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Novel Solid Phase Synthesis Supports for the Preparation of Oligonucleotides Containing 3'-Alkyl Amines.

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Abstract: The synthesis and application of photolabile supports for solid phase oligonucleotide synthesis that release oligonucleotides containing 3'-alkyl amines is described. The alkyl amine functionality can be revealed without removing any other protecting groups throughout the biopolymer. 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 as the initiation site for oligonucleotide synthesis and the latent alkyl amine. Eicosameric oligonucleotides are obtained in yields ranging from 70-98% under photolysis conditions that are known to produce less than 3% thymidine-thymidine photodimers. Longer oligonucleotides (30 and 40 nucleotides in length) are cleaved from these solid phase synthesis supports in lower yields.

Oligonucleotides are often prepared so as to contain alkyl amines which can then be used to conjugate the biopolymer to other supports and/or molecules that are free in solution.¹ The resulting biopolymers are useful as diagnostic probes and as potential therapeutic devices. Commercially available phosphoramidite reagents allow one to incorporate alkyl amines at the 5' and 3'-termini as well as internally.² Recently, solid phase oligonucleotide synthesis supports which yield fully deprotected oligonucleotides containing 3'-alkyl amines have been reported.³ Carrying out conjugation on the fully deprotected oligonucleotides obtained via these methods suffers from the possibility of competing reactions between the alkyl amine and other nucleophiles present in the biopolymer.⁴⁻⁶ An alternative strategy involves conjugating the alkyl amine prior to incorporation in the biopolymer, and subsequently introducing the conjugated alkyl amine in the form of a phosphoramidite.⁷ However, this linear strategy requires utilizing a large excess of the conjugating moiety in a manner that makes it difficult to recover the unincorporated material.⁸ We report herein a family of solid phase oligonucleotide



synthesis supports (1) that enable one to synthesize oligonucleotides containing 3'-alkyl amines, in which the alkyl amine moiety can be unveiled under conditions in which the phosphate and exocyclic amine protecting groups are unperturbed (Scheme 1). Conjugation of oligonucleotides released from such supports should not be susceptible to the side reactions observed during the reaction of deprotected oligonucleotides.^{4,6}





We previously described the synthesis and utility of photolabile oligonucleotide synthesis supports that employ the *o*-nitrobenzyl photoredox reaction (eqn. 1).^{9,10} When used in conjunction with Pd(0) labile phosphoramidites, **2** and **3** can be used to site specifically incorporate alkaline labile nucleosides into oligonucleotides.¹¹ While supports **2** and **3** release oligonucleotides containing the relatively unreactive 3'hydroxyl group, support **4** yields oligonucleotides containing 3'-alkyl carboxylic acids.¹² The oligonucleotide synthesis supports that reveal carboxylic acids (4) serve as the basis for the design of **1**.



RESULTS AND DISCUSSION

Design and Synthesis of 1a-c. Supports 1a-c utilized the veratrole substituted *o*-nitrobenzyl chromophore found in 4, which we and others have found to be more efficient than the parent *o*-nitrobenzyl moiety.^{10,12,13} Synthesis of 4 was less efficient than desired, because of problems associated with the three carbon tether between the aromatic chromophore and the solid phase support. The three carbon linker was found to be susceptible to β -elimination of the phenolate moiety. The four carbon tether was incorporated into 1 to eliminate this problem. The carbamate linkage also enabled us to avoid having to introduce the tether in a different oxidation state. Finally, two general aspects were taken into consideration while designing the syntheses of 1a-c:

1. Loading of the CPG support was carried out in the final step in order to minimize the introduction of impurities to the resin.

2. A convergent approach towards the synthesis of 1 was taken in order to facilitate the alteration of the length of the alkyl amine tether.



^aKey: (a) Methyl 4-chlorobutyrate, K_2CO_3 , n-Bu₄N^{+1⁻}, CH₃CN, reflux (b) Furning HNO₃, AcOH, 25°C (c) NaBH₄, EtOH, 0°C.

