Oxalyl-CPG: a labile support for synthesis of sensitive oligonucleotide derivatives

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ABSTRACT

A procedure is described for linking nucleosides covalently to controlled pore glass or cross-linked polystyrene supports by means of an oxalyl anchor. Though stable to triethylamine and diisopropylamine, the nucleoside-oxalyl link can be cleaved within a few minutes at room temperature with ammonium hydroxide in methanol. This new anchor can be used in automated synthesis of conventional oligonucleotides. The primary value, however, is that it enables one to employ solid support methodology to synthesize a variety of base-sensitive oligonucleotide derivatives, as illustrated here by synthesis of oligomers with base protecting groups intact and with methyl phosphotriester groups at the internucleoside links.

INTRODUCTION

Current methodology for oligonucleotide synthesis utilizes nucleosides anchored to a controlled pore glass (CPG) support by a succinyl linker (1). In a terminal chemical step the oligomer is released with concentrated ammonium hydroxide under conditions that lead to extensive or complete removal of the NH_2 and P-O protecting groups. For work with certain oligonucleotide derivatives it would be helpful, and in some cases necessary, to have linkers available that could be cleaved under milder conditions, so that sensitive substituents at phosphorus and/or base protecting groups could be retained in the freed oligomer. We describe herein an oxalyl connector that enables this to be accomplished. Convenient procedures are reported for preparing the nucleoside-oxalyl-CPG supports and examples are provided to illustrate use of these supports in synthesizing oligonucleotides and alkali sensitive oligonucleotide derivatives.

METHODS

Synthetic procedures

Synthetic reactions involving solid supports were carried out on a 1 μmole scale either manually, in a gas tight syringe equipped with a plug of glass wool at the base, or automatically on a Biosearch 8600 synthesizer. Nucleoside β-cyanoethyl phosphoramidites (MilliGen/Biosearch), nucleoside methyl phosphoramidites (Glen Research), nucleoside hydrogen phosphonates (Glen Research), long chain alkylamine controlled pore glass supports (80−100 mesh, 500A; Sigma Chemical Company), and aminomethylpolystyrene resin (1% divinylbenzene; Sigma Chemical Company) were used as obtained commercially. Acetonitrile, tetrahydrofuran, and pyridine were dried over calcium hydride. Unless otherwise stated, standard procedures for oligonucleotide synthesis *via* phosphoramidite (1) or hydrogen phosphonate chemistry (2) were employed. Care was taken to avoid unnecessary exposure of oligonucleotide methyl phosphotriester derivatives to basic conditions. Thus, after cleavage of the oxalyl anchor by ammonia or amines in methanol, the methanol solution was immediately expelled from the syringe and evaporated under vacuum. Products were then taken up in water and purified by reversed phase HPLC. Oligonucleotide hydrogen phosphonate intermediates were oxidized to the phosphodiesters with 0.2M iodine in water/pyridine, 1/9 v/v, (20 minutes). Triethylamine was eliminated from the standard recipe (2) to avoid premature cleavage of the oxalyl anchor. Iodine oxidation of the methyl and β-cyanoethyl phosphite derivatives was carried out with 0.1M iodine in tetrahydrofuran/pyridine/water, 20/9/1 v/v/v, (2 minutes).

Isolation and characterization procedures

C-18 Reversed phase chromatography was performed on a Hewlett-Packard 1090 chromatograph or a Dionex chromatograph equipped with a Hypersil ODS 5μ column, 100×2.1 mm, or a Nucleosil ODS 5μ column, 200×4.6 mm, with a gradient of acetonitrile (increasing 0.5%/min or 1.0%/min) and 0.03M triethylammonium acetate (pH 7.0), increasing 1%/min in acetonitrile. The flow rate was 0.5 ml/min for the 100 mm column and 1.0 ml/min for the 200 mm column.

Gel electrophoresis (PAGE) was carried out with 20% denaturing gels (acrylamide/bisacrylamide, 28.4/1 w/w; 170×14×2 mm plate) run for 2 hours at 400 volts.

