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(2-Acetoxyphenoxy)ethyl (APOE) as a Phosphate Protecting Group in Solid-Phase Synthesis of Oligonucleotides via the Phosphoramidite Approach

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Abstract:

The (2-acetoxyphenoxy)ethyl (APOE) group could be an alternative to the conventional 2-cyanoethyl group for phosphate protection in solid-phase oligonucleotide synthesis to circumvent DNA alkylation by acrylonitrile generated under basic condition. This group is stable during oligonucleotide synthesis and can be removed under mild conditions using aqueous ammonium hydroxide. Multiple phosphorothioate oligodeoxyribonucleotides and 2′-*O*-methoxyethyl-modified oligoribonucleotide chimera were synthesized and characterized extensively. The deprotection of this group follows an intramolecular attack on the α-carbon adjacent to phosphate oxygen to liberate the oligonucleotide and an innocuous cyclic ether as side product. No modification of nucleobases was observed during deprotection of this group.

Introduction

With the advent of 2-cyanoethyl-protected nucleoside phosphoramidites,[1] rapid and highly efficient solid-phase syntheses of oligodeoxyribonucleotides and their analogues are now available and have, in the past two decades, revolutionized the therapeutic field as shown by numerous drugs being evaluated in the clinic.[2] Synthetic scales range from nanomole to almost a mole, and currently it takes less than 8 h to synthesize a phosphorothioate oligonucleotide of 20-mer in length at 750 mmol scale on GE Amersham OligoProcess synthesizer. Synthesis involves monomeric building blocks containing a 2-cyanoethyl group for phosphate protection. This group is eventually removed using an amine or other conditions.[3] Care must be taken to avoid addition of acrylonitrile formed by β-elimination of cyanoethyl group to nucleobases. The potent DNA alkylating properties of acrylonitrile have been investigated by many laboratories.[4] In addition, storage of solutions of cyanoethyl-protected phosphoramidites for extended periods of time undergo an Arbuzov-type of rearrangement leading to depletion of the desired concentration. Even though methods have been taken to circumvent the DNA-alkylation issue, it is desirable to have a phosphate protecting group which will not have such side reactions. Several phosphate protecting groups have been reported by many laboratories in the last several years.[5] Still there is a need to develop a group that will meet all of the requirements of a good phosphate protecting group, viz. stability and compatibility with various reagents, scalability, absence of side reactions, low cost, and versatility. Here we report (2-acetoxyphenoxy)ethyl (APOE) as an alternative to the 2-cyanoethyl group for the efficient synthesis of oligonucleotides.

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(1) (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett*. **1981**, *22*, 1859−1862. (b) McBridge, L. J.; Caruthers, M. H. *Tetrahedron Lett*. **1983**, *24*, 245−248. (c) Sinha, N. D.; Biemat, J.; Koster, H. *Tetrahedron Lett*. **1983**, *24*, 5843−5846. (d) Sinha, N. D.; Biemat, J.; McManus, J.; Koster, H. *Nucleic Acids Res*. **1984**, *12*, 4539−4557. (e) Gasparutto, D.; Molko, D.; Teoule, R. *Nucleosides Nucleotides* **1990**, *9*, 1087−1098. (f) Lyttle, M. H.; Wright, P. B.; Sinha, N. D.; Bain, J. D.; Chamberlin, A. R. *J. Org. Chem*. **1991**, *56*, 4608−4615. (g) Beaucage, S. L.; Caruthers, M. H. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.; John Wiley & Sons: New York, 1999; pp 3.3.1−3.3.20.

(2) A number of second-generation phosphorothioate oligonucleotides are in various stages of preclinical and clinical trials against ApoB-100, PTP-1B, VLA4, TRPM2, survivin, STAT-3, eIF-4E, etc. for the treatment of a variety of diseases such as cancer, psoriasis, diabetes, asthma, arthritis, multiple sclerosis, etc. Refer to website: www.isispharm.com for additional information.

(3) (a) Capaldi, D. C.; Gaus, H.; Krotz, A. H.; Arnold, J.; Carty, R. L.; Moore, M. N.; Scozzari, A. N.; Lowery, K.; Cole, D. L.; Ravikumar, V. T. *Org. Process Res. Dev*. **2003**, *7*, 832−838. (b) Umemoto, T.; Wada, T. *Tetahedron Lett*. **2005**, *46*, 4251−4253.