In practice, alcohol 7 (Scheme 2), which is a common intermediate in the syntheses of 1a-c, was prepared in 3 steps from vanillin in 42% overall yield. Contrary to results obtained using similar substrates, electrophilic aromatic nitration of 5 produced only the desired isomer.^{12,13} The crude nitration product (6) was carried on to 7, which was purified by column chromatography.



^aKey: (a) Phosgene (1 M) in toluene, THF, 25°C (b) Amino alcohol, iPr_2NEt , THF, 25°C (c) DMTCl, pyridine, 35°C (d) LiOH, dioxane, 25°C (e) 2,4,5-Trichlorophenol, DCC, CH_2Cl_2 , 25°C (f) LCAA-CPG, DMF. The carbamates (8a-c) were formed via the crude chloroformate of 7, whose formation was analyzed for by ¹H NMR, as previously described (Scheme 3).¹³ We found that it was unnecessary to transiently protect the alcohol of the amino alcohols, as high yields of 8 were obtained. None of the product resulting from nucleophilic attack by the hydroxyl group of the amino alcohols was detected. Following dimethoxytritylation, the methyl esters (9a-c) were selectively hydrolyzed using LiOH (~ 0.25 M) at room temperature. The carboxylic acids were then activated as their trichlorophenyl esters (11a-c), which were purified and fully characterized prior to their being used to load the controlled pore glass support. Typical loadings were determined by 4,4'-dimethoxytrityl cation analysis, and were found to range between 49-54 µmol/g.



Figure 1. Isolated yields of T_{20} using a high pressure Hg/Xe lamp (solid bar) and Rayonet photoreactor (striped bar) as a function of irradiation time. Photocleavage was carried out on detrivulated material, prior to NH4OH deprotection. Photolytically cleaved oligonucleotide was subsequently deprotected with NH4OH and isolated by denaturing gel electrophoresis. Isolated yields are expressed relative to yield of oligonucleotide obtained without irradiation, but with identical NH4OH cleavage.

Photolytic Release of 3'-Alkyl Amine Tethered Oligonucleotides 1a-c. Photolysis conditions were optimized using detritylated, but otherwise protected eicosameric polythymidylate (T_{20}) that was prepared on 1b using standard automated oligonucleotide synthesis protocols (Figure 1). Photolyses were carried out on deaerated CH₃CN/H₂O (9:1) solutions, using the band pass filtered (λ_{max} = 400 nm) output of a

high pressure Hg/Xe lamp (800 W), or a Rayonet RPR-100 photoreactor equipped with black lamps (λ_{max} = 350 nm). 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 ammonium hydroxide cleavage. Maximum yields were obtained for 3 h photolyses using both irradiation sources. Longer irradiation led to a reduction in yield of isolated oligonucleotides. Previous experiments using tritiated nucleotides indicate that these photolysis conditions produce less than 2% thymine+thymine photodimers.⁹

Realizing that the above irradiation sources are not commonly available in biological laboratories, we recently investigated the feasibility of utilizing long wavelength (365 nm) transilluminators to effect photochemical cleavage of the protected oligonucleotides from the solid phase supports.¹³ Optimum irradiation periods were found to be 2 h for an eicosameric polythymidylate (T_{20}) prepared on 3. It was also determined that polymers containing other commonly utilized nucleosides were obtainable, provided the exocyclic amines were not protected as their benzamides. Applying these same irradiation conditions to T_{20} prepared on 1a-c produced very good yields of purified product (Table 1). One should note that experiments utilizing tritium enriched oligonucleotides showed that less than 3% photodimers are formed under these photolysis conditions.¹³ Furthermore, upon photolysis under these same conditions, 12 was obtained in 83 ± 9% yield from material prepared on 1a using commercially available fast deprotecting phosphoramidites.

Table 1. Isolated Yields of Completely Deprotected T20 as a Function of Alkyl Amine Tether Using a Transilluminator.*

Solid Phase Support	% Yield
1 a	98 ± 6.4
1 b	70 ± 12.4
1c	94 ± 8.1

"Yields are determined relative to nonphotolyzed material (see text).