Thin layer chromatography (TLC) was performed on Silica G Brinkmann plates using chloroform/methanol (9/1 v/v; useful in characterizing the uncharged oligonucleotide triester derivatives), or with 1-propanol/ammonium hydroxide/water (70/10/20 v/v/v, useful in characterizing the negatively charged oligomers).

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FAB positive ion mass spectra were obtained by Dr. D. Hung on a VG-70-250SE spectrometer.

For enzymatic hydrolysis of the N-protected oligonucleotide, 0.5 A_{260} Units of the 11-mer in 100 μl buffer (32 mM Tris-Cl, 12 mM $MgCl_2$, pH 7.5) was treated with phosphodiesterase from Crotalus durissus (Boehringer Mannheim, 45 μl as supplied) and alkaline phosphatase (Boehringer Mannheim, 8 μl, as supplied), and the volume was brought to 250 μl with distilled water. The mixture was incubated at 37°C for 7 hours, then heated 4 min. at 70°C, diluted to 400 μl with water, filtered and subjected to HPLC.

Preparation of nucleoside-oxalyl-CPG

Oxalyl chloride (20 μl, 0.23 mmol) was added to a solution of 1,2,4-triazole (77 mg, 1.1 mmol) in acetonitrile (2 ml). A small amount of precipitate formed but disappeared after addition of pyridine (0.1 ml). The appropriate DMT-nucleoside (0.23 mmol) in acetonitrile (1 ml) and pyridine (0.5 ml) was added, and after one hour the solution was drawn into a syringe containing the long chain aminoalkyl controlled pore glass (CPG) (400 mg). This mixture was allowed to stand for 15 min.; then the liquid was ejected and the solid in the syringe was treated successively with acetonitrile (wash), dry methanol (to cap residual oxalyl triazolide groups), acetonitrile (wash), an equivolume mixture of tetrahydrofuran solutions of dimethylaminopyridine (0.3M) and acetic anhydride (0.6M) (to cap any residual amino groups), pyridine (wash), and acetonitrile (wash). The nucleoside loading averaged about 50 μmoles/gram, as determined by absorbance of the dimethoxytrityl cation liberated on treatment of an aliquot with 3% dichloroacetic acid in methylene chloride.

An aminomethylpolystyrene resin was loaded with d(DMT-T) in the same way, yielding in this case a resin with approximately 110 μmoles DMT-thymidine per gram resin.

Alternatively, the support (aminoalkyl-CPG) could be treated first with oxalyl triazolide (30 minutes) and then with the DMT, N-protected nucleoside and dimethylaminopyridine (2 hours), with washing and capping as before. This sequence afforded a bound nucleoside suitable for synthetic extension; however, the extent of loading was relatively low (16−24 μmoles/gram after two loading cycles).

Preparation of d(T[OMe]T)

The preparation of this compound is representative of the procedures used. A solution containing the DMT-thymidine methyl phosphoramidite reagent (20 mg) and tetrazole (8.5 mg) in acetonitrile (0.25 ml) was drawn into a well dried syringe (2.5 ml, equipped with a glass wool filter at the base) containing dT-oxalyl-CPG (20 mg, 0.7 μmole of dT). After 2 minutes the solution was expelled and the solid washed with pyridine/acetonitrile (1/4) and dichloromethane. The contents of the syringe were then successively treated with 0.5M t-butyl hydroperoxide in dichloromethane (1 ml, 2 minutes), dichloromethane (wash), 3% dichloroacetic acid in dichloromethane, pyridine/dichloromethane (1/4; wash), methanol (wash), and 5% ammonium hydroxide in methanol (3 minutes). The ammoniacal solution was collected, the solid washed with methanol, and the combined methanolic solutions immediately evaporated under vacuum to give 10.7 A_{260} units of reaction products, 99% of which is attributable to d(T[OMe]T) (see HPLC profile in Figure 2; 0.63 μmole). Subsequent treatment of the support in the syringe with concd. ammonium hydroxide afforded an additional 0.7 A_{260} units of nucleotidic material, showing that 94% of the nucleotides originally bound to the support had been removed by the dilute ammonium hydroxide/methanol solution.

