Synthesis of Oligonucleotides Containing 3′-Alkyl Carboxylic Acids Using Universal, Photolabile Solid Phase Synthesis Supports†

Dong Jin Yoo and Marc M. Greenberg*

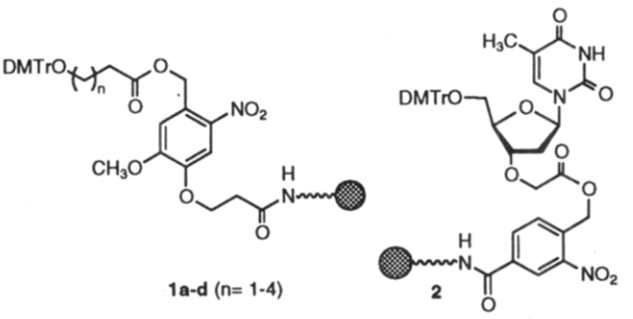
Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received January 31, 1995⊗

The synthesis and application of photolabile supports for solid phase oligonucleotide syntheses that release oligonucleotides containing 3′-alkyl carboxylic acids is described. The carboxylic acid functionality is revealed without removing any other protecting groups throughout the biopolymer, and the protected oligonucleotides are amenable to reverse phase HPLC. The solid phase synthesis supports do not contain a nucleoside, making it possible to use a single support for the synthesis of oligonucleotides independent of their sequence. Individual solid phase synthesis supports differ according to the length of the alkyl tether between the 4,4′-dimethoxytrityl group which serves as the initiation site for oligonucleotide synthesis and the latent carboxylic acid. Eicosameric oligonucleotides are obtained in as high as 92% yield, under photolysis conditions that are known to produce less than 1% thymidine·thymidine photodimers.

Covalent attachment of oligonucleotides to solid supports and/or other molecules in solution is finding an increasingly larger array of applications.[1] A number of methodologies for modifying oligonucleotides at the 5′-terminus exist. Furthermore, cleverly designed phosphoramidites enable one to functionalize specific sites throughout the biopolymer and even the 3′-terminus.[2] Development of the methodology for functionalization of the 3′-terminus of oligonucleotides has generally lagged behind that of chemistries which facilitate conjugation at other sites within these biopolymers. Recently, we and others have reported on a series of modified solid phase synthesis supports that enable one to synthesize oligonucleotides containing functional groups at the 3′-terminus that are amenable to carrying out oligonucleotide conjugation chemistry.[3,4] The supports previously described by us have an additional advantage. Since oligonucleotides synthesized on them are cleaved photolytically, at room temperature and neutral pH, the biopolymers can be released into solution without affecting the phosphodiester and nucleobase protecting groups. It has been suggested that the synthesis of protected oligonucleotides containing a single reactive moiety at either terminus will facilitate improvements in oligonucleotide conjugation chemistry.[5] We report on the first examples of orthogonal, universal oligonucleotide synthesis supports that enable one to efficiently synthesize oligonucleotides containing 3′-alkyl carboxylic acids with or without protecting groups throughout the biopolymer.[6]

The impetus for the design and synthesis of resins **1a−d** was derived from results obtained using **2**. We



found that eicosameric oligonucleotides containing a 3′-glycolate group prepared on **2** were obtained in ≤62% yield via 3 h irradiation (400 nm), followed by ammonium hydroxide deprotection.[3b] Eicosamers were chosen as a calibration point, because a biopolymer of this length is sufficient for single site recognition in chromosomal DNA and is therefore directly relevant to the design of antisense therapeutics. In designing the current solid phase synthesis supports, we sought to address the following issues: (1) increasing the yields of isolated oligonucleotides containing 3′-terminal carboxylic acids compared to those obtained using **2**,[3b] (2) decreasing photolysis times in order to further reduce the photodamage imparted upon the biopolymer,[3a] and (3) increasing the versatility of individual solid phase synthesis supports, so as to enable one to utilize a single support to synthesize oligonucleotides, independent of the nature of the 3′-terminal nucleotide.

† Presented, in part, at the 208th ACS National Meeting, Washington, DC, August 21−25, 1994.

⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1995.

(1) (a) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165. (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925. (c) de la Torre, B. G.; Avino, A.; Tarrason, G.; Piulats, J.; Albericio, F.; Eritja, R. *Tetrahedron Lett.* **1994**, *35*, 2733. (d) Leonetti, J.-P.; Degols, G.; Lebleu, B. *Bioconjugate Chem.* **1990** *1*, 149. (e) Letsinger, R. L.; Zhang, G.; Sun, D. K.; Ikeuchi, T.; Sarin, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6553. (f) Lund, V.; Schmid, R.; Rickwood, D.; Hornes, E. *Nucleic Acids Res.* **1988**, *16*, 10861. (g) Ghosh, S. S.; Musso, G. F. *Nucleic Acids Res.* **1987**, *15*, 5353.

(2) (a) Nelson, P. S.; Kent, M.; Muthini, S. *Nucleic Acids Res.* **1992**, *20*, 6253. (b) Theisen, P.; McCollum, C.; Upadhya, K.; Jacobson, K.; Vu, H.; Andrus, A. *Tetrahedron Lett.* **1992**, *33*, 5033.

(3) (a) Greenberg, M. M.; Gilmore, J. L. *J. Org. Chem.* **1994**, *59*, 746. (b) Greenberg, M. M. *Tetrahedron* **1995**, *51*, 29.

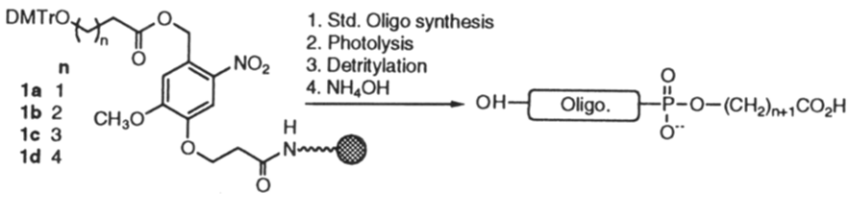
(4) (a) Hovinen, J.; Guzaev, A.; Azhayev, A.; Lönnberg, H. *Tetrahedron* **1994**, *50*, 7203. (b) Gottikh, M.; Asseline, U.; Thuong, N. T. *Tetrahedron Lett.* **1990**, *31*, 6657. (c) Gupta, K. C.; Sharma, P.; Kumar, P.; Sathyanarayana, S. *Nucleic Acids Res.* **1991**, *19*, 3019.

(5) Miller, P. S.; Thaden, J. *Bioconjugate Chem.* **1993**, *4*, 395.

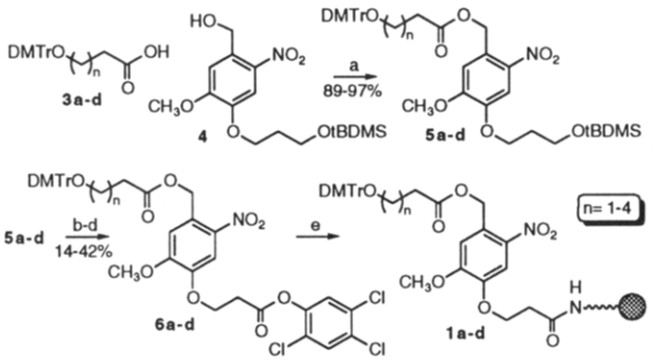
(6) The term orthogonal is meant to imply that the conditions used to cleave the oligonucleotides from the solid phase support do not affect the protecting groups throughout the oligonucleotide. To our knowledge, this term was first applied to oligonucleotide synthesis in: Zon, G.; Geiser, T. G. *Anti-Cancer Drug Des.* **1991**, *6*, 539. The term universal is meant to imply that the sequences of oligonucleotides preparable using the solid phase supports are not limited by the nature of the 3′-terminal nucleotide. This term has been applied previously in oligonucleotide synthesis as well (ref 4c).