Thus far, oligonucleotides synthesized have contained 20 nucleotides. This length biopolymer was chosen, because it represents an appropriate size target for devising potential inhibitors of genetic expression. Other applications could warrant longer oligonucleotides. Yet, the effect of oligonucleotide length on the photocleavage process had not been addressed previously. Consequently, two oligonucleotides 30 and 40 nucleotides long respectively were prepared on 1a using fast deprotecting phosphoramidites. Anticipating greater



competition for the light between the oligonucleotide and the *o*-nitrobenzyl chromophore, resins containing 13 and 14 were irradiated for 3 h with a transilluminator. Irradiating under these conditions, 13 and 14 were obtained in $72 \pm 11\%$ and $62 \pm 8\%$ yield respectively. These results indicate that longer oligonucleotides decrease the yields of photocleavage. This is not surprising, since as the oligonucleotides become longer, they have larger extinction coefficients, and absorb more light. It is possible that yields could be increased by irradiating for longer periods of time. However, this would result in greater amounts of random damage to the biopolymer. The fairly large standard deviations are attributed to the large number of manipulations involved during the isolation of the oligonucleotides.

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.^{9,13} Alternate forms of damage in 12 were investigated by enzymatic digestion. At the outset of these experiments, we were uncertain as to whether gel purified oligonucleotides synthesized on 1a would be digested by snake venom phosphodiesterase. Fortunately, 12 was completely digested to nucleosides by snake venom phosphodiesterase in the presence of calf intestine alkaline phosphatase. Two extraneous peaks appear in the chromatogram of the digest from the photolyzed material that are not present in the control (Figure 2). Coinjection of deoxyuridine, which could be derived from the photohydrate of deoxycytidine, eliminated this possibility for the peak eluting at ≈ 14.2 min. It is not known at this time, what compounds the peaks eluting at 14.2 and 27 min correspond to.

When added together, these peaks correspond to slightly less than 1% of the area corresponding to the four expected nucleosides.



Figure 2. Reverse phase HPLC analysis of nucleosides obtained upon enzymatic digestion of 12 A) Obtained via direct subjection of resin to NH4OH. B) Obtained via photolysis, followed by NH4OH.

Summary. These results demonstrate that the photolabile solid phase synthesis supports described above can be used to synthesize oligonucleotides up to 40 nucleotides long that contain 3'-alkyl amines. Photocleavage of the protected biopolymers is carried out under conditions that induce minor amounts of photodamage on the biopolymers, using light sources that are routinely available in chemistry and biology laboratories. The protected oligonucleotides obtained directly upon photolysis are amenable to purification by reverse phase HPLC.¹² Efforts to develop methodology for preparing oligonucleotide bioconjugates using these solid phase supports are currently underway.

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EXPERIMENTAL

General Methods. ¹H NMR spectra were collected at 270 or 300 MHz. All reactions were carried out in oven dried glassware, under a nitrogen atmosphere, unless specified otherwise. DMF, pyridine and CH₃CN were distilled from CaH₂. THF was distilled from Na/benzophenone ketyl. Long chain alkyl amine controlled pore glass support (CPG) was purchased from Sigma. Solid phase support loading, as well as oligonucleotide synthesis and purification were carried out as described previously.^{9,12} Snake venom phosphodiesterase was obtained from Boehringer Mannheim and calf alkaline phosphatase was from New England Biolabs. Enzymatic digestions were carried out as previously described.¹²

HPLC analyses were carried out on a Rainin Microsorb-MV C₁₈ (4.6 x 250 mm) reverse phase column. Photolyses carried out with an Oriel high pressure Hg/Xe lamp at 800 W, utilized a band pass filter from Oriel ($\lambda_{max} = 400$ nm, #59820). The Rayonet photoreactor (RPR-100) was from the Southern New England Ultraviolet Company. Photolyses carried out using a transilluminator utilized a UVP dual wavelength transilluminator containing 4 x 8 W lamps.

Oligonucleotides were synthesized using an Applied Biosystems Incorporated 380B automated synthesizer. Standard ABI synthesis cycles were used to prepare all oligonucleotides. "Fast deprotecting" phosphoramidites were from Pharmacia. All other DNA synthesis reagents were from Glen Research.