When the oxidation was carried out (2 min) with 0.1M iodine in tetrahydrofuran/pyridine/water (20/9/1 v/v/v) or tetrahydrofuran/pyridine/methanol (20/9/1 v/v/v), d(TpT) was observed as a product as well as the methyl triester (see Figure 2). About 95% of the loaded nucleotides was removed from the support by the brief treatment with 5% ammonium hydroxide in methanol in each case.

d(TpT) was characterized by co-elution on RP-C18 HPLC and co-migration on TLC (silica, 1-propanol/ammonium hydroxide/water 70/10/20 v/v/v) with an authentic sample of d(TpT). d(T[OMe]T) was characterized by FAB mass spectrometry ($M+H^+$, 561); migration on silica on TLC with methanol/chloroform (1/9 v/v) (Rf 0.35; the anionic dinucleoside phosphate does not migrate in this system); and conversion to d(TpT) by treatment with ammonium hydroxide.

RESULTS AND DISCUSSION

Labile anchors

Exploratory experiments were carried out with three structural variations in the anchor linking the 3'O of a nucleoside to the amino group of an aminoalkylsilica support: (i) $ROC(O)CH_2OCH_2C(O)...$, (ii) $ROC(O)C(O)...$, and (iii) $ROC(O)CH_2CH_2C(O)CH_2CHC(O)...$, where R represents a nucleoside group (3). Selection of these structures was based on literature precedents showing that, for reactions of esters in solution, the rate of cleavage by nucleophiles is accelerated by an α-alkoxy substituent (4) or a neighboring carbonyl group (5,6), and the rate of hydrazinolysis is enhanced by a gamma-keto group (7). We found that nucleosides could be loaded satisfactorily on an aminoalkyl-silica support *via* each of these linkers (3); however, only for the oxalic derivative (ii) was the increase in rate of release of nucleoside from the support sufficiently great relative to that for the succinyl derivatives to look promising for solid supported syntheses.

A convenient procedure for preparing a nucleoside-oxalyl-support is to treat oxalyl chloride successively with triazole, an appropriate N-protected DMT-nucleoside, and a solid support bearing amino groups (aminoalkyl-CPG or aminomethylpolystyrene resin). The overall process, including capping steps and washings, can be carried out in less than two hours.

Reactivity of the oxalyl anchor was examined with a CPG-support loaded with DMT-Thymidine (DMT-T). It was found that DMT-T was released quantitatively in less than five minutes by treatment with wet triethylamine, n-propylamine/dichloromethane (1/5 v/v), 40% trimethylamine in methanol, or 5% ammonium hydroxide in methanol. Under the same conditions less than 5% of the DMT-thymidine was released from a loaded succinyl-CPG support. Titanium isopropoxide (8) and cesium fluoride (0.5M) in methanol were also found to be effective in cleaving the oxalic ester in this system (~90% cleavage within 15 minutes).

Of equal interest with respect to synthetic applications is the stability of the oxalyl anchor. DMT-T-oxalyl-CPG proved to be stable to dry pyridine, triethylamine, or diisopropylamine for at least 15 hours at room temperature. In addition, it was also resistant to a mixture of pyridine, triethylamine, and thiophenol, the reagent employed in demethylating nucleoside phosphotriesters on solid supports.