(4) (a) Wilk, A.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem*. **1999**, *64*, 7515−7522. (b) Wilk, A.; Grajkowski, A.; Srinivasachar, K.; Beaucage, S. L. *Antisense Nucleic Acid Drug Dev*. **1999**, *9*, 361−366. (c) Crippa, S.; Di Gennaro, P.; Lucini, R.; Orlandi, M.; Rindone, B. *Gazz. Chim. Ital*. **1998**, *123*, 197−203. (d) Prokopczyk, B.; Bertinato, P.; Hoffman, D. *Carcinogenesis* **1988**, *9*, 2125−2128. (e) Mag, M.; Engels, J. W. *Nucleic Acids Res*. **1988**, *16*, 3525−3543. (f) Chambers, R. W. *Biochemistry* **1965**, *4*, 219−226.

(5) Phosphate protecting groups: *3-(2-pyridyl)-1-propyl and 2-[N-methyl-N-(2-methyl)]aminoethyl*: Cleslak, J.; Beaucage, S. L. *J. Org. Chem*. **2003**, *68*, 10123−10129. *3-(N-tert-Butylcarboxamido)-1-propyl*: Wilk, A.; Chmielewski, M. K.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem*. **2002**, *67*, 6430−6438. *4-[N-Methyl-N-(2,2,2-trifluoroacetyl)-amino]butyl*: Wilk, A.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem*. **1999**, *64*, 7515−7522. *4-Cyano-2-butenyl:* Ravikumar, V. T.; Cheruvallath, Z. S.; Cole, D. L. *Tetrahedron Lett*. **1996**, *37*, 6643−6646. *Allyl:* (a) Hayakawa, Y.; Uchiyama, M.; Kato, H.; Noyori, R. *Tetrahedron Lett*. **1985**, *26*, 6505−6508. (b) Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. *J. Org. Chem*. **1986**, *51*, 2400−2402. (c) Hayakawa, Y.; Kato, H.; Nobori, T.; Noyori, R.; Imai, J. *Nucleic Acids Res. Ser*. **1986**, *17*, 97−100. (d) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc*. **1990**, *112*, 1691−1696. (e) Hayakawa, Y.; Hirose, M.; Noyori, R. *J. Org. Chem*. **1993**, *58*, 5551−5555. (f) Hayakawa, Y.; Hirose, M.; Noyori, R. *Nucleosides Nucleotides* **1994**, *13*, 1337−1345. (g) Bergmann, F.; Kueng, E.; Laiza, P.; Bannwarth, W. *Tetrahedron Lett*. **1995**, *51*, 6971−6976. *2-(Trimethylsilyl)ethyl:* Wada, T.; Sekine, M. *Tetrahedron Lett*. **1994**, *35*, 757−760. *(2-Diphenylmethylsilyl)ethyl*: (a) Ravikumar, V. T.; Sasmor, H.; Cole, D. L. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 2637−2640. (b) Ravikumar, V. T.; Wyrzykiewicz, T. K.; Cole, D. L. *Tetrahedron* **1994**, *50*, 9255−9266. (c) Ravikumar, V. T.; Cole, D. L. *Gene* **1994**, *149*, 157−161. (d) Krotz, A. H.; Cole, D. L.; Ravikumar, V. T. *Tetrahedron Lett*. **1996**, *37*, 1999−2002. (e) Krotz, A. H.; Wheeler, P.; Ravikumar, V. T. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 2406−2409. (f) Krotz, A. H.; Cheruvallath, Z. S.; Cole, D. L.; Ravikumar, V. T. *Nucleosides Nucleotides* **1998**, *17*, 2335−2338. *2,2,2-Trichloro-1,1-dimethylethyl*: (a) Letsinger, R. L.; Groody, E. P.; Tanaka, T. *J. Am. Chem. Soc*. **1982**, *104*, 6805−6806. (b) Letsinger, R. L.; Groody, E. P.; Lander, N.; Tanaka, T. *Tetrahedron* **1984**, *40*, 137−143. *p-Nitrophenylethyl*: (a) Schwarz, M. W.; Pfleiderer, W. *Tetrahedron Lett*. **1984**, *25*, 5513−5516. (b) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59−72. (c) Schwarz, M. W.; Pfleiderer, W. *Nucleosides Nucleotides* **1985**, *4*, 291−292. (d) Hamamoto, S.; Takaku, H. *Chem. Lett*. **1986**, 1401−1404.