0022-3263/95/1960-3358$09.00/0

Scheme 1

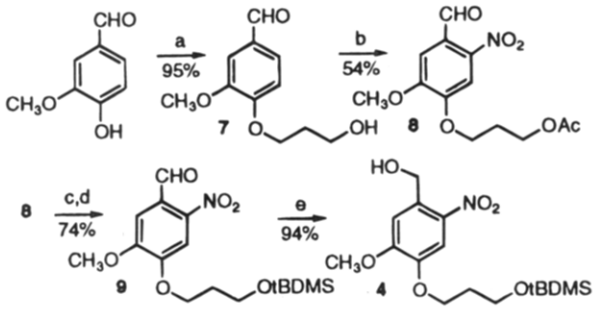


Scheme 2[a]



[a] Key: (a) DCC, CH_2Cl_2; (b) TBAF, HOAc, THP, (c) PDC, DMF; (d) 2,4,5-trichlorophenol, DCC, CH_2Cl_2; (e) LCAA–CPG, DMF.

Scheme 3[a]



[a] Key: (a) 3-bromopropanol, K_2CO_3, CH_3CN; (b) HNO_3, AcOH; (c) K_2CO_3, MeOH; (d) tBDMSCl, imidazole, DMF; (e) $NaBH_4$, EtOH.

Design and Synthesis of Solid Phase Synthesis Supports. The last of the above three points was addressed by preparing synthesis supports composed of *o*-nitrobenzyl esters linked via alkyl chains of varying length (three to six carbons) to a dimethoxytrityl ether. One consequence of this design is that a phosphodiester moiety is introduced between the 3′-terminal nucleotide and the alkyl carboxylic acid (Scheme 1). We anticipated that the efficiency and proficiency of photocleavage could be improved by introducing the more efficient veratrole moiety into the photolabile *o*-nitrobenzyl component of the solid phase synthesis support.[7]

Syntheses of **1a–d** were carried out by coupling the respective carboxylic acids (**3**) to benzyl alcohol **4** (Scheme 2). The benzyl alcohol (**4**) was prepared from vanillin (Scheme 3). In order to moderate the reactivity of the nitric acid, we found it necessary to carry out the nitration in glacial acetic acid. Under these conditions, acetylation of the primary alcohol occurred. Acetylation required that further protecting group manipulation be carried out in order to avoid cleaving the *o*-nitrobenzyl ester in **5** (Scheme 2). This was accomplished by saponifying the acetate and reprotecting the alcohol as the silyl ether prior to coupling **3** to the benzyl alcohol (**4**). Activated esters (**6**) used to load controlled pore glass (CPG) support were obtained following desilylation of **5** via conventional methods. The CPG loading was carried out in the final step, in order to minimize the introduction of impurities on the solid support. Unreacted amines were capped with acetic anhydride and DMAP in pyridine. Typical loadings were determined by 4,4′-dimethoxytrityl cation analysis and were found to range between 28 and 36 μmol/g.[8]

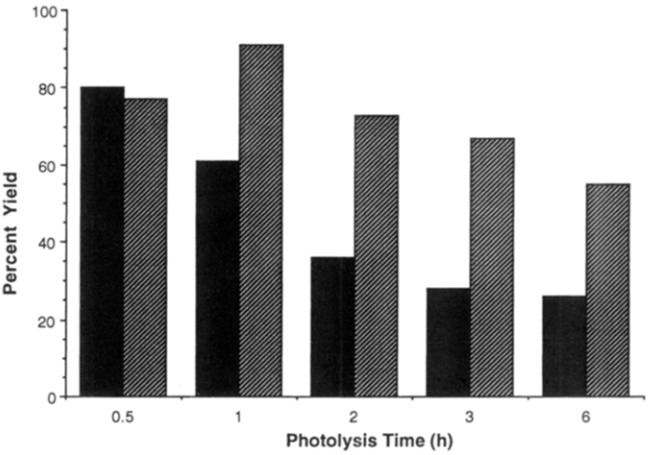
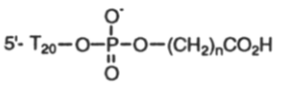


Figure 1. Isolated yields of **10b** (striped bar) and **11b** (solid bar) as a function of irradiation time. Photocleavage was carried out on detritylated material, prior to NH_4OH deprotection. Photolytically cleaved oligonucleotide was subsequently deprotected with NH_4OH and isolated by denaturing gel electrophoresis. Isolated yields are expressed relative to the yield of oligonucleotide obtained without irradiation, but with identical NH_4OH cleavage.

Photolytic Release of Oligonucleotides from Solid Phase Synthesis Supports. Photolysis conditions were optimized using detritylated but otherwise protected eicosameric oligonucleotides (**10** and **11**) that were prepared on **1b** (Figure 1) using standard automated oligonucleotide synthesis protocols. Photolyses were carried out on deaerated CH_3CN/H_2O (9:1) solutions, using the band-pass-filtered (λ_{max} = 400 nm) output of a high-



10a-d (n=2-5)

11a-d (n=2-5)

(7) (a) Pallai, V. N. R. In *Organic Photochemistry;* Padwa, A., Ed.; Marcel Dekker: New York, 1987; Vol. 9. (b) Amit, B.; Zehavi, U.; Patchornik, A. *J. Org. Chem.* **1974**, *39*, 192. (c) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6335.

(8) Atkinson, T.; Smith, M. In *Oligonucleotide Synthesis a Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984.

Table 1. Isolated Yields of Completely Deprotected Oligonucleotides[a]

	10		11	
support (*n*)	5′-ODMTr	5′-OH	5′-ODMTr	5′-OH
1a (1)	70	70	76	77
1b (2)	83	91	72	80
1c (3)	85	92	70	71
1d (4)	71	69	87	70

[a] Yields are isolated yields expressed relative to the amount of oligonucleotide obtained without irradiation, but with the identical NH_4OH treatment.

pressure Hg/Xe lamp (800 W). Yields are expressed in terms of isolated oligonucleotide obtained via photolysis followed by ammonolysis, relative to oligonucleotide obtained from the same synthesis which was not irradiated but was subjected to identical ammonium hydroxide cleavage. Yields of both oligonucleotides were found to decrease upon prolonged irradiation. However, the yield of heteropolymer **11b** decayed more rapidly as a function of photolysis time than did that of the poly-T (**10b**).