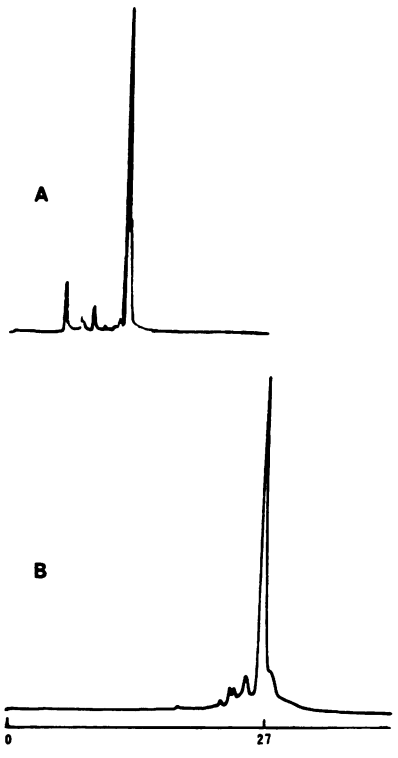


Figure 1. HPLC profile of products obtained by treating d(bzC[CE]bzC[CE]T[CE]bzA[CE]ibG[CE]bzC[CE]T[CE]T[CE]bzC[CE]bz-C-[CE]T-oxalyl-CPG, where [CE] represents the β-cyanoethyl phosphotriester internucleoside link, with: **A**, concd. NH_4OH, 55°C (19 h); **B**, $(i\text{-}Pr)_2NH$, room temperature (14 h) (to convert the cyanoethyl phosphotriester to diester links) followed by washing the support with acetonitrile and cleavage of the oxalic ester bond by 20% NH_4OH in MeOH, room temperature (2 min). The 100×2.1 mm column was used (see Methods section for conditions). d(CCTAGCTTCCT) elutes as the main peak in (**A**) at 10.9 min and d(bzCbzCTbzAibGbzCTTbzCbzCT) as the main peak in (**B**) at 26.8 min.

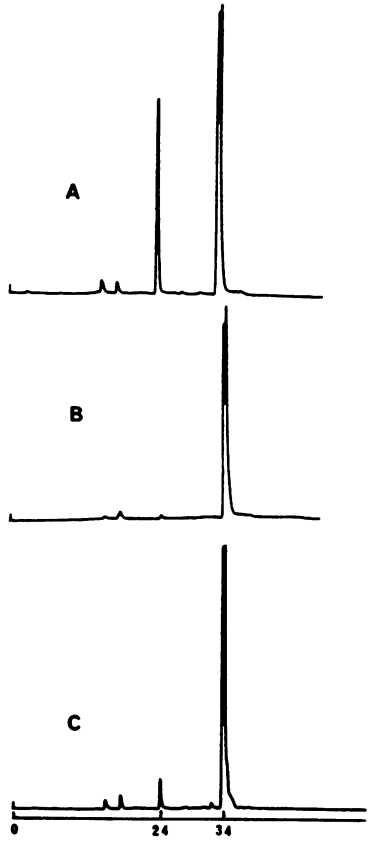


Figure 2. HPLC profile of products from oxidation of the phosphite precursor of dDMT-(T[OMe]T)-oxalyl-CPG with: **A**, 0.1M I_2 in THF/pyridine/H_2O (20/9/1 v/v/v; 2 min); **B**, 1M t-BuOOH in CH_2Cl_2 (2 min); **C**, I_2 in MeOH/pyridine (9/1 v/v; 2 min). Following oxidation, the products were released from the support by 5% NH_4OH in MeOH at room temperature (10 min), the solvent was evaporated, and the products were taken up in water and chromatographed (200×4.6 mm column). d(TpT) elutes at 24 min and d(T[OMe]T) at 34 min in this system.

Oligonucleotide synthesis

As a test of the feasibility of using the oxalyl anchor in automated syntheses, d(ACACCCAATTCTGAAAATGG) was prepared twice on a Biosearch 8600 instrument, one time with an oxalyl-CPG support and the other time with a conventional succinyl-CPG support. β-Cyanoethyl phosphoramidite chemistry was employed in both cases. The yields, measured by release of the dimethoxytrityl cation, averaged 96% for couplings on the oxalyl support and 97% for couplings on the succinyl support, and the oligonucleotides isolated from the two runs were identical as judged by elution time on HPLC (12.1 min; Hypersil ODS column), the ultraviolet spectrum (λ max 260 nm, λmin 238 nm), PAGE (Rf rel. to bromophenol blue 0.29), and the Tm values for the complexes formed with the complementary strand (Tm 70°C, pH 7.0, 1.0M NaCl)

Also, a manual synthesis of a decamer, d(TTTTTTTTTT), was carried out successfully using dT-oxalyl-CPG and hydrogen phosphonate chemistry, demonstrating that the oxalyl anchor is compatible with this approach as well.