Preparation of 5. To vanillin (2.28 g, 15.0 mmol) in CH₃CN (40 mL), was added K₂CO₃ (4.98 g, 36.0 mmol), tetrabutylammonium iodide (1.12 g, 3.0 mmol) and methyl 4-chlorobutyrate (2.46 g, 18.0 mmol). The solution was refluxed for 17 h, at which time the precipitate was filtered and washed with EtOAc (3 x 20 mL). The solvents were removed in vacuo. The solid was dissolved in Et₂O (40 mL) and extracted with H₂O (3 x 10 mL). The organic layer was removed in vacuo, and 2.85 g (75%) of 5 was crystallized from EtOAc (50 mL). m.p. 68-70 °C; ¹H NMR (CDCl₃) δ 9.82 (s, 1H), 7.42-7.34 (m, 2H), 6.95 (d, 2H, J = 2 Hz), 3.91 (s, 3H), 3.67 (s, 3H), 2.52 (t, 2H, J = 7 Hz), 2.16 (m, 2H). IR (film) 2952, 1733, 1682, 1586, 1510, 1468, 1425, 1398, 1340, 1267, 1196, 1170, 1136, 1096, 867 cm⁻¹.

Preparation of 6. To 5 (2.70 g, 10.70 mmol) was added a mixture of HNO₃ (7 mL) and glacial acetic acid (27 mL) at 0°C. The mixture was allowed to warm to room temperature and stirred for 6 h. The reaction mixture was poured over crushed ice. The aqueous mixture was extracted with Et₂O (3 x 100 mL). The combined organics were washed with H₂O (50 mL), neutralized with successive saturated NaHCO₃ washings (several 50 mL volumes), washed with brine (50 mL), and dried over MgSO₄. The solution, which showed one

spot on TLC (EtOAc:Hexanes; 1:2) was concentrated in vacuo to yield 2.70 g (85%) of 6 as a yellow solid. The nitrated product was used without further purification. m.p. 76-78 °C; ¹H NMR (CDCl₃) δ 10.43 (s, 1H), 7.60 (s, 1H), 7.39 (s, 1H), 4.22 (t, 2H, J = 6 Hz), 3.94 (s, 3H), 3.71 (s, 3H), 2.59 (t, 2H, J = 7 Hz), 2.24 (m, 2H). IR (film) 2953, 1732, 1537, 1439, 1368, 1338, 1286, 1229, 1046.7 cm⁻¹.

Preparation of 7. Sodium borohydride (0.477 g, 12.62 mmol) was added to 6 (2.5 g, 8.41 mmol) in EtOH (50 mL) at room temperature. After 3 h, the EtOH was removed in vacuo. The residue was suspended in H₂O (30 mL) and extracted with Et₂O (3 x 50 mL). The combined organics were washed with brine (50 mL) and dried over MgSO₄. Flash chromatography (EtOAc:Hexanes; 1:2) yielded 1.66 g (66%) of 7. m.p. 98-100 °C; ¹H NMR (CDCl₃) δ 7.66 (s, 1H), 7.14 (s, 1H), 4.92 (s, 2H), 4.09 (t, 2H, J = 6 Hz), 3.94 (s, 3H), 3.67 (s, 3H), 2.53 (t, 2H, J = 7 Hz), 2.16 (m, 2H); IR (thin film) 1734, 1576, 1520, 1325, 1273, 1214, 1068, 668 cm⁻¹.