While the source of the decrease in yields of oligonucleotides is uncertain at this time, direct photodegradation of the biopolymers is not believed to be the cause of the decrease in yield as a function of the length of irradiation. If this were the case, we would expect the yield of the poly-T oligonucleotide to be more susceptible to the length of irradiation. Furthermore, examination of a 5′-^{32}P-labeled oligonucleotide containing a nonnative nucleoside, in addition to all four native deoxyribonucleotides, which was subjected to similar irradiation under anoxic conditions, followed by Pd(0) deprotection, shows no evidence of strand scission following piperidine treatment.[9,10] It is possible that degradation of the protected oligonucleotide is caused by reaction(s) between the biopolymer and the nitroso aldehyde moiety which is produced upon photocleavage (and remains bound to the resin). Purine-containing oligonucleotides are expected to be more susceptible to such reactions, because of the presence of nucleophilic nitrogens in the purine rings. Diminution of the yields of free amines obtained from *o*-nitrobenzyl precursors has been observed. Unfortunately, the reagents (e.g. H_2SO_4 and hydrazine) typically employed to prevent such side reactions are incompatible with nucleic acids.[7b] Studies directed toward eliminating these degradative processes are warranted. These shortcomings aside, it is important to note that, using the identical photolysis conditions, the irradiation times for the efficient photocleavage of oligonucleotides from **1** are 3 (poly-T) to 6 (heteropolymers) times shorter than those employed for supports previously reported by us.[3] Optimal photolysis times for poly-T (**10b**) and **11b** were determined to be 60 and 30 min, respectively. Extrapolation of ^{3}H-labeling results from these prior experiments indicates that the cleavage of oligonucleotides from the present solid phase synthesis supports will result in the formation of significantly less than 1% thymine-thymine photodimers.[3a]

The irradiation conditions for the respective polymers having been optimized, the isolated yields of identical sequences prepared on **1a–d** were determined for polymers with and without trityl groups (Table 1). Although these data show that there is some variation in yield with respect to alkyl chain length, there is no apparent trend. Importantly, each of these solid phase synthesis supports provides better yields of oligonucleotides containing 3′-terminal carboxylic acids than **2**.

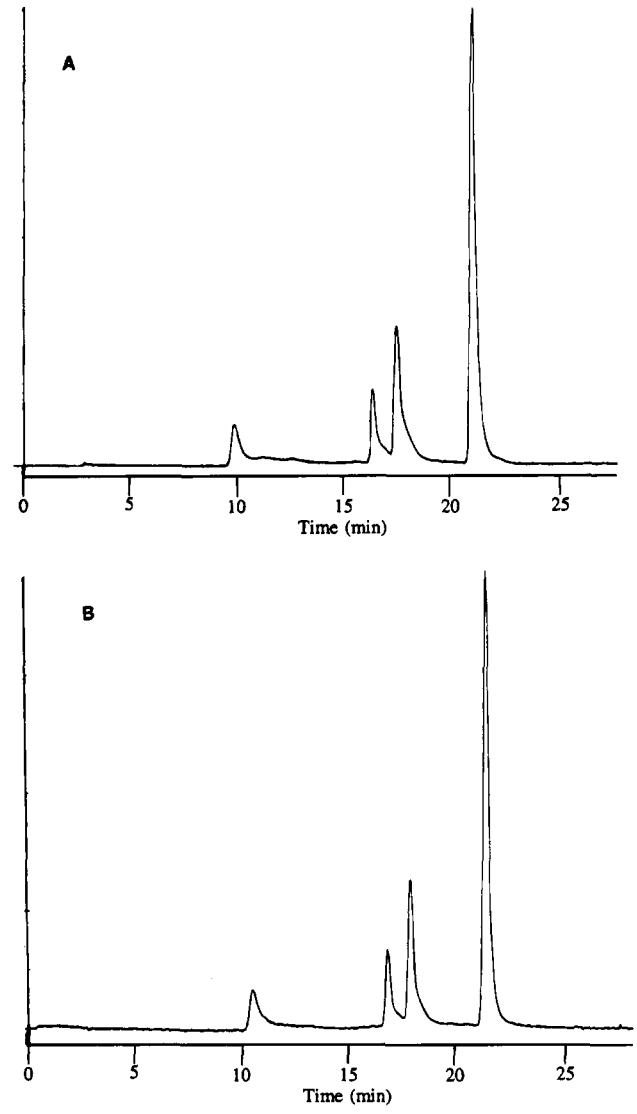


Figure 2. Reverse phase HPLC analysis of nucleosides obtained upon enzymatic digestion of **11b** (A) obtained via direct subjection of resin with NH_4OH and (B) obtained via photolysis, followed by ammonolysis.

Characterization of the Oligonucleotides. As described above, extrapolation of previous tritium experiments enables us to discount thymidine-thymidine dimer formation as a concern in these experiments.[3a] Alternate forms of damage in **11b** were investigated by enzymatic digestion. At the outset of these experiments, we were uncertain as to whether gel-purified oligonucleotides synthesized on **1b** would be digested by snake venom phosphodiesterase. Fortunately, **11b** was completely digested to nucleosides by snake venom phosphodiesterase in the presence of calf intestine alkaline phosphatase. No extraneous nucleosides were observed by HPLC analysis of the enzymatic digestion of the heteropolymer which was photolytically cleaved from the solid phase support prior to base and phosphodiester deprotection or of those which were removed from the resin during ammonolysis (Figure 2). Furthermore, we were unable to detect any difference in relative peak areas between the differently treated samples. These

(9) (a) Matray, T. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1994**, *116*, 6931. (b) Barvian, M. R.; Greenberg, M. M. Unpublished results.
(10) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691.

results could indicate that any nucleobase damage produced during photolysis results in the formation of shorter oligonucleotides due to strand scission during ammonolysis. This damage would not be detected by the above assay, because the shorter fragments are removed during gel electrophoretic purification of the oligonucleotide. We find this explanation to be unlikely because, as was mentioned above, gel electrophoresis results from radioactively labeled oligonucleotides that were subjected to longer periods of irradiation than those currently under discussion, followed by Pd(0) deprotection, are not susceptible to piperidine.[9]

Evidence in support of the intact nature of the phosphodiester and alkyl carboxylic acid was obtained using anion exchange HPLC (Figure 3). The photolytically cleaved/ammonium hydroxide-deprotected product (**10d**) eluted almost 4 min later than a T_{20} oligonucleotide containing 5′- and 3′-hydroxy termini. Proof that the oligonucleotide obtained from the photolytic cleavage was not a 3′-phosphorylated T_{20} was obtained by independent synthesis of this oligonucleotide. The 3′-phosphorylated eicosamer eluted approximately 0.8 min later than **10d** under identical chromatographic conditions (Figure 3C).

Finally, we previously chromatographed photolytically cleaved oligonucleotides that retain their phosphodiester and nucleobase protecting groups on normal phase HPLC columns.[3b] The results were less than satisfying, as the oligonucleotides eluted as very broad peaks. However, the protected oligonucleotides prepared herein are readily chromatographable on reverse phase HPLC columns (Figure 4).[11] Proof that the peak observed corresponds to nucleobase- and phosphate-protected **10b** was obtained by collecting the peak, treating the concentrated residue with concentrated NH_4OH, and analyzing the material by denaturing polyacrylamide gel electrophoresis. The oligonucleotide comigrated on the gel with **10b** which had not been subjected to reverse phase HPLC prior to ammonolysis.

These results demonstrate that the photolabile solid phase synthesis supports described above can be used to synthesize any oligonucleotide sequence from a single synthesis support under conditions that will inflict miniscule amounts of photodamage on the biopolymers.[3a] Such versatility, along with the option to cleave protected biopolymers containing a single reactive moiety at their 3′-termini, suggests that orthogonal, photolabile synthesis supports should be useful tools for preparing oligonucleotide bioconjugates. Further improvements in the photochemical process and development of conjugation chemistry using these supports are underway.

