparison of cupric chloride vs cupric acetate and zinc chloride vs zinc acetate showed that acetate was superior to chloride in enhancing the deprotection rate With cupric chloride, but not the other salts, approximately 5 to 10% of the sample was detritylated during the time required for removal of the *o*-bromophenyl group. Selective removal of the o-bromophenyl group with cupric acetate was analyzed more carefully using reverse phase high performance liquid chromatography. Using conditions

Table 2 Removal of the *o*-Bromophenyl Group from $(MeO)_2$Trd(T*p*T-Bz) Using Metal Salts [a]

Salt	mole salt/mole dinucleotide	Time (h) for 100% Rx	Solvent (9 1, v/v)
$Cu(CH_3CO_2)_2$	4	2[b]	pyridine H_2O
$CuCl_2$	4	6	pyridine H_2O
	4	10	2,6-lutidine H_2O
$Zn(CH_3CO_2)_2$	4	10	pyridine H_2O
$ZnCl_2$	4	18	pyridine·H_2O
$NiCl_2$	4	72	2,6-lutidine H_2O
$CoCl_2$	4	72	2,6-lutidine H_2O

[a]Reactions were at room temp. and analyzed by chromatography on silica gel thin layer plates.

[b]Detectable internucleotide bond cleavage was not observed even after 18 hours with cupric acetate in pyridine H_2O

for complete removal of the *o*-bromophenyl group (2 h) from $(MeO)_2$Trd(TpT-Bz), internucleotide bond cleavage was not observed (less than 1%) and the product was the expected dinucleotide. These results were comparable to similar experiments completed simultaneously on the same dinucleotide using N^1, N^1, N^3, N^3-tetramethylguanidinium *syn*-*p*-nitrobenzaldoxime. Analysis of $(MeO)_2$TrdT, $(MeO)_2$TrdbzC, $(MeO)_2$TrdbzA, and $(MeO)_2$TrdibG in copper acetate as a pyridine water (9 1, v/v) solution at room temperature for seven days indicated that all four protected deoxynucleosides were stable to these reaction conditions. Other controls were also investigated Concentrated ammonium hydroxide caused more than 50% internucleotide bond cleavage Furthermore a dinucleotide of thymidine containing a *p*-chlorophenyl triester was stable to the copper acetate solution for at least one week These results suggest that copper acetate complexed with the *o*-bromophenyl group has a strong directing effect on the reaction We speculate that the reaction proceeds via a concerted deprotection mechanism as shown in Figure 2 Selectivity is presumably due to formation of a bromine, phenol oxygen and cupric ion complex Anion attack on this complex would then lead to elimination of the *o*-bromophenoxide Presumably the rate determining step is formation of the metal ion-phosphotriester complex, attack of anion on this complex, or hydrolysis of the resulting mixed anhydride. Quite possibly the rate difference between chloride and acetate could be due to the rapid hydrolysis of the mixed anhydride formed from a phosphate diester and acetate anion We have not, however, attempted to establish either a mechanism for this reaction or a rate determining step Recent independently derived results using 5-chloro-8-quinolyl as the phosphotriester protecting group have been interpreted similarly (11) Deprotection using $ZnCl_2$ has been postulated to occur via the same proposed mechanism