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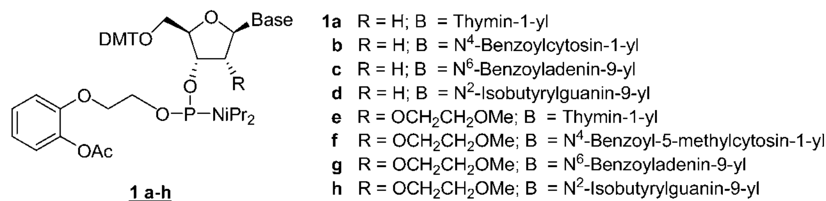
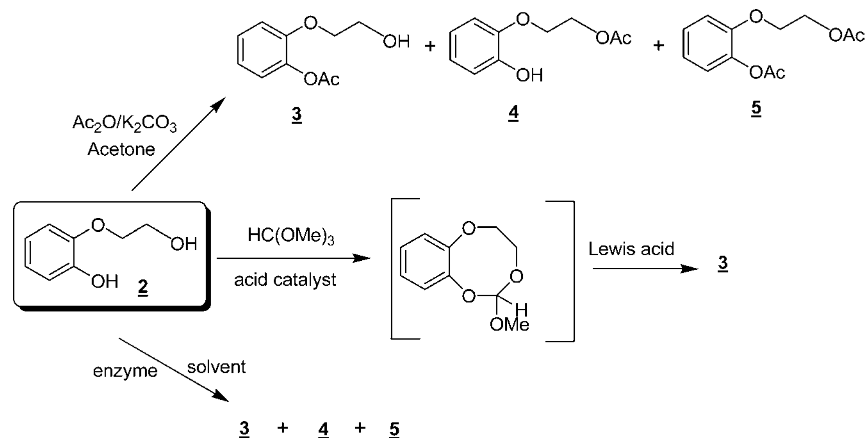


Figure 1. **Various APOE-protected phosphoramidites synthesized.**

Scheme 1. **Synthesis of 2-(2-acetoxyphenoxy)ethanol**



Earlier, we reported the use of deoxyribonucleotide phosphoramidites in the solid-phase synthesis of oligonucleotides having the 2-diphenylmethylsilylethyl (DPSE) group as a phosphate protecting group.[5] This group is easily removed from oligonucleotides by simple treatment with tetrabutylammonium fluoride (TBAF). DPSE had many of the favorable properties of a good phosphate protecting group; however, the starting material, 2-diphenylmethylsilylethanol is relatively expensive even in bulk quantities. Because of this these phosphoramidites might not cost-effectively compete with conventional 2-cyanoethyl amidites.

In an effort to reduce the cost of therapeutic oligonucleotides, we initiated a search for an inexpensive molecule which could serve as an efficient phosphate protecting group. We now propose the use of (2-acetoxyphenoxy)ethyl (APOE) for enhanced phosphate protection and report its incorporation into oligonucleotides via the nucleoside phosphoramidites **1a**−**h** (Figure 1). The usefulness of this group is demonstrated in the solid-phase synthesis of several first- and second-generation phosphorothioate oligonucleotides. The rapid and facile removal of the APOE group under standard ammonia deprotection conditions is shown, and the rates of deprotection kinetics under different conditions are addressed.

Synthesis of (2-Acetoxyphenoxy)ethanol

2-(2-Hydroxyphenoxy)ethanol is an inexpensive starting material ($70/kg) and is available in bulk from multiple vendors. Protection of the phenolic hydroxyl group could be achieved by different routes. The key to choosing the best route is the one which will afford the maximum yield with chemospecific protection of the phenoxy hydroxyl group and not the aliphatic hydroxyl group.

Chemical Synthesis. Direct acetylation of 2-(2-hydroxyphenoxy)ethanol with acetic anhydride under various conditions gave different selectivities. A key to obtaining high yield and quality is by slowly adding acetic anhydride to a vigorously stirred mixture of 2-(2-hydroxyphenoxy)ethanol in acetone and powdered potassium carbonate. The reaction is fast and complete within 4 h. Slow addition of anhydride and fast mechanical stirring is very important for achieving best results. The desired product was obtained as a pale-yellow viscous oil in >80% chemical yield and quantitative chemoselectivity after purification (based on HPLC analysis) (Scheme 1). The other regioisomeric acetate **4** and bis-acetate **5** were obtained as colorless solids.