Experimental Section

General Methods. All reactions were carried out in oven-dried glassware, under a nitrogen atmosphere, unless specified otherwise. DMF was distilled from CaH_2. THF was distilled from Na/benzophenone ketyl. Acetonitrile was purified according to literature procedures.[12] Long chain alkyl amine CPG support was purchased from Sigma. Solid phase support loading, as well as oligonucleotide synthesis and purification, was carried out as described previously.[3a] Snake venom phosphodiesterase was obtained from Boehringer Mannheim and calf alkaline phosphatase from New England Biolabs.

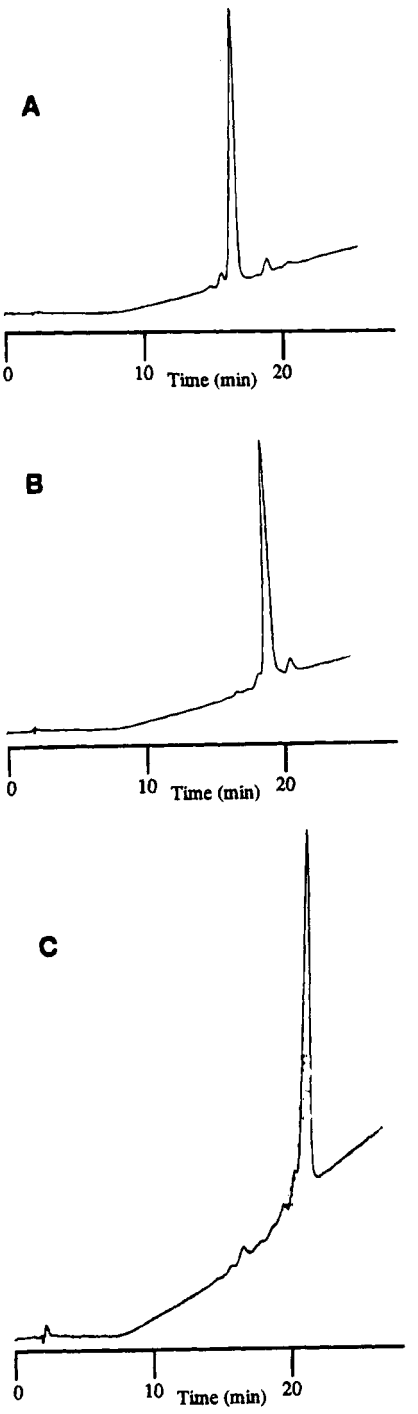


Figure 3. Anion exchange HPLC analysis of **10d**: (A) control T_{20}, (B) **10d**, and (C) control 5′-T_{20}-OPO_3H.

HPLC columns that were used were column A, Rainin Microsorb MV C_{18} (4.6 × 250 mm); and column B, Vydac weak anion exchange oligonucleotide column (4.6 × 250 mm). Photolyses were carried out using the band-pass-filtered output of an Oriel 1000 W high-pressure Hg/Xe lamp. The band-pass filter was from Oriel (λ_{max} = 400 nm, no. 59820). Quantities of oligonucleotides are expressed in OD units. One OD equals that amount of oligonucleotide which when dissolved in 1 mL of H_2O has an absorbance of 1.0 at 260 nm.

Preparation of 7. 3-Bromo-1-propanol (5.49 g, 39.43 mmol) was refluxed in CH_3CN (60 mL) with vanillin (5.00 g, 32.86 mmol), K_2CO_3 (11 g, 78.86 mmol), and KI (100 mg) for 9 h. The mixture was filtered after cooling, and the filter cake was washed with EtOAc. Following removal of the solvent in vacuo, the residue was crystallized from EtOAc and hexanes

(11) Alul, R. H.; Singman, C. N.; Zhang, G.; Letsinger, R. L. *Nucleic Acids Res.* **1991**, *19*, 1527.
(12) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergammon Press: New York, 1988.

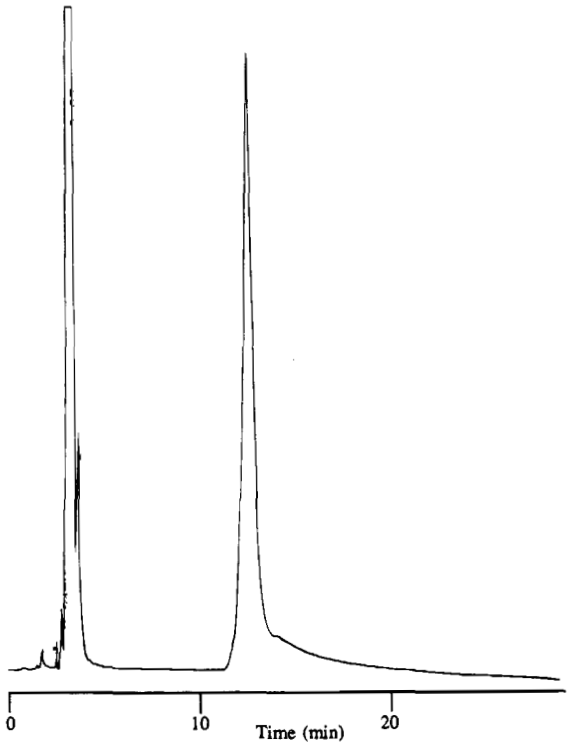


Figure 4. Reverse phase HPLC analysis of detritylated, β-cyanoethyl-protected **10b**.

(19:1) to yield 6.57 g (95%) of **7**. Mp: 81–82.5 °C. ^{1}H NMR ($CDCl_3$): δ 9.76 (s, 1H), 7.36 (dd, 1H, J = 2, 9 Hz), 7.32 (d, 1H, J = 2 Hz), 6.91 (d, 1H, J = 9 Hz), 4.20 (t, 2H, J = 6 Hz), 3.83 (s, 3H), 3.81 (t, 2H, J = 6 Hz), 2.71 (s, 1H), 2.06 (tt, 2H, J = 6, 6 Hz). ^{13}C NMR ($CDCl_3$): δ 190.9, 153.6, 149.6, 130.0, 126.6, 111.3, 109.0, 67.3, 60.3, 55.8, 31.5. IR (KBr): 3270, 3082, 3007, 2943, 2883, 2853, 1697, 1680, 1584, 1509, 1471, 1426, 1410, 1277, 1159, 1131, 1050, 1033 cm^{-1}.

Preparation of 8. Fuming nitric acid (6 mL) was added dropwise to **7** (6.0 g, 28.54 mmol) in glacial acetic acid (60 mL) at 0 °C. The mixture was allowed to warm to room temperature and stir overnight. The mixture was poured over crushed ice and extracted with EtOAc (3 × 200 mL). The combined organics were washed with H_2O (100 mL), saturated $NaHCO_3$ (3 × 150 mL), and H_2O (100 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 1:3) yielded 4.37 g (54%) of **8** as a yellow solid. Mp: 70–71.5 °C. ^{1}H NMR ($CDCl_3$): δ 10.42 (s, 1H), 7.59 (s, 1H), 7.38 (s, 1H), 4.25 (m, 4H), 3.98 (s, 3H), 2.21 (tt, 2H, J = 6, 6 Hz), 2.05 (s, 3H). IR (film): 3091, 2968, 1738, 1690, 1573, 1514, 1468, 1403, 1335, 1285, 1166, 1061 cm^{-1}.