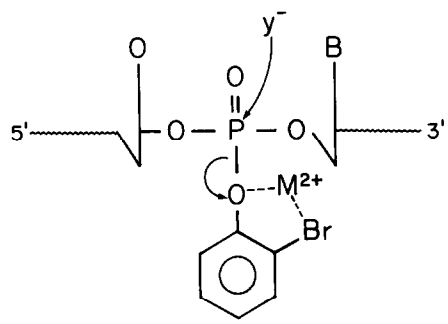


Figure 2

As a test of the cupric acetate deprotection procedure, a partially purified sample (silica gel column purification only) of $(MeO)_2$Trd[*CpCpGpAp*T*pAp*A*pC*p*A*p*A*p*T*pT-Bz] was converted to the completely deprotected deoxyoligonucleotide. The dodecanucleotide (6 6 mg, 1 μmole) was dissolved in 0 2 ml pyridine H_2O (9 1, v/v) containing 0 3 M $Cu(CH_3CO_2)_2$ and the reaction mixture was kept at room temperature for 8 h 1 M Ammonium hydroxide was added and the reaction mixture was kept at room temperature for 2 h The solvent was removed *in vacuo* and the residue was treated with concentrated ammonium hydroxide (1 ml) at 50°C for 6 h After removal of solvent *in vacuo* the residue was treated with 80% acetic acid for 10 min at room temperature Ammonium sulphide was added (approx 9 2 ml of a 6 M solution) and the cupric sulphide precipitate was removed by centrifugation The supernatant was concentrated to dryness The residue was dissolved in water (1 5 ml), washed with ether three times, concentrated to dryness *in vacuo*, and redissolved in 0 1 M triethylammonium acetate, pH 7.0 (0 5 ml). The deoxydodecanucleotide was isolated from this solution by chromatography on a reverse phase C-18 u-bondapak column (Waters Associates) using as eluant 89% 0.1 M triethylammonium acetate and 11% acetonitrile The elution profile is reproduced in Figure 3 The minor peaks are not due to degradation via cupric acetate. These minor peaks correspond to the normal impurities present in any

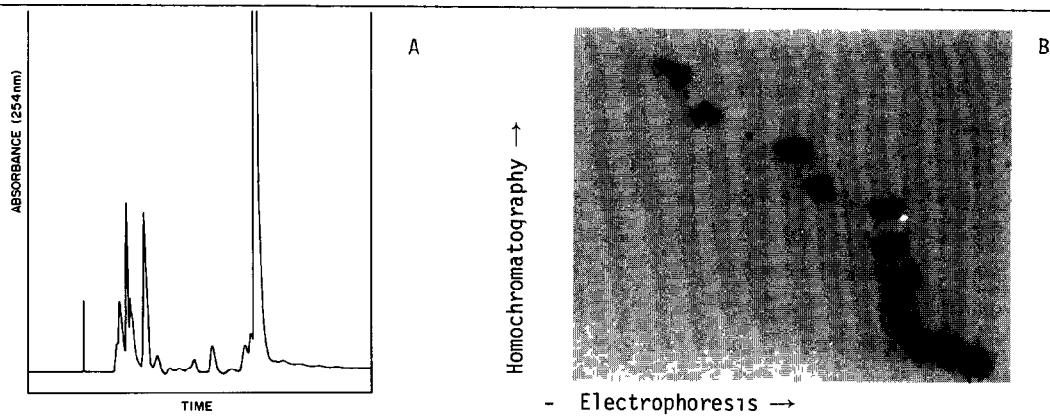


Figure 3. Part A HPLC elution Profile of a Completely Deprotected Sample of d(C-C-G-A-T-A-A-C-A-A-T-T), Part B Two Dimension Analysis of the Material Isolated from the Major Peak of the HPLC Profile Shown in Part A

sample of a deoxyoligonucleotide triester that has been partially purified through the first silica gel column The major peak was collected, lyophylized, redissolved in 10 mM triethylammonium bicarbonate, pH 7 0, and enzymatically characterized as the dodecanucleotide by two dimension analysis (Figure 3) A major series of spots corresponding in sequence to the expected product was observed

This phosphotriester deprotection procedure appears very promising Copper acetate catalyzed cleavage is rapid and specific for removal of the *o*-bromophenyl protecting group. Reaction conditions are extremely mild and no side products attributed to the deprotection have been observed Additional experiments using different metal salts and various substituted phenols could further enhance the usefulness of this approach

ACKNOWLEDGEMENTS

This is paper 7 in a series on Nucleotide Chemistry Paper 6 is M. H Caruthers, Y Stabinsky, Z Stabinsky, M Peters in *Proceedings of the Symposium on Promoters Structure and Function*, Monterey, CA, May, 1981, Praeger Scientific The research was supported by NIH (GM21120 and GM25680) and a Career Development Award (1 K04 GM00076) to M H C

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(Received in USA 23 September 1981)