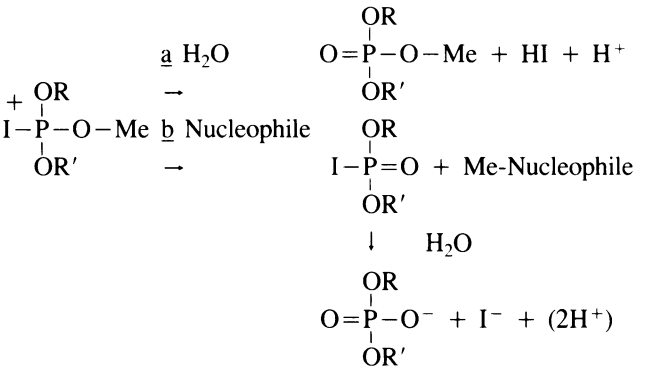
With these favorable results, attention was directed to two classes of compounds that have not been generally accessible *via* solid support methodology: N-protected oligonucleotides and oligonucleotide analogues bearing methyl phosphotriester internucleoside links. Both types of compounds are sensitive to the ammoniacal conditions used in cleaving the conventional succinyl anchor.

N-Protected oligonucleotides

d(bzCbzCTbzAibGbzCTTbzCbzCT) was selected as a synthetic target to explore selectivity in cleaving the oxalyl anchor, the β-cyanoethyl phosphotriesters, and the N-acyl protecting groups. For this purpose the fully protected oligomer was prepared using nucleoside β-cyanoethyl phosphoramidites and an oxalyl-CPG support loaded with thymidine. Treatment of a portion of the sample with concentrated ammonium hydroxide (5 hours, 55°C) afforded the fully deprotected oligomer (Figure 1A). Treatment of another portion under much milder conditions (20% ammonium hydroxide in methanol, 2 min) liberated the oligomers, but a complex mixture of products was found, indicative of non-specific partial removal of the cyanoethyl and N-protecting groups. The cyanoethyl phosphotriester groups in a short oligomer are quite sensitive to ammonium hydroxide (9); however, as negative charge is built up along the backbone of the longer oligomers, decyanoethylation of the remaining triesters becomes increasingly difficult (10).

Fortunately, a highly selective reaction with diisopropylamine was found that enables one to handle this problem. As a relatively

strong, sterically hindered base, the dry amine is effective in decyanoethylating the phosphotriesters, but it does not cleave the oxalyl-linker or the N-protecting groups over a period of 15 hours. Consequently, treatment of the support-bound oligomer with diisopropylamine (14 hours, room temperature), followed by washing with acetonitrile and reaction with 20% ammonium hydroxide in methanol (2 minutes), afforded the desired N-protected oligomer in high yield (Figure 1B) (11). Essentially no oligomer was lost from the support during the treatment with diisopropylamine, yet 97% of the support-bound material was liberated by the ammonium hydroxide reaction. That all triester groups had been converted to phosphodiesters and all base-protecting groups were still intact was demonstrated by hydrolyzing the purified oligomer with aqueous snake venom phosphodiesterase and alkaline phosphatase. The products were the protected nucleosides: dbzA, dbzC, dibG and dT (12).



Scheme 1. Pathway For Iodine Oxidation

d[T(OMe)T]

The oligonucleotide methyl phosphotriesters serve as prototypes for oligonucleotide analogues bearing functional groups at phosphorus sensitive to conventional cleaving reagents. In addition, interest in the methyl esters has recently been stimulated by reports that compounds of this class exhibit unexpected hybridization properties (13). Short oligomer strands containing methyl phosphotriesters were prepared by solution-phase chemistry many years ago (14,15), and a procedure has been described for obtaining oligonucleotides bearing some methyl triester links, stabilized by neighboring phosphodiesters, using solid phase methodology (10).

We explored the potential of the oxalyl anchor in syntheses of methyl triester derivatives by study of the dimer, d(T[OMe]T), where [OMe] represents the internucleoside methyl phosphotriester link. Thus, the 5'DMT-3'methyl phosphoramidite reagent of thymidine was condensed with thymidyl-oxalyl-CPG; then the intermediate phosphite was oxidized under several different conditions. In each case the resulting products were cleaved from the support with 5% ammonium hydroxide in methanol (3 minutes) and separated by reversed phase chromatography.