Preparation of 9. K_2CO_3 (3.1 g, 22.31 mmol) and **3** (3.16 g, 11.16 mmol) were stirred in MeOH (100 mL) at room temperature for 3 h. H_2O (150 mL) was added, and the MeOH was removed in vacuo. The aqueous residue was extracted with CH_2Cl_2 (3 × 100 mL). The combined organics were washed with brine (75 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 1:1) yielded 2.66 g (93%) of deacetylated alcohol. Mp: 136–141 °C. ^{1}H NMR ($CDCl_3$): δ 10.42 (s, 1H), 7.62 (s, 1H), 7.38 (s, 1H), 4.31 (t, 2H, J = 6 Hz), 3.98 (s, 3H), 3.88 (t, 2H, J = 6 Hz), 2.14 (m, 2H), 1.93 (bds, 1H). IR (film): 3242, 2952, 1682, 1571, 1523, 1224, 1052 cm^{-1}.

tert-Butyldimethylsilyl chloride (2.0, 13.23 mmol), imidazole (1.90 g, 27.57 mmol), and the alcohol from above (2.66 g, 10.42 mmol) were stirred in DMF (25 mL) at room temperature for 6 h. The mixture was poured into H_2O (75 mL) and extracted with diethyl ether (3 × 100 mL). The organic layers were washed with brine (50 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 1:4) yielded 3.03 g (79%) of **9**. Mp: 57–59 °C. ^{1}H NMR ($CDCl_3$): δ 10.40 (s, 1H), 7.60 (s, 1H), 7.37 (s, 1H), 4.24 (t, 2H, J = 6 Hz), 3.97 (s, 3H), 3.79 (t, 2H, J = 6 Hz), 2.06 (m, 2H), 0.84 (s, 9H), 0.01 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 187.7, 153.4, 152.0, 143.9, 125.2, 109.8, 108.0, 66.5, 58.9, 56.6, 31.8, 25.8, 18.3, 5.5. IR (film): 2955, 2930, 2884, 2857, 1685, 1601, 1570, 1516, 1472, 1406, 1327, 1299, 1256, 1226, 1102, 1062 cm^{-1}. Anal. Calcd for $C_{17}H_{27}NO_6Si$: C, 55.26; H, 7.37; N, 3.79. Found: C, 55.43; H, 7.52; N, 3.78.

Preparation of 4. Sodium borohydride (476 mg, 12.59 mmol) was added to **9** (3.0 g, 8.12 mmol) in EtOH (40 mL) at 0 °C. After 4 h, H_2O (10 mL) was added, and the solvents were removed in vacuo. The residue was resuspended in H_2O (30 mL) and extracted with Et_2O (3 × 50 mL). The combined organics were washed with brine (50 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 1:3) yielded 2.84 g (94%) of **4**. Mp: 69–70 °C. ^{1}H NMR ($CDCl_3$): δ 7.66 (s, 1H), 7.13 (s, 1H), 4.90 (s, 2H), 4.14 (t, 2H, J = 6 Hz), 3.92 (s, 3H), 3.78 (t, 2H, J = 6 Hz), 2.92 (bds, 1H), 2.02 (tt, 2H, J = 6, 6 Hz), 0.83 (s, 9H), −0.01 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 154.2, 147.3, 139.5, 132.2, 110.8, 109.3, 66.1, 62.6, 59.2, 56.3, 31.9, 25.8, 18.2, −5.5. IR (film): 3416, 2954, 2930, 2884, 2857, 1578, 1520, 1472, 1326, 1274, 1215, 1069 cm^{-1}. Anal. Calcd for $C_{17}H_{29}NO_6Si$: C, 54.96; H, 7.87; N, 3.77. Found: C, 54.80; H, 8.06; N, 3.76.

Preparation of 5a. Reaction of **4** (605 mg, 1.63 mmol), carboxylic acid **3a** (959 mg, 2.44 mmol), DCC (504 mg, 2.28 mmol), and DMAP (20 mg, 0.17 mmol) as described for the preparation of **5b** yielded 1.17 g (96%) of **5a**. ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H), 7.41 (d, 2H, J = 6 Hz), 7.17–7.32 (m, 7H), 6.98 (s, 1H), 6.79 (d, 4H, J = 6 Hz), 5.54 (s, 2H), 4.18 (t, 2H, J = 6 Hz), 3.82 (m, 5H), 3.74 (s, 6H), 3.44 (t, 2H, J = 6 Hz), 2.69 (t, 2H, J = 6 Hz), 2.04 (m, 2H), 0.88 (s, 9H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 171.0, 158.3, 153.7, 147.6, 144.7, 139.7, 135.8, 129.8, 127.9, 127.6, 126.6, 126.5, 112.9, 110.4, 109.2, 86.0, 77.2, 66.0, 63.1, 59.0, 56.0, 54.9, 35.3, 31.9, 25.7, 18.1, −5.6. IR (film): 2953, 2932, 2856, 1744, 1608, 1581, 1523, 1510, 1464, 1446, 1386, 1330, 1279, 1252, 1219, 1177, 1068, 1035, 1004 cm^{-1}.

Preparation of 5b. Alcohol **4** (798 mg, 2.15 mmol) was added to a solution of **3b** (1.18 g, 2.90 mmol), DCC (552 mg, 2.68 mmol), and DMAP (27 mg, 0.22 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was allowed to stir and warm to room temperature overnight. The mixture was filtered and the DCU washed with cold CH_2Cl_2 (5 mL). Flash chromatography (EtOAc:hexanes, 1:4) yielded 1.45 g (89%) of **5b** as a foam. ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 1H), 7.39 (dd, 2H, J = 2, 6 Hz), 7.14–7.29 (m, 7H), 6.94 (s, 1H), 6.78 (d, 4H, J = 6 Hz), 5.46 (s, 2H), 4.18 (t, 2H, J = 6 Hz), 3.87 (s, 3H), 3.81 (t, 2H, J = 6 Hz), 3.76 (s, 6H), 3.12 (t, 2H, J = 6 Hz), 2.55 (t, 2H, J = 7.5 Hz), 1.93–2.09 (m, 4H), 0.88 (s, 9H), 0.04 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 172.8, 158.4, 153.7, 147.7, 145.1, 139.9, 136.3, 129.9, 128.1, 127.7, 126.8, 126.6, 112.9, 110.5, 109.4, 85.9, 66.1, 63.1, 62.2, 59.2, 56.2, 55.1, 32.0, 31.4, 25.9, 25.5, 18.3, −5.5. IR (film): 2954, 2932, 2856, 1741, 1654, 1608, 1580, 1522, 1509, 1464, 1445, 1386, 1330, 1279, 1251, 1219, 1175, 1068, 1035, 1006 cm^{-1}.

Preparation of 5c. Reaction of **4** (620 mg, 1.67 mmol), carboxylic acid **3c** (1.05 g, 2.50 mmol), DCC (516 mg, 2.5 mmol), and DMAP (20 mg, 0.17 mmol) as described for the preparation of **5b** yielded 1.23 g (97%) of **5c**. ^{1}H NMR ($CDCl_3$): δ 7.73 (s, 1H), 7.40 (d, 2H, J = 6 Hz), 7.15–7.33 (m, 7H), 6.95 (s, 1H), 6.80 (d, 4H, J = 6 Hz), 5.49 (s, 2H), 4.17 (t, 2H, J = 7 Hz), 3.87 (s, 3H), 3.81 (t, 2H, J = 6 Hz), 3.76 (s, 6H), 3.07 (t, 2H, J = 6 Hz), 2.41 (t, 2H, J = 7.5 Hz), 2.04 (tt, 2H, J = 6 Hz), 1.77 (m, 2H), 1.67 (m, 2H), 0.88 (s, 9H), 0.04 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 172.8, 158.3, 153.7, 147.7, 145.2, 139.9, 136.4, 129.9, 128.1, 127.6, 126.8, 126.5, 112.9, 110.4, 109.4, 85.7, 66.1, 63.1, 62.7, 59.1, 56.2, 55.1, 34.0, 31.9, 29.5, 25.8, 22.0, 18.2, −5.5. IR (film): 3059, 3000, 2954, 2933, 2857, 1742, 1608, 1581, 1521, 1510, 1464, 1446, 1385, 1330, 1279, 1251, 1218, 1175, 1156, 1068, 1035 cm^{-1}.