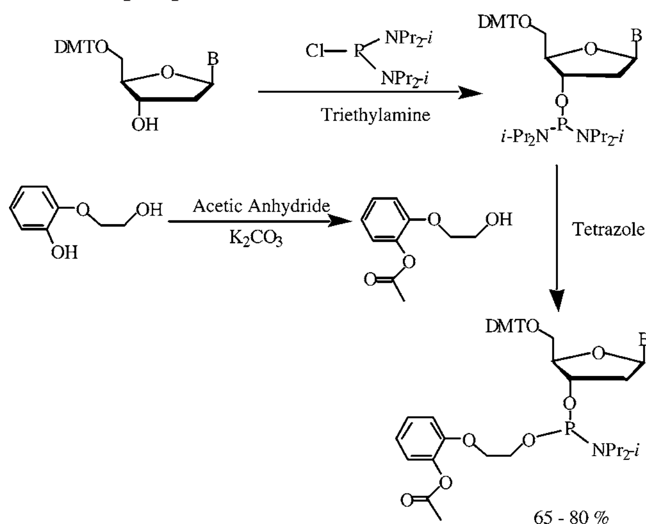
An alternative choice to obtaining monoprotection is formation of an acetal with triethyl orthoformate and chemoselective ring opening with a Lewis acid such as $AlCl_3$ or DIBALH. Multiple attempts at optimizing the reaction conditions did not afford any better yield and chemoselectivity as compared to those from direct acetylation (Scheme 1).

Enzymatic Synthesis. Monoacetylation was also achieved via the use of lipase enzymes. However, after screening several enzymes, it was not found to be better than direct chemical acylation and was not pursued further (Scheme 1).

Synthesis of APOE-Protected Phosphoramidites

Using Nucleoside Bisamidite Approach. Treatment of a DMT-protected nucleoside with bis(diisopropylamino)-chlorophosphine in the presence of triethylamine as base in anhydrous acetonitrile or dichloromethane gave the bisamidite which, upon further reaction with 2-(2-acetoxyphenoxy)ethanol, gave after workup and purification the corresponding phosphoramidites as colorless, foamy solids. ^{31}P

Scheme 2. **Various syntheses of APOE-protected nucleoside phosphoramidites**



NMR of deoxyribonucleoside phosphoramidites synthesized through this approach showed the characteristic signals (~146 ppm) corresponding to diastereoisomeric mixtures, and no 3′,3′-dinucleoside or bis-APOE phosphites could be detected. The phosphoramidites **1 a−d** were prepared in 65−80% yields (Scheme 2).

Using APOE Phosphitylating Reagent 6. Alternatively, treatment of 2-(2-acetoxyphenoxy)ethanol with bis(diisopropylamino)chlorophosphine in the presence of triethylamine under anhydrous conditiond gave the phosphitylating reagent **6** as a colorless solid which, on reaction with DMT-protected nucleosides in the presence of 1*H*-tetrazole, gave (after work up and column chromatography purification) the desired phosphoramidites in 60−82% overall yield (Scheme 3).[6]

Oligonucleotide Synthesis. The applicability of phosphoramidites **1 a-d** were demonstrated by the synthesis of four heterodimers d(TpsC), d(CpsT), d(GpsA), and d(ApsG) on solid support (yield >99%). The integrity and authenticity of these compounds were confirmed by comparing with identical hetrodimers independently synthesized using 2-cyanoethyl protection of the phosphorothioate backbone.

Synthesis of 20-mer Phosphorothioates on Solid Support

First-Generation Oligonucleotides. To evaluate the coupling efficiency of the APOE group, a 20-mer phosphorothioate oligodeoxyribonucleotide (5′-TCC-CGC-CTG-TGA-CAT-GCA-TT) (ISIS 5132) was chosen as an example. The synthesis was carried out on a 160 μmole scale using Pharmacia OligoPilot II synthesizer. Pharmacia's HL30 primary support (90 μmol/g loading) with 2.5 molar excess of amidites was used. Phenylacetyl disulfide (0.2 M, CH_3CN/3-picoline, 1:1 v/v) was used for sulfurization. Following the synthesis, the oligonucleotide was deprotected with concentrated aqueous ammonium hydroxide for 12 h at 55 °C. The sample was cooled, filtered, concentrated, and analyzed by CGE.[7] Subsequently, the material was purified by C-18 reversed-phase HPLC; all DMT-on fractions were collected, detritylated, and lyophilized to a colorless hygroscopic powder. The purified oligonucleotide was analyzed by CGE, anion-exchange HPLC, and ^{31}P NMR spectroscopy.[8] These data clearly indicate that APOE is a suitable phosphate protecting group. This excellent performance of the APOE group was exemplified with another sequence (5′-GCC-CAA-GCT-GGC-ATC-CGT-CA) synthesized under identical conditions.

