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Photochemical Cleavage of Oligonucleotides From Solid Phase Supports

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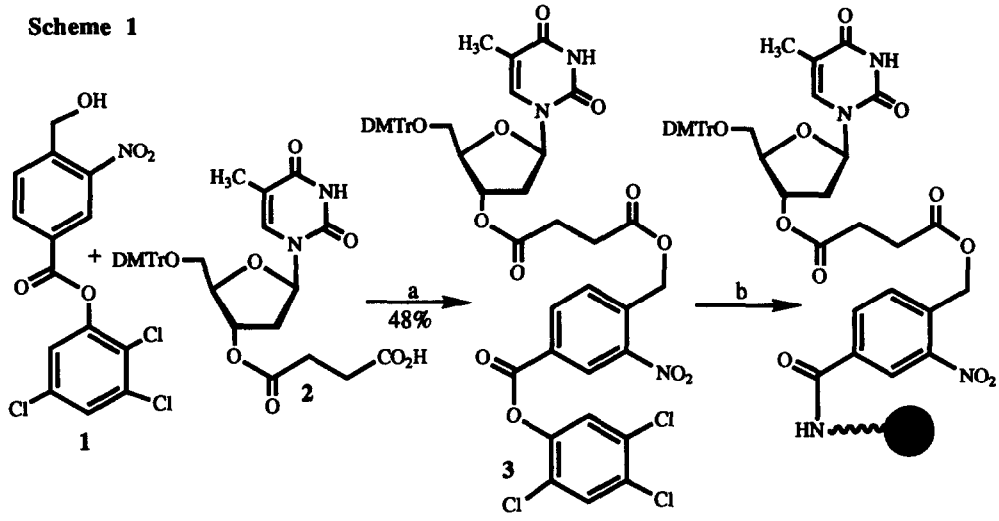
Abstract: 5'-O-Dimethoxytrityl-3'-O-succinatothymidine is covalently linked to long chain alkyl amine controllled pore glass support (LCAA-CPG) via an *o*-nitrobenzyl group. The modified solid phase synthesis support is compatible with standard automated phosphoramidite based oligonucleotide synthesis. Cleavage from the solid support is achieved via $CuSO_4$ filtered photolysis. Isolated yields are comparable with those obtained from other oligonucleotide supports.

Modified oligodeoxynucleotides are of tremendous use in mechanistic bioorganic chemistry and show great promise as therapeutic devices.[1,2] Chemical methods that permit the incorporation of unnatural and/or base sensitive functional groups are evolving at a rapid rate.[3] A variety of methods have been developed to deprotect chemically synthesized oligonucleotides. Improvements include alkali labile nucleobase protecting groups, as well as those removable under reductive conditions.[4,5] A variety of linkers between the oligonucleotide and solid support that exhibit enhanced base lability, or are cleaved under reductive conditions have also been developed.[6,7] This letter reports the first photochemical method for cleaving an oligonucleotide from its solid support. The photocleavage is accomplished at room temperature, at neutral pH, and with wavelengths of light which do not damage the biopolymer. When used in conjunction with previously published methods for deprotecting nucleobases and phosphodiesters, this method enables the complete deprotection of an oligonucleotide under extremely mild conditions.

Severing of the oligonucleotide-biopolymer linkage was accomplished using the photolabile *o*-nitrobenzyl moiety. The *o*-nitrobenzyl group has been used successfully as a photolabile protecting group for a variety of functional groups, as well as a photocleavable linker in solid phase peptide and oligosaccharide synthesis.[8,9] While the *o*-nitrobenzyl moiety has been used as a nucleoside protecting group,[3a] it has yet to be employed as a photolabile solid phase support linker in oligonucleotide synthesis. Such a support was constructed by covalently linking the 3'-terminus nucleoside to the long chain alkylamine controlled pore glass (LCAA-CPG) support via its succinate ester, which was in turn bound to the photolabile moiety (Scheme 1). 5'-O-Dimethoxytrityl-3'-O-succinatothymidine (**2**) was coupled in solution to the trichlorophenyl ester of 4-hydroxymethyl-3-nitrobenzoic acid (**1**).[8a] Activated ester **3**[10] was then coupled to the LCAA-CPG support in the presence of 1-hydroxybenzotriazole hydrate. The activated ester was present in twenty fold excess with respect to free amine on the resin. After shaking the heterogeneous mixture for 24 hours, quantitative ninhydrin analysis of an aliquot of the resin indicated that less than 5% of the original amount of free amine remained. Unreacted amine was capped using acetic anhydride, pyridine and catalytic DMAP. The resin was determined to contain 35 micromoles of the dimethoxytrityl moiety per gram of resin by treating a portion of resin with 0.1 N p-toluenesulfonic acid in acetonitrile, and quantitating the dimethoxytrityl cation released by absorption spectroscopy (λ_{max} = 498 nm, ε = 7 x 10^4 $M^{-1}cm^{-1}$).