When the oxidation was carried out with 0.1M iodine in tetrahydrofuran/pyridine/water (20/9/1 v/v/v, 2 minutes), two major products were observed in the HPLC profile (Figure 2A). These were identified as the phosphodiester d(TpT) and the phosphotriester d(T[OMe]T) and were obtained in the ratio d(TpT)/d(T[OMe]T) = 0.25 (see Methods section for characterization). When the iodine oxidation was carried out in the presence of a low concentration of a hindered base (0.1M iodine and 0.24M 2,6-lutidine in tetrahydrofuran/water, 2/1 v/v), less, but still an appreciable amount, of the phosphodiester observed was found (d(TpT)/d(T[OMe]T) = 0.16).

The appearance of d(TpT) was somewhat surprising since the iodine reagent had been shown to convert analogous internucleoside trichloroethyl phosphites cleanly to the triester (16) and it has generally been assumed that long oligomer chains prepared on CPG supports *via* methyl phosphoramidite chemistry contain only phosphotriester links prior to deprotection with thiophenoxide. In principle, d(TpT) could have arisen (a) in the course of the iodine/water oxidation reaction, (b) subsequent to oxidation, by nucleophilic attack on the methyl group of the phosphotriester, or (c) during treatment of the triester derivative with ammonium hydroxide/methanol in the oxalyl cleavage step.

Several lines of evidence render alternatives b and c improbable as significant factors in generating d(TpT). Model experiments conducted in solution showed that d(T[OMe]T) is stable in the iodine/water reagent and in the dilute ammonium hydroxide/methanol solution under the conditions used in the synthetic reactions. Also, when an oxalyl-CPG support bearing the dinucleoside phosphite was exposed to the iodine reagent for 30 minutes and then worked up, essentially the same ratio of d(TpT)/d(T[OMe]T) was obtained as in the case of the 2 minute oxidation reaction, demonstrating that, even when attached covalently to a CPG support, a nucleoside methyl phosphotriester is resistant to the nucleophiles in the oxidation mixture. Especially revealing, however, was an experiment in which the loaded dimer at the phosphite stage was oxidized with t-butyl hydroperoxide in dichloromethane, a reagent reported to oxidize phosphites to phosphates rapidly and efficiently (17,18). Work-up as in the case of the iodine oxidations afforded the methyl phosphotriester with only a trace of the diester product (see Figure 2B; d(TpT)/d(T[OMe]T) = 0.006). This experiment indicates that t-butyl hydroperoxide is the reagent of choice in syntheses where a methyl phosphotriester is to be recovered and that the methyl phosphotriester is relatively stable to ammonium hydroxide/methanol in the environment of the CPG support.

We conclude from these experiments that formation of the phosphodiester (Figure 1A) is directly coupled to the oxidation reaction. A plausible pathway leading to the observed products is shown in Scheme 1 (19). Path a proceeds by attack of water at a phosphonium ion (generated by reaction of iodine with the phosphite) and leads ultimately to the methyl phosphotriester. Path b proceeds by attack of a nucleophile (e.g. pyridine, water, hydroxide, or iodide) at the methyl group in the phosphonium ion and affords the phosphodiester by hydrolysis of the intermediate phosphoryl iodide (or its equivalent). In line with this picture, we found that oxidation of the phosphite with 0.1M iodine in tetrahydrofuran/pyridine/methanol (20/9/1) gave the methyl triester with little diester (d(TpT)/d(T[OMe]T) = 0.06; see Figure 2C for the HPLC profile).

Evidence that the silica support *per se* is not a significant factor in determining the product distribution was obtained by synthesizing d(T[OMe]T) from thymidine loaded on an oxalyl-aminopolystyrene resin in place of oxalyl-CPG. Oxidation of separate aliquots of the resin bearing dimer at the phosphite stage with the iodine reagent, t-butyl hydroperoxide, and iodine/methanol, and work-up as in the previous cases, afforded products exhibiting HPLC profiles similar to those in Figure 2.

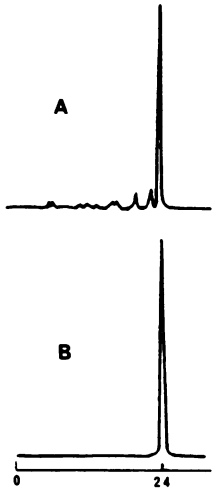


Figure 3. HPLC profile of d((T[OMe])$_5$T): **A**, crude product recovered from the solid support; **B**, rechromatography of product obtained from **A**; 100×2.1 mm column.