Preparation of 5d. Reaction of **4** (630 mg, 1.70 mmol), carboxylic acid **3d** (1.11 g, 2.54 mmol), DCC (525 mg, 2.54 mmol), and DMAP (20 mg, 0.17 mmol) as described for the preparation of **5b** yielded 1.18 g (90%) of **5d**. ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H), 7.42 (d, 2H, J = 6 Hz), 7.18–7.32 (m, 7H), 6.95 (s, 1H), 6.81 (d, 4H, J = 7 Hz), 5.49 (s, 2H), 4.17 (t, 2H, J = 6 Hz), 3.90 (s, 3H), 3.81 (t, 2H, J = 6 Hz), 3.76 (s, 6H), 3.04 (t, 2H, J = 6 Hz), 2.40 (t, 2H, J = 7.5 Hz), 2.05 (t,

2H, J = 6 Hz), 1.63 (m, 4H), 1.43 (m, 2H), 0.87 (s, 9H), 0.04 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 172.9, 158.3, 153.7, 147.7, 145.3, 139.9, 136.5, 129.9, 128.1, 127.6, 126.8, 126.5, 112.9, 110.5, 109.4, 85.6, 66.1, 63.1, 59.1, 56.2, 55.1, 34.2, 31.9, 31.5, 29.7, 25.9, 24.8, 22.6, 18.2, 14.1. IR (film): 2857, 1743, 1608, 1581, 1524, 1511, 1463, 1445, 1330, 1279, 1251, 1218, 1176, 1155, 1068, 1035 cm^{-1}.

Preparation of 6a. Desilylation of **5a** (1.20 g, 1.61 mmol) as described for **5b** yielded 746 mg (74%) of the requisite primary alcohol. Mp: 46–48 °C. ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 1H), 7.37 (d, 2H, J = 7 Hz), 7.16–7.27 (m, 7H), 6.96 (s, 1H), 6.76 (dd, 4H, J = 3, 9 Hz), 5.53 (s, 2H), 4.23 (t, 2H, J = 6 Hz), 3.86 (m, 2H), 3.77 (s, 3H), 3.75 (s, 6H), 3.41 (t, 2H, J = 6 Hz), 2.66 (t, 2H, J = 6 Hz), 2.13 (bds, 1H), 2.05 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 171.1, 158.4, 153.7, 147.3, 144.8, 139.8, 136.0, 129.9, 128.0, 127.7, 127.2, 126.8, 113.0, 110.5, 109.4, 86.2, 68.0, 63.2, 60.6, 59.1, 56.2, 55.2, 35.5, 31.5. IR (film): 3400, 2937, 2837, 1741, 1608, 1581, 1522, 1510, 1464, 1446, 1386, 1279, 1251, 1177, 1067, 1034 cm^{-1}. Anal. Calcd for $C_{35}H_{37}NO_{10}$: C, 66.55; H, 5.90; N, 2.22. Found: C, 66.32; H, 5.76; N, 2.06.

Oxidation to the Carboxylic Acid. Reaction of PDC (1.32 g, 3.51 mmol) and the primary alcohol (370 mg, 0.59 mmol) as described for the preparation of **6b** yielded 252 mg (68%) of the carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H), 7.37 (d, 2H, J = 6 Hz), 7.13–7.28 (m, 7H), 6.95 (s, 1H), 6.78 (d, 4H, 7 J = Hz), 5.51 (s, 2H), 4.33 (t, 2H, J = 6 Hz), 3.79 (s, 3H), 3.76 (s, 6H), 3.41 (t, 2H, J = 7 Hz), 2.89 (t, 2H, J = 7 Hz), 2.65 (t, 2H, J = 7 Hz).

Final Preparation of 6a. Reaction of 2,4,5-trichlorophenol (118 mg, 0.60 mmol), DCC (123 mg, 0.60 mmol), DMAP (5 mg, 0.04 mmol), and the carboxylic acid (257 mg, 0.40 mmol) as described for the preparation of **6b** yielded 117 mg (36%) of **6a**. Mp: 55–58 °C. ^{1}H NMR ($CDCl_3$): δ 7.77 (s, 1H), 7.54 (s, 1H), 7.38 (d, 2H, J = 6 Hz), 7.17–7.31 (m, 8H), 6.99 (s, 1H), 6.75 (d, 4H, J = 6 Hz), 5.53 (s, 2H), 4.45 (t, 3H, J = 6 Hz), 3.81 (s, 3H), 3.77 (s, 6H), 3.42 (t, 2H, J = 6 Hz), 3.16 (t, 2H, J = 6 Hz), 2.67 (t, 2H, J = 6 Hz). ^{13}C NMR ($CDCl_3$): δ 171.1, 167.7, 158.4, 153.9, 146.9, 145.6, 144.8, 139.7, 131.5, 131.1, 130.0, 128.0, 127.9, 127.8, 126.8, 125.3, 113.0, 110.8, 110.2, 86.2, 64.6, 63.2, 59.1, 56.2, 55.2, 35.5, 34.0, 33.9, 25.6, 24.9. IR (film): 2934, 1775, 1742, 1608, 1581, 1522, 1509, 1463, 1330, 1280, 1250, 1220, 1176, 1120, 1066, 1034 cm^{-1}. Anal. Calcd for $C_{41}H_{36}NO_{11}Cl_3$: C, 59.68; H, 4.40; N, 1.70. Found: C, 59.72; H, 4.41; N, 1.82.

Preparation of 6b. Silyl ether **5b** (1.45 g, 1.91 mmol) was added to a THF (15 mL) solution containing 4 equiv of TBAF and acetic acid at 0 °C. The mixture was allowed to stir and warm to room temperature over 24 h. The reaction was quenched with NH_4HCO_3 (10 mL), and the mixture was diluted with H_2O (30 mL) and extracted with $CHCl_3$ (2 × 50 mL). Flash chromatography (EtOAc:hexanes, 1:2) yielded 1.13 g (92%) of the primary alcohol. ^{1}H NMR ($CDCl_3$): δ 7.71 (s, 1H), 7.39 (dd, 2H, J = 2, 6 Hz), 7.26 (m, 6H), 7.19 (m, 1H), 6.94 (s, 1H), 6.79 (d, 4H, J = 6 Hz), 5.45 (s, 2H), 4.22 (t, 2H, J = 6 Hz), 3.80–3.86 (m, 5H), 3.75 (s, 6H), 3.12 (t, 2H, J = 6 Hz), 2.54 (t, 2H, J = 7.5 Hz), 2.40 (bds, 1H), 2.07 (m, 2H), 1.97 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.8, 158.3, 153.6, 147.3, 145.0, 139.7, 136.2, 129.9, 128.0, 127.6, 127.2, 126.6, 112.9, 110.4, 109.3, 85.8, 67.7, 63.0, 62.1, 60.3, 56.2, 55.1, 31.5, 31.3, 25.4. IR (film): 3549, 3418, 3058, 2935, 2877, 2837, 1739, 1608, 1580, 1522, 1510, 1464, 1445, 1382, 1330, 1278, 1250, 1218, 1175, 1067, 1034 cm^{-1}. Anal. Calcd for $C_{36}H_{39}NO_{10}$: C, 66.96; H, 6.09; N, 2.17. Found: C, 67.15; H, 6.26; N, 2.18.