Second-Generation Oligonucleotides. To extend the utility of the APOE protecting group to the synthesis of 2′-*O*-methoxyethyl-modified oligoribonucleotide phosphorothioates, a 20-nucleotide hemi-mer [5′-d($G^{me}C^{me}C^{me}CAAG^{me}$-$CTGG^{me}C$)-r($A^{me}U^{me}C^{me}CG^{me}U$)] [ISIS 15839] (where ^{me}dC and MOE ^{me}C are methylated at the 5-position of the nucleobase, r = 2′-*O*-methoxyethylribonucleoside) was chosen as an example. Synthetic conditions similar to those for first-generation phosphorothioate oligonucleotides were followed. The oligonucleotide was purified similarly to afford the desired colorless hygroscopic powder. The purified oligonucleotide was analyzed by CGE, anion-exchange HPLC, and ^{31}P NMR spectroscopy. The oligonucleotide was further confirmed by mass spectroscopy, demonstrating that APOE is a suitable phosphate protecting group for modified sequences also.

Experimental Section

Materials and Methods. 1*H*-Tetrazole was purchased from American International Co. Standard 5′-*O*-4,4′-dimethoxytrityl-protected deoxyribonucleosides were purchased from Yamasa, Japan, and 2′-*O*-methoxyethylribonucleosides (benzoyl dA and MOE A, benzoyl dC and MOE meC, isobutyryl dG and MOE G, T and 5meU) were purchased from customs manufacturers, viz. Innovasynth Chemical Co, India, Sai Dru Syn, India, and Shasun, India. Phenylacetyl disulfide (PADS) was purchased from Acharya Chemicals, Dombivili, India, and dichloroacetic acid, from Clariant Life Sciences, Germany. Derivatized polystyrene supports loaded with the corresponding nucleoside were obtained from GE Amersham Biosciences, Uppsala, Sweden.

Acetylation of 2-(2-Hydroxyphenoxy)ethanol. 2-(2-Hydroxyphenoxy)ethanol (308 g; 2 mol) was taken up in a 5-L Erlenmeyer flask fitted with mechanical stirrer. Anhydrous acetone (dried with K_2CO_3) (4 L) and potassium carbonate powder (290 g; 2.1 mol) were added to it and stirred vigorously. Acetic anhydride (207 mL; 2.2 mol) was taken up in an additional funnel and added slowly over a period of 1 h. Stirring was continued for 3−4 h. TLC (CH_2Cl_2/MeOH, 9:1, v/v) showed the disappearance of the starting material. The reaction mixture was filtered, the solid was washed thoroughly with acetone (1 L). The combined fractions were concentrated and purified by silica gel chromatography, eluting with hexane and ethyl acetate (0%

(6) Gukathasan, R.; Massoudipour, M.; Gupta, I.; Chowdhury, A.; Pulst, S.; Ratnam, S.; Sanghvi, Y. S.; Laneman, S. A. *J. Organmet. Chem.* **2005**, *690*, 2603−2607.

(7) (a) Srivatsa, G. S.; Batt, M.; Schuette, J.; Carlson, R. H.; Fitchett, J.; Lee, C.; Cole, D. L. *J. Chromatogr.* **1994**, *680*, 469−477. (b) Cummins, L. L.; Winniman, M.; Gaus, H. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1225−1230.

(8) Srivatsa, G. S.; Klopchin, P.; Batt, M.; Feldman, M.; Carlson, R. H.; Cole, D. L. *J. Pharm. Biomed. Anal.* **1997**, *16*, 619−630.

Scheme 3. Synthesis of APOE-protected nucleoside phosphoramidites

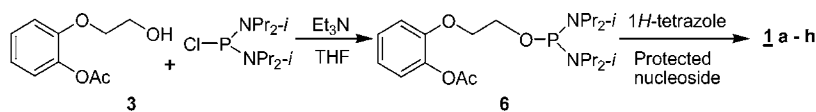


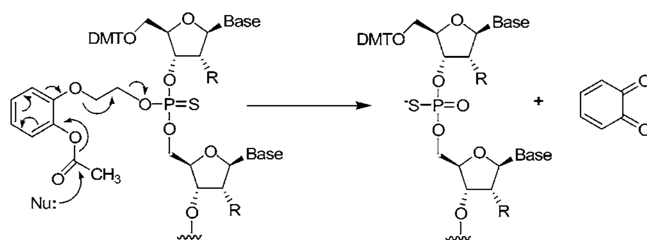
Table 1. Synthesis parameters of cycle used on Pharmacia OligoPilot II DNA/RNA synthesizer