Scheme 1



a. DCC, CH_2Cl_2 b. HOBt•H_2O, LCAA-CPG, DMF

Oligonucleotides consisting of polythymidine were synthesized using unaltered automated synthesis cycles. In order to demonstrate the tolerance of the resin for a range of deprotection strategies, nucleoside β-cyanoethyl phosphoramidites were used for preparing an eicosamer of polythymidine (T_{20}), and nucleoside methyl phosphoramidites were employed during the synthesis of a dodecamer of polythymidine (T_{12}). No differences in coupling efficiency (as determined from trityl responses) were detected between the modified CPG support and commercial succinate columns. Methyl groups were removed from phosphotriesters using thiophenol and triethylamine in dioxane (1:2:2 by volume) for 1 hour at 25°C. β-Cyanoethyl containing phosphotriesters were unmasked using neat diisopropylamine (15 hours, 25°C).[6a] Phosphate protecting groups could be removed before, or after photolytic cleavage. However, when employing methyl phosphoramidites it was more convenient to remove the phosphate protecting groups while the biopolymer was still on the resin. In this way the thiol could be conveniently removed by filtration. Photolyses were carried out under nitrogen in acetonitrile:water (90:10, v:v) at 800 W for three hours using the $CuSO_4$ filtered output of a Hg/Xe lamp. Samples were mechanically stirred throughout the photolysis. Stirring a mixture of resin bound oligonucleotide and the aforementioned solvents in the dark did not release any biopolymer, indicating that cleavage does not result from mechanical shearing of the biopolymer from the support.

In order to gauge the proficiency of the photocleavage process, yields of photochemically cleaved biopolymers were determined relative to resin from the same synthesis which was subjected to the same phosphate deprotection conditions, but which was cleaved from the resin using ammonium hydroxide. Oligonucleotides were quantitated by UV-VIS spectroscopy following purification of fully deprotected oligonucleotides by 20% denaturing (45% urea) polyacrylamide gel electrophoresis (PAGE), elution from the gel, and desalting using C_{18} reverse phase cartridges. Yields per milligram of resin of photochemically cleaved oligonucleotide were greater than 86% those obtained by standard ammonium hydroxide treatment.

The structure and chemical integrity of the unpurified biopolymers were investigated further by anion exchange HPLC (Figure 1) and PAGE analysis of 5'-^{32}P labeled material. The retention time of the oligonucleotide which was deprotected in the conventional manner (concentrated NH_4OH, 55°C) was approximately two minutes shorter than the dodecamer that was deprotected photochemically. This supports the assumption that the photocleavage results in release of an oligonucleotide containing a free carboxylic acid moiety at its 3'-terminus via oxidation of the benzylic position followed by hydrolysis (Figure 2).[9] The additional negative charge associated with the free carboxylate increases the retention time of the oligonucleotide by approximately the same amount as an additional nucleotide and its accompanying phosphodiester. Autoradiograms of gel electrophoresis experiments did not reveal the presence of slower migrating bands