Oxidation to the Carboxylic Acid. PDC (1.24 g, 3.30 mmol) and the primary alcohol (355 mg, 0.55 mmol) were stirred in DMF (3 mL) at room temperature for 48 h. The reaction was quenched with H_2O (20 mL), and the mixture was extracted with diethyl ether (3 × 50 mL). The organic layers were washed with brine (50 mL) and dried over Na_2SO_4. Flash chromatography (EtOAc:CH_2Cl_2, 1:1) yielded 191 mg (52%) of the carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.39 (dd, 2H, J = 2, 6 Hz), 7.25 (m, 7H), 6.95 (s, 1H), 6.80 (dd, 4H, J = 3, 6 Hz), 5.46 (s, 2H), 4.34 (t, 2H, J = 6 Hz), 3.86 (s, 3H), 3.76 (s, 6H), 3.14 (t, 2H, J = 6 Hz), 2.91 (t, 2H, J = 6 Hz), 2.56 (t, 2H, J = 7.5 Hz), 1.98 (m, 2H). IR (film): 3640, 3058, 3002, 2936, 2837, 1738, 1710, 1608, 1582, 1510, 1463, 1445, 1413, 1382, 1331, 1280, 1250, 1219, 1176, 1116, 1066, 1034 cm^{-1}.

Final Preparation of 6b. 2,4,5-Trichlorophenol (68 mg, 0.35 mmol) was added to a solution of the carboxylic acid (190 mg, 0.29 mmol), DCC (71 mg, 0.35 mmol), and DMAP (4 mg, 0.03 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The reaction mixture was filtered and the DCU washed with CH_2Cl_2 (5 mL). Flash chromatography (EtOAc:hexanes, 1:3) yielded 163 mg (67%) of **6b**. ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.54 (s, 1H), 7.39 (dd, 2H, J = 1.5, 6 Hz), 7.28 (m, 8H), 6.97 (s, 1H), 6.79 (d, 4H, J = 7 Hz), 5.47 (s, 2H), 4.45 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.76 (s, 6H), 3.14 (m, 4H), 2.55 (t, 2H, J = 7.5 Hz), 1.97 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.8, 167.7, 158.3, 153.8, 146.8, 145.6, 145.1, 139.7, 136.2, 131.5, 131.0, 129.1, 128.1, 127.9, 127.7, 126.6, 126.1, 125.3, 113.1, 113.0, 110.8, 110.1, 85.9, 64.5, 63.0, 62.6, 56.3, 55.1, 33.9, 31.4, 25.5. IR (film): 2936, 2837, 1776, 1740, 1608, 1581, 1522, 1510, 1462, 1280, 1250, 1219, 1174, 1120, 1081, 1066, 1034 cm^{-1}. Anal. Calcd for $C_{42}H_{38}NO_{11}Cl_3$: C, 60.12; H, 4.56; N, 1.67. Found: C, 60.08; H, 4.72; N, 1.80.

Preparation of 6c. Desilylation of **5c** (440 mg, 0.58 mmol) as described for **5b** yielded 283 mg (74%) of the primary alcohol. ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 1H), 7.41 (d, 2H, J = 7.5 Hz), 7.15–7.31 (m, J = 7 Hz), 6.96 (s, 1H), 6.79 (d, 4H, J = 7.5 Hz), 5.48 (s, 2H), 4.22 (t, 2H, J = 6 Hz), 3.87 (m, 5H), 3.76 (s, 6H), 3.06 (t, 2H, J = 6 Hz), 2.40 (t, 2H, J = 7 Hz), 2.09 (m, 2H), 1.76 (m, 2H), 1.65 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.9, 158.3, 153.6, 147.3, 145.2, 139.8, 136.4, 129.9, 128.1, 127.7, 126.6, 112.9, 110.4, 109.4, 85.7, 67.9, 63.0, 62.7, 62.1, 60.5, 56.3, 55.1, 34.0, 31.5, 29.5, 22.0. IR (film): 3400, 2936, 1734, 1684, 1653, 1608, 1579, 1559, 1522, 1508, 1278, 1250, 1218, 1175, 1067, 1034 cm^{-1}.

Oxidation to the Carboxylic Acid. Reaction of PDC (780 mg, 2.12 mmol) and the primary alcohol (233 mg, 0.35 mmol) as described for the preparation of **6b** yielded 179 mg (75%) of the carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 7.73 (s, 1H), 7.43 (d, 2H, J = 7 Hz), 7.15–7.36 (m, 7H), 6.96 (s, 1H), 6.82 (d, 4H, J = 7 Hz), 5.46 (s, 2H), 4.34 (t, 2H, J = 6 Hz), 3.87 (s, 3H), 3.76 (s, 6H), 3.09 (t, 2H, J = 6 Hz), 2.88 (t, 2H, J = 6 Hz), 2.42 (t, 2H, J = 7.5 Hz), 1.81 (m, 2H), 1.68 (m, 2H). IR (film): 3497, 3061, 3003, 2936, 2837, 1739, 1651, 1608, 1582, 1511, 1463, 1446, 1415, 1385, 1332, 1280, 1250, 1218, 1177, 1066, 1034 cm^{-1}.

Final Preparation of 6c. Reaction of 2,4,5-trichlorophenol (78 mg, 0.40 mmol), DCC (82 mg, 0.40 mmol), DMAP (3.2 mg, 0.03 mmol), and the carboxylic acid (178 mg, 0.26 mmol) as described for the preparation of **6b** yielded 92 mg (41%) of **6c**. ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.52 (s, 1H), 7.39 (d, 2H, J = 7 Hz), 7.15–7.31 (m, 8H), 6.97 (s, 1H), 6.79 (d, 4H, J = 7 Hz), 5.49 (s, 2H), 4.43 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.76 (s, 6H), 3.15 (t, 2H, J = 6 Hz), 3.06 (t, 2H, J = 6 Hz), 2.40 (t, 2H, J = 7 Hz), 1.75 (m, 2H), 1.64 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.8, 167.7, 158.3, 153.9, 146.9, 145.6, 145.2, 139.8, 136.5, 131.5, 131.1, 129.9, 128.0, 127.7, 126.6, 126.1, 125.3, 113.0, 110.8, 110.2, 85.8, 64.6, 63.4, 63.0, 62.7, 56.3, 55.2, 34.1, 32.8, 29.5, 22.0, 19.9. IR (film): 3088, 2937, 1774, 1740, 1608, 1581, 1522, 1509, 1459, 1280, 1249, 1219, 1174, 1119, 1081, 1034 cm^{-1}. HRMS FAB (M^+) calcd 851.1667, found 851.1672.