step	reagent	volume (mL)	time (min)
detritylation	10% dichloroacetic acid/ toluene	18	3
coupling	phosphoramidite (0.2M) and 1*H*-tetrazole (0.4 M) in CH_3CN	2.2, 2.2	3
sulfurization	PADS (0.2M) in 3-picoline-CH_3CN (1:1, v/v)	11	3
capping	Ac_2O/pyridine/ CH_3CN, NMI/CH_3CN	7.5, 7.5	2.5

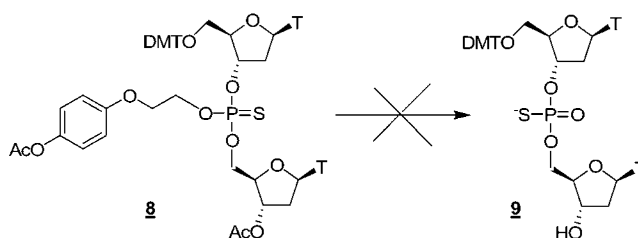
to 35% EtOAc; v/v). The product was obtained as a colorless, viscous oil. Yield 295−302 g (80−82%). Purification: 180 cm (length) × 100 cm (diameter) column was packed with silica gel (ca. 900 g) made into a slurry with 100% hexanes. The crude reaction material was loaded onto the column gently without disturbing the top bed, eluting started with 10% EtOAc/hexanes (6 L), increased gradually to 15% EtOAc/hexanes (6 L), and then to 20% EtOAc/hexanes when the diacetate started to come off. The same 20% EtOAc/hexanes gradient was used until all the diacetate came off. Then the polarity was increased to 35% EtOAc/hexanes to get the product out. The product fractions were collected in 1000 mL sizes, checked by TLC (10% MeOH/methylene chloride), pooled, and concentrated to afford the product as a colorless liquid. A total of ca. 30 L of solvent was used for the column purification. These are not optimized conditions, and the amount of silica gel as well as solvent volume consumption could be reduced further on larger scale.

5′-*O*-DMT-*N*⁴-Benzoyl-2′-deoxycytidine-3′-*O*-(2-acetoxy phenoxyethyl)-*N*,*N*-diisopropyl Phosphoramidite. Bis(diisopropylamino)chlorophosphine (46.96 g; 0.176 mol) was dissolved in anhydrous dichloromethane (700 mL) under nitrogen at room temperature. Triethylamine (22.26 g; 30.67 mL) was added, followed by 5′-*O*-DMT-N^4-benzoyl-2′-deoxycytidine (92.52 g; 0.146 mol) as a solid. After 60 min, the solution was evaporated in a vacuum to remove dichloromethane and excess triethylamine. The residue was dissolved in anhydrous acetonitrile (1200 mL) and stirred at room temperature. 2-Acetoxyphenoxyethanol (31.36 g; 0.16 mol) was dissolved in acetonitrile (300 mL) and added, followed immediately by 1*H*-tetrazole (5.6 g; 0.08 mol). After 2 h, the reaction mixture was evaporated to foam and redissolved in dichloromethane (1000 mL). The solution was washed with cold, saturated sodium bicarbonate (250 mL) and cold, saturated sodium chloride (4 × 200 mL) and was dried over magnesium sulfate for 3 h. After filtration and evaporation, the residue was purified by silica gel chromatography. Purification: 2.75 kg silica gel; column dimensions: 15 cm diameter × 95 cm height; bed height 36 cm. The crude product was mixed with 120 g of silica gel and 200 mL of dichloromethane for loading onto column;

Scheme 4. Mechanism of APOE group deprotection



Scheme 5. Attempted deprotection of 2-(4-acetoxyphenoxy)ethyl-protected TpsT dimer