Oligonucleotide methyl phosphotriesters

A number of remarkable properties have been reported for the methyl phosphotriester derivative d((T[OMe])$_5$T) (13). These include self-association in water (or ethanol/water) to form a parallel stranded duplex exhibiting Tm values between 60°C and 70°C at oligomer concentrations ranging from 10^{-2} to 10^{-5}M.

In extending the scope of the solid supported oligomer syntheses and as a check on the reported dimerization, we synthesized this hexamer using a CPG support with the oxalyl anchor. Standard methyl phosphoramidite chemistry was employed in the couplings and t-butyl hydroperoxide (18) was used to oxidize the phosphite intermediates to the phosphate. Cleavage from the support was effected with 5% ammonium hydroxide in methanol (5 minutes). The synthesis proceeded well, as indicated by the HPLC profiles for the crude reaction mixture and the isolated product (Figure 3); 84% of the UV absorbing material liberated from the support was in the major peak. This compound was characterized by FAB mass spectroscopy ($M+H^+$ ion, 1833), conversion to d((Tp)$_5$T) by thiophenol and triethylamine, and migration on silica TLC with methanol/chloroform, 2/8 v/v (Rf 0.6). It may be noted, in accord with data for the dimer, that oxidation with iodine in tetrahydrofuran/pyridine/water afforded much less of the fully methylated hexamer (25% of the UV absorbing material that was eluted from the support) and several peaks (5 major ones) eluting faster than d((T[OMe])$_5$T on reversed phase chromatography. These products probably reflect varying degrees of demethylation.

For comparison, a hexanucleotide analogue containing four methyl triester groups and one phosphodiester link, d(Tp(T[OMe])$_4$T), was prepared. The synthetic sequence was the same as that used for d((T[OMe])$_5$T), with oxidation with t-butyl hydroperoxide, except that the final coupling utilized hydrogen phosphonate chemistry (2) and oxidation with 0.2M iodine in pyridine/water, 9/1 v/v. The synthesis proceeded well; 81% of UV absorbing material in crude reaction mixture corresponded to the target compound. In agreement with expectations, this compound did not migrate on a TLC silica plate (solvent: chloroform/methanol, 9/1 v/v), eluted on reversed phase HPLC faster (21.4 minutes) than the pentamethyl derivative (24.2 minutes), and showed a $M+H^+$ ion in the FAB mass spectrum at 1819. The difference in the properties of these two hexamers provides strong additional evidence that all methyl phosphotriester groups are indeed intact in the oligomer assigned the structure d((T[OMe])$_5$T).

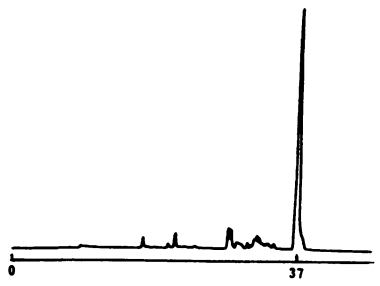


Figure 4. HPLC profile of crude product mixture obtained directly from synthesis of d(bzA[OMe]bzC[OMe]ibG); 200×4.6 column. The product elutes at 37 min.

No hyperchromic change (<0.3% variation in A_{260}) was observed when d((T[OMe])$_5$T) was warmed from 0°C to 90°C in water. In contrast to the previous report (13), therefore, we saw no evidence for self-association or any unusual hybridization properties for this modified oligonucleotide.

Oligonucleotide blocks protected at NH_2 and P-O

Fully protected short oligomers might serve as useful intermediates in block synthesis of polynucleotides and their derivatives. As a test of use of the oxalyl linker in making oligomers containing both P-O and NH_2 protecting groups, we prepared d(bzA[OMe]bzC[OMe]ibG). The synthesis, which employed dibG-oxalyl-CPG, nucleoside methylphosphoramidite reagents, oxidation with t-butyl hydroperoxide, and cleavage 5% ammonium hydroxide in methanol, proceeded satisfactorily, affording the targeted oligomer as the major product (80% of the A_{260} units released from the support; see Figure 4 for HPLC profile). This oligomer was characterized by the mass spectrum (parent ion 1176), ultraviolet spectrum (λ max 262, λ min 232), and conversion by ammonium hydroxide to the corresponding unprotected phosphodiester, which was identical to an authentic sample of d(ACG) prepared on a succinyl-CPG support in the conventional way.