Preparation of 6d. Desilylation of **5d** (570 mg, 0.74 mmol) as described for **5b** yielded 407 mg (82%) of the primary alcohol. ^{1}H NMR ($CDCl_3$): δ 7.71 (s, 1H), 7.41 (d, 2H, J = 7 Hz), 7.14–7.32 (m, 7H), 6.96 (s, 1H), 6.78 (d, 4H, J = 7.5 Hz), 5.47 (s, 2H), 4.22 (t, 2H, J = 6 Hz), 3.89 (s, 3H), 3.85 (t, 2H, J = 6 Hz), 3.76 (s, 6H), 3.04 (t, 2H, J = 6 Hz), 2.42 (t, 2H, J = 6 Hz), 2.35 (bds, 1H), 2.08 (m, 2H), 1.65 (m, 4H), 1.42 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.9, 158.3, 158.2, 153.6, 147.3, 145.2, 136.5, 136.2, 129.9, 128.0, 127.2, 126.5, 112.9, 110.4, 109.4, 85.6, 67.8, 63.0, 60.3, 56.2, 55.1, 34.1, 32.2, 31.5, 29.6, 25.8, 24.8. IR (film): 3524, 2936, 2869, 1739, 1608, 1580, 1522, 1509, 1464, 1445, 1383, 1330, 1279, 1250, 1218, 1176, 1154, 1067, 1034 cm^{-1}.

Oxidation to the Carboxylic Acid. Reaction of PDC (1.13 g, 3.00 mmol) and the primary alcohol (405 mg, 0.60 mmol) as described for the preparation of **6b** yielded 354 mg (86%) of the carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 7.75 (s, 1H), 7.41 (d, 2H, J = 7.5 Hz), 7.13–7.32 (m, 7H), 6.97 (s, 1H), 6.80 (d,

4H, J = 6 Hz), 5.48 (s, 2H), 4.33 (t, 2H, J = 6 Hz), 3.89 (s, 3H), 3.76 (s, 6H), 3.02 (t, 2H, J = 6.5 Hz), 2.89 (t, 2H, J = 6 Hz), 2.39 (t, 2H, J = 6.5 Hz), 1.65 (m, 4H), 1.42 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 175.1, 173.0, 158.5, 158.2, 145.2, 139.4, 136.5, 129.9, 128.1, 127.7, 127.6, 127.0, 126.5, 112.9, 110.3, 110.1, 85.6, 81.4, 77.2, 64.7, 63.0, 55.1, 34.1, 33.9, 29.7, 25.9, 24.8. IR (film): 3300, 2937, 2837, 1738, 1710, 1607, 1582, 1512, 1463, 1445, 1384, 1331, 1280, 1250, 1219, 1177, 1066, 1034 cm^{-1}.

Final Preparation of 6d. Reaction of 2,4,5-trichlorophenol (151 mg, 0.76 mmol), DCC (157 mg, 0.76 mmol), DMAP (6.2 mg, 0.05 mmol), and the carboxylic acid (350 mg, 0.51 mmol) as described for the preparation of **6b** yielded 261 mg (60%) of **6d**. ^1H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.54 (s, 1H), 7.41 (d, 2H, J = 7 Hz), 7.15–7.31 (m, 8H), 6.98 (s, 1H), 6.78 (d, 4H, J = 7.5 Hz), 5.49 (s, 2H), 4.44 (t, 2H, J = 6 Hz), 3.91 (s, 3H), 3.76 (s, 6H), 3.16 (t, 2H, J = 6 Hz), 3.03 (t, 2H, J = 6 Hz), 2.40 (t, 2H, J = 7.5 Hz), 1.67 (m, 4H), 1.43 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.9, 167.7, 158.3, 153.9, 146.9, 145.6, 145.3, 139.8, 136.6, 131.5, 131.1, 130.8, 129.9, 128.1, 128.0, 127.7, 126.6, 126.1, 125.3, 113.0, 110.8, 110.2, 85.7, 64.6, 63.0, 56.3, 55.2, 34.2, 34.0, 29.7, 25.9, 24.9. IR (film): 2936, 1774, 1740, 1608, 1582, 1522, 1509, 1462, 1349, 1329, 1280, 1249, 1219, 1175, 1153, 1120, 1082, 1066, 1034 cm^{-1}. HRMS FAB (M^+) calcd 865.1823, found 865.1797.

General Procedure for Photolytic Cleavage of Oligonucleotides. Oligonucleotide bound to support (~1.5 mg, ~50 nmol) was added to a Pyrex tube containing a magnetic stir bar and 3 mL of ACN/H_2O (9:1 by volume). The tube was fitted with a rubber septum and sparged with N_2 for 20 min. Photolysis was carried out using band-pass-filtered (λ_{max} = 400 nm) output of a high-pressure Hg/Xe lamp (800 W) for the prescribed period under dynamic N_2 with stirring. (Following sparging, the needle is raised well above the surface of the solvent, in order to avoid evaporation during photolysis.) The solution was filtered through a 0.45 μm membrane filter. The tube was washed with CH_3CN (3 × 1 mL), followed by H_2O (3 × 1 mL). Each washing was filtered through the membrane. The washings were combined and concentrated in vacuo, after which they were subjected to NH_4OH deprotection. Deprotected oligonucleotides were purified via 20% nondenaturing polyacrylamide gel electrophoresis.

Reverse Phase HPLC Characterization of Protected Oligonucleotides. Column A was employed using a gradient: eluent A, 0.03 M Et_3NHOAc (pH 7.0), 2% CH_3CN (v:v); eluent B, CH_3CN (v:v); 3–100% B linearly over 20 min. Maintain 100% B for 5 min. Flow rate: 1.0 mL/min.

Anion Exchange HPLC Characterization of Fully Deprotected Oligonucleotides. Column B was employed using a gradient: eluent A, 0.1 M $(NH_4)_2HPO_4$ (pH 6.7), 20% CH_3CN (v:v); eluent B, 0.3 M $(NH_4)_2HPO_4$ (pH 6.7), 20% CH_3CN (v:v); 0–100% B linearly over 20 min. Flow rate: 1.5 mL/min.

Enzymatic Digestion of 5′-dTAC GCA ATG CTA GAT CTA AT-glycolate. Snake venom phosphodiesterase (2 μL, 0.03 unit/μL) and calf alkaline phosphatase (1 μL, 1 unit/μL) were added to **11b** (0.3 OD) in 47 μL of Tris-acetate buffer (0.1 M, pH 8.75) and $MgCl_2$ (0.015 M). The tube was vortexed, spun briefly, and immersed in a 37 °C water bath for 12 h. The sample was precipitated via the addition of 20% NaOAc (5 μL) and EtOH (180 μL) and freezing (−78 °C). The solution was centrifuged at 14 000 rpm for 10 min. The supernatants were carefully removed so as to not perturb the salt pellet at the bottom of the tube. The sample was reprecipitated via the addition of EtOH (350 μL) to the supernatant from the first precipitation. The supernatants were separated from the salt pellet as described above, combined with those from the first precipitation, and removed in vacuo (Savant Speed Vac). The residue was resuspended in H_2O (50 μL). Samples were passed through 0.45 μm filters prior to HPLC analysis (column A). Gradient conditions: A, 0.01 M KH_2PO_4 (pH 6.8), 2.5% MeOH; B, 0.01 M KH_2PO_4 (pH 6.8), 20% MeOH; 0–100% B linearly over 15 min. Maintain 100% B for 20 min. Flow rate: 1.0 mL/min.

Acknowledgment. Partial support of this research by the Colorado Advanced Technology Institute (CATI) is appreciated. D.J.Y. is grateful for partial support in the form of a KOSEF postdoctoral fellowship.

JO950188I