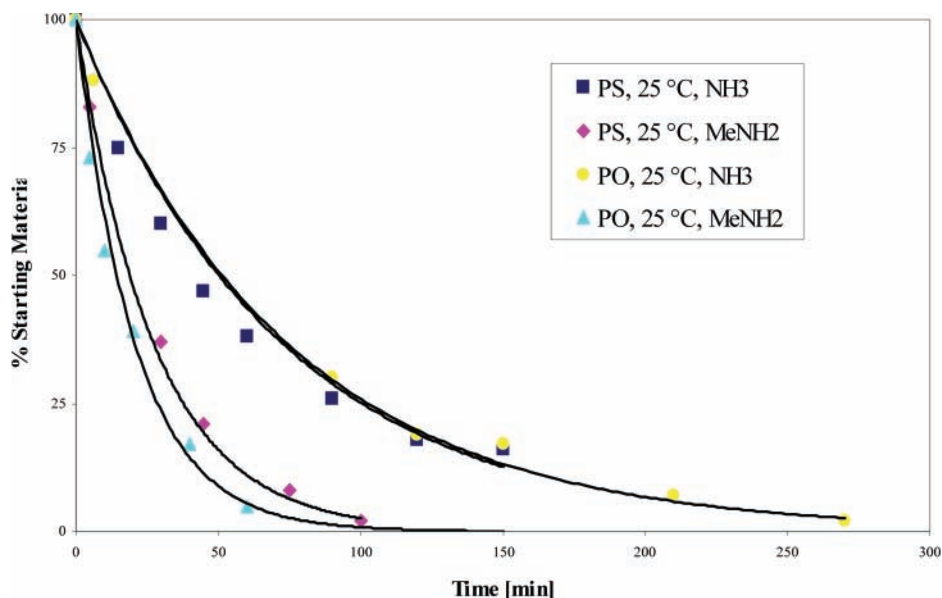
eluant: hexane/ethyl acetate (1:1) (packed with 0.5% triethylamine); run column with 0.25% triethylamine; void volume: 7 L; upper impurities 4 L, no product; collected 4 L product. Solutions were evaporated and azeotroped with dry acetonitrile to give 86 g (70%) of the desired product.

Oligonucleotide Synthesis. Syntheses were performed on a Amersham Pharmacia Biotech OligoPilot II DNA/RNA synthesizer. The support was tightly packed in a stainless steel column (volume 6.33 mL). Details of the synthesis cycle are given in Table 1. Dichloroacetic acid (10%) in toluene was used for deblocking of the dimethoxytrityl (DMT) groups from 5′-hydroxyl group of the nucleotide.[9]

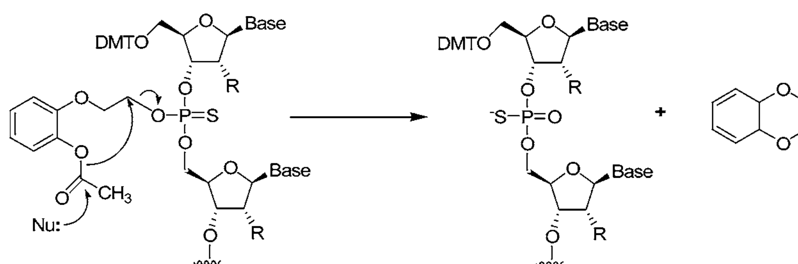
1*H*-Tetrazole (0.4 M) in acetonitrile was used as activator during the coupling step. Low-water acetonitrile (water content <10 ppm) was used for preparing phosphoramidite and activator solutions. 2.5 equiv of amidites (both deoxy- and 2′-*O*-methoxyethylribonucleosides) and a ratio of 40:60 (v/v) of amidite to activator solution was used during the coupling step. Phenylacetyl disulfide (PADS) (0.2 M in 3-picoline/CH_3CN, 1:1, v/v) was used as the sulfur transfer reagent.[10] Capping reagents were made to the recommended GE Amersham Bioscience receipe: Cap A: *N*-methylimidazole-CH_3CN (1:4 v/v), Cap B: acetic anhydride/pyridine/CH_3CN (2:3:5, v/v/v). Subsequently, the solid support