CONCLUSIONS

Nucleosides can be linked easily to controlled pore glass or cross-linked polystyrene supports through an oxalyl anchor. The nucleoside-oxalyl link, which is much more sensitive to bases than the nucleoside-succinyl link conventionally used in support syntheses of oligonucleotides, can be cleaved readily with alcoholic solutions containing amines or ammonia, with a primary amine in absence of an alcohol, with cesium fluoride in methanol, or with titanium(IV) alkoxides. Dilute solutions of ammonium hydroxide in methanol (5−20%) are especially convenient since cleavage is complete within a few minutes at room temperature and the product can be quickly and easily recovered from the reagent. In contrast, the nucleoside-oxalyl link is relatively stable to dry tertiary amines, to hindered secondary amines (e.g. diisopropylamine), and to thiophenol in the presence of triethylamine.

The oxalyl anchor can be used in synthesizing oligonucleotides by means of either phosphoramidite or hydrogen phosphonate reagents. It also opens the way to synthesis of base-sensitive oligonucleotide derivatives on solid supports, as illustrated by preparation of an oligomer bearing N-protecting groups, d(bzCbzCTbzAibGbzCTTbzCbzCT), oligomers with methyl phosphotriester internucleoside links, d(T[OMe]T), d((T[OMe]$_5$T), and d(Tp(T[OMe]$_4$T), and an oligomer with both N-protecting groups and internucleoside methyl phosphotriester links, d(bzA[OMe]bzC[OMe]ibG). In contrast to a previous report (13), no evidence of self association of d((T[OMe]$_5$T) in water was found. The work with the methyl triesters revealed that phosphodiesters are formed as well as phosphotriesters when the methyl phosphite intermediates are oxidized with an iodine/water reagent. t-Butyl hydrogen peroxide is a preferred oxidizing reagent when one wishes to isolate the triesters. In the synthesis of d(bzCbzCTbzAibGbzCTTbzCbzCT) it was found advantageous to cleave the β-cyanoethyl groups by treatment with diisopropylamine prior to release of the oligomer from the support.

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11. In developing this reaction sequence we found that the tetramer, d(TbzCbzCT), could be recovered efficiently from the solid-supported intermediate, d(T[CE]bzC[CE]bzC[CE]T)-oxalyl-CPG), by treatmentment with neat triethylamine (15 hours, to remove cyanoethyl groups) followed by triethylamine/methanol (1/1) (15 minutes, to cleave the oxalyl group); however, these reagents were not satisfactory for work with the undecamer derivative (only partial decyanoethylation and about 50% cleavage from the support).
12. It was shown in a control with a mixture of the four nucleosides and the three N-protected nucleosides that a baseline separation of all seven compounds was obtained on HPLC (200 mm column). The chromatogram of the hydrolyzed N-protected oligomer showed the expected dT, dibG, dibC and dbzA. A barely detectable amount of dC was observed (< 1% of the dbzC peak), and no dA or dG was found.
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19. Transformations of this type have also been reported for the iodine/water oxidation of internucleoside phosphite esters containing a 2-cyano-1,1-dimethylethyl group [Nielsen, J., and Caruthers, M.H. (1988) J. Am. Chem. Soc., **110**, 6275–6276] and a 2-methylbenzyl group [Caruthers, M.H., Kierzek, R., and Tang, J.-Y. (1987), Biophosphates and their Analogs, Synthesis, Structure, Metabolism and Activity, Bruzik, K.S., and Stec, W., Eds., Elsevier, Amsterdam, p 3]. Also pertinent is the conversion of triethyl phosphite to diethyl phosphoroiodite by iodine in ether at 0°C [McCombie, H., Saunders, B.C., and Stacy, G.J. (1945) J. Chem. Soc., 921].