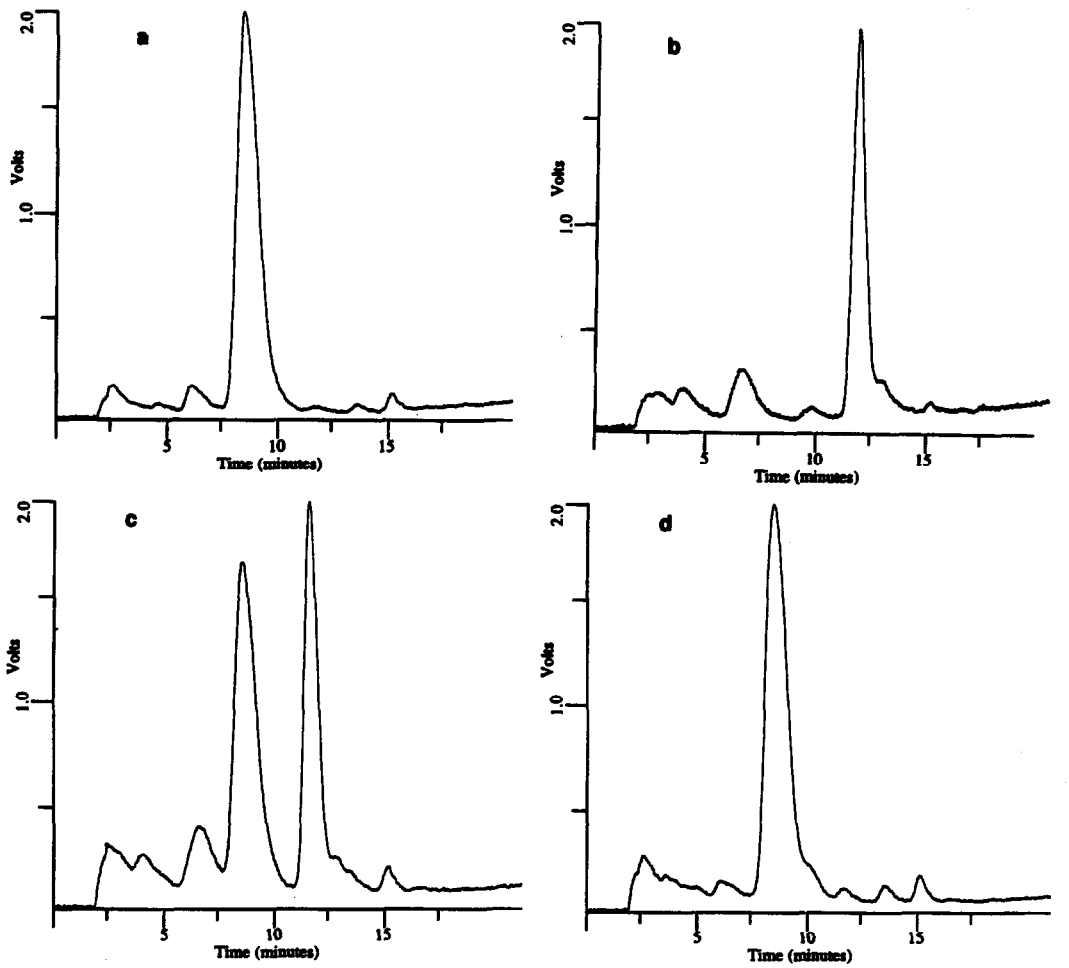


Figure 1. Ion exchange HPLC analysis of crude T_{12}.[11] a) Dodecamer deprotected using concentrated NH_4OH at 55°C for 4 hours. b) Dodecamer treated with thiophenol and then photolyzed for 3 hours. c) Coinjection of concentrated NH_4OH and photolytically deprotected T_{12}. d) Photolytically deprotected T_{12} subsequently treated with concentrated NH_4OH.

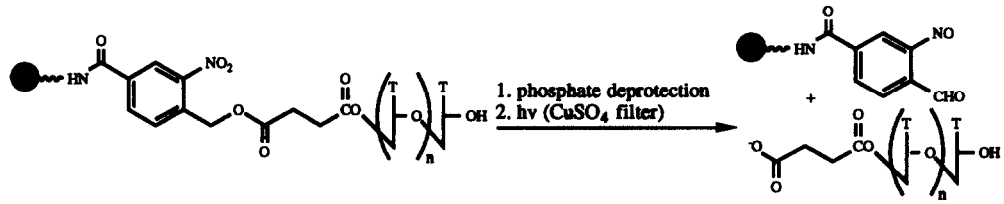


Figure 2. Formation of oligonucleotide containing a 3'-succinate terminus upon photolysis of *o*-nitrobenzyl linked oligonucleotide.

(corresponding to thymine-thymine photodimers) in lanes containing the photocleaved polymers, indicating that photolysis does not damage the biopolymer.[12]

In conclusion, this letter presents a method which enables the removal of oligonucleotides from their solid phase support in high yield under conditions that do not degrade the biopolymer. This method will be amenable to the synthesis of heterogeneous oligonucleotide sequences when used in conjunction with the appropriate nucleobase protecting groups.[5] By eliminating the concentrated ammonium hydroxide treatment, this strategy should facilitate the incorporation of novel, base sensitive nucleosides.[13] Furthermore, it is potentially advantageous over previous modified cleavage protocols in that it will enable the conjugation of oligonucleotides to other biomolecules through dissulfide linkers.[7]

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10. **3**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.80 (bd s, 1H), 8.42 (d, 1H, J = 6 Hz), 7.83 (d, 1H, J = 6 Hz), 7.60 (s, 1H), 7.57 (m, 2H), 7.40 (s, 1H), 7.38 (m, 1H), 7.27 (m, 7H) 6.82 (m, 4H), 6.44 (m, 1H), 5.65 (s, 2H), 5.50 (m, 1H), 4.13 (m, 1H), 3.78 (s, 6H), 3.45 (m, 2H), 2.72-2.84 (m, 4H), 2.42 (m, 2H), 1.38 (s, 3H). IR (cm^{-1}) 3325, 1737, 1690, 1623. MP 165-168°C (dec). Exact mass: $C_{49}H_{42}N_3O_{14}Cl_3Na$(calcd.) 1026.1600 (found).1026.1602
11. HPLC was carried out on a VYDAC 0.46 x 25 cm weak anion exchange column. Elution was accomplished using a linear gradient consisting of 0-100% B over 30 minutes. A: 0.1 M pH 6.7 ammonium phosphate, 20% acetonitrile. B: 0.3 M pH 6.7 ammonium phosphate, 20% acetonitrile.
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