(9) (a) Krotz, A. H.; Cole, D. L.; Ravikumar, V. T. *Bioorg. Med. Chem.* **1999**, *7*, 435−439. (b) Krotz, A. H.; Cart, R. L.; Moore, M. N.; Scozzari, A. N.; Cole, D. L.; Ravikumar, V. T. *Green Chem.* **1999**, 277−281. (c) Krotz, A. H.; Carty, R. L.; Scozzari, A. N.; Cole, D. L.; Ravikumar, V. T. *Org. Process Res. Dev.* **2000**, *4*, 190−193. (d) Gaus, H.; Olsen, P.; Van Sooy, K.; Rentel, C.; Turney, B.; Walker, K. L.; McArdle, J. V.; Capaldi, D. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4118−4124. (e) Capaldi, D. C.; Gaus, H. J.; Carty, R. L.; Moore, M. N.; Turney, B. J.; Decottignies, S. D.; MaArdle, J. V.; Scozzari, A. N.; Ravikumar, V. T.; Krotz, A. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4683−4690. (f) Krishna Kumar, R.; Ravikumar, V. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3426−3429. (g) Cieslak, J.; Austin, C.; Chmielewski, M. K.; Kauffman, J. S.; Snyder, J.; Del-Grosso, A.; Beaucage, S. L. *J. Org. Chem.* **2005**, *70*, 3303−3306.

Chart 1. Rates of deprotection of APOE-protected dimers under different conditions



Scheme 6. Probable mechanism of deprotection of APOE group



containing oligonucleotide was incubated with concentrated aqueous ammonium hydroxide at 55 °C for 14 h to complete cleavage from the support and deprotection of the base-protecting groups. The crude DMT-on oligonucleotide was concentrated to remove ammonia and taken up in a known volume by addition of water with the final concentration checked and analyzed by CGE. The crude material was purified by reversed-phase C18 silica HPLC chromatography, and all DMT-on fractions were collected, detritylated, and lyophilized to a colorless, amorphous, hygroscopic solid.

Results and Discussion

Investigation on the Deprotection of APOE Protecting Group. To check the stability of this new protecting group in the presence of aqueous ammonium hydroxide, the APOE-protected phosphate and phosphorothioate heterodimers d(TC), d(CT), d(GA), and d(AG) were synthesized in solution, purified, and subsequently treated with aqueous ammonium hydroxide dissolved in ethanol (1:1, v/v) as well as in aqueous methylamine. The deprotection rates were studied at 25 °C and 55 °C by ^{31}P NMR spectroscopy. Chart 1 shows the rates of deprotection under various conditions. At 55 °C, the deprotection of all APOE-protected dimers was complete within 10 min.

Mechanism of Deprotection. Initially, we thought the mechanism involves a nucleophilic attack of the base on the acetyl group to liberate the phenolic hydroxyl group which undergoes subsequent fragmentation to liberate the nucleotide, ethylene, and *o*-quinone (Scheme 4).

To investigate this proposed mechanism, synthesis of an analogue of APOE protecting group, viz. 2-(4-acetoxyphenoxy)ethyl-protected TpsT dimer in solution, was carried out and treated with ammonium hydroxide under identical conditions (55 °C). No measurable deprotection occurred as judged by the absence of change in the chemical shift in ^{31}P NMR spectroscopy (Scheme 5).

Hence, a probable mechanistic pathway could be a direct attack on the α-carbon adjacent to the phosphate oxygen, leading to the formation of unreactive bicyclic ether as a side product (Scheme 6).

Conclusion

In summary, the APOE is a suitable protecting group for internucleotidic phosphate protection. Our initial experiments comparing the APOE with the cyanoethyl group indicated that the two groups had equal coupling efficiency (based on CGE analyses and isolated yields) besides the obvious elimination of cyanoethyl adduct formation (data not shown). The APOE phosphitylating reagent as well as the phosphoramidites **1e−h** were scaled up to multi-hundreds of grams

(10) (a) Cheruvallath, Z. S.; Wheeler, P. D.; Cole, D. L.; Ravikumar, V. T. *Nucleosides Nucleotides* **1999**, *18*, 485−492. (b) Cheruvallath, Z. S.; Carty, R. L.; Moore, M. N.; Capaldi, D. C.; Krotz, A. H.; Wheeler, P. D.; Turney, B. J.; Craig, S. R.; Gaus, H. J.; Scozzari, A. N.; Cole, D. L.; Ravikumar, V. T. *Org. Process Res. Dev.* **2000**, *4*, 199−204. (c) Krotz, A. H.; Gorman, D.; Mataruse, P.; Foster, C.; Godbout, J. D.; Coffin, C. C.; Scozzari, A. N. *Org. Process Res. Dev.* **2004**, *8*, 852−858.

with no major hurdles. Several synthetic applications of this versatile protecting group in oligonucleotide synthesis are currently being developed, including the scale-up of phosphorothioate oligonucleotides for potential therapeutic applications.

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Supporting Information Available

^{1}H NMR and ^{31}P NMR spectra, HPLC analyses, CGE analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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