Preparation of 8a. A solution of phosgene in toluene (3.22 mL, 1.93 M) was added to 7 (0.48 g, 1.60 mmol) in THF (7.2 mL). The reaction was stirred at room temperature for 3 h and sparged with N₂ for 1 h. Solvents were removed in vacuo. To formed oil under N₂ was added diisopropylethyl amine (0.46 g, 3.52 mmol), THF (5 mL), and 4-amino-1-butanol (0.31 g, 3.52 mmol). The reaction mixture was stirred at room temperature and taken up in EtOAc (20 mL). The mixture was extracted with H₂O (3 x 5 mL). Organics were combined, washed with brine (5 mL), and dried with MgSO4. Flash chromatography (CH₂Cl₂:EtOAc; 1:1) gave 0.5 g (89%) of 8a as a solid. m.p. 115-116 °C; ¹H NMR (CDCl₃) δ 7.66 (s, 1H), 6.96 (s, 1H), 5.45 (s, 2H), 4.09 (t, 2H, J = 6 Hz), 3.92 (s, 3H), 3.67 (s, 3H), 3.64 (m, 2H), 3.23 (t, 2H, J = 3 Hz), 2.52 (t, 2H, J = 7 Hz), 2.16 (m, 2H), 1.59 (m, 4H); IR (thin film) 3312, 2941, 1734, 1717, 1700, 1684, 1653, 1558, 1540, 1521, 1506, 1472, 1457, 1275, 1064, 875 cm⁻¹.

Preparation of 8b. The same procedure was followed as described for 8a, except 5-amino-1-pentanol (0.36 g, 3.52 mmol) was used in place of 4-amino-1-butanol to give 0.64 g (94%) of 8b. m.p. 99-102 °C; ¹H NMR (CDCl₃) δ 7.65 (s, 1H), 6.95 (s, 1H), 5.44 (s, 2H), 4.08 (t, 2H, J = 6 Hz), 3.91 (s, 3H), 3.66 (s, 3H), 3.60 (t, 2H, J = 6 Hz), 3.19 (t, 2H, J = 7 Hz), 2.52 (t, 2H, J = 7 Hz), 2.15 (t, 2H, J = 7 Hz), 1.57 - 1.42 (m, 6H); IR (thin film) 3315, 2938, 2863, 1724, 1697, 1580, 1524, 1378, 1321, 1274, 1221, 1065, 883, 815 cm⁻¹.

Preparation of 8c. The same procedure was followed as described for 8a, except 6-amino-l-hexanol (0.41 g, 3.52 mmol) was used in place of 4-amino-l-butanol to give 0.51 g (72%) of 8c. m.p. 96-98 °C; ¹H NMR (CDCl₃) 7.60 (s, 1H), 6.55 (s, 1H), 5.44 (s, 2H), 4.11 (t, 2H, J = 6 Hz), 4.25 (s, 3H), 3.66 (s, 3H),

3.62 (t, 2H, J = 7 Hz), 3.21 (m, 2H), 2.53 (t, 2H, J = 7 Hz), 2.14 (m, 2H), 1.59 - 1.31 (m, 8H). IR (film) 2938, 1724, 1695, 1523, 1320, 1274, 1220, 1064 cm⁻¹.

Preparation of 9a. To **8a** (0.46 g, 1.10 mmol) was added pyridine (10 mL) and dimethoxytritylchloride (1.12 g, 3.30 mmol). The solution was stirred for 1 h at 50°C then quenched with MeOH (1 mL). H₂O (35 mL) was added, and the solution extracted with Et₂O (3 x 20 mL). Combined organics were washed with brine (20 mL), saturated NaHCO₃ solution (20 mL), and dried with MgSO₄. Flash chromatography (EtOAc:Hexanes; 1:2) yielded 0.79 g (100%) of **9a.** ¹H NMR (CDCl₃) δ 7.68 (s, 1H), 7.42-7.10 (m, 9H), 6.92 (s, 1H), 6.80 (d, 4H, J = 9 Hz), 5.46 (s, 2H), 4.09 (t, 2H, J = 6 Hz), 3.89 (s, 3H), 3.77 (s, 6H), 3.68 (s, 3H), 3.19 (m, 2H), 3.06 (t, 2H, J = 7 Hz), 2.53 (t, 2H, J = 7 Hz), 2.16 (m, 2H), 1.61 (m, 4H); IR (film) 2942, 1753, 1520, 1508, 1278, 1250, 1066, 1034, 828 cm⁻¹; Anal. calcd. for C₃₉H₄₄O₁₁N₂. C, 65.35; H, 6.19; N, 3.91. Found: C, 65.50; H, 6.40; N, 3.90.

Preparation of 9b. The reaction of 8b (0.47 g, 1.10 mmol) and dimethoxytritylchloride as described for preparation of 9a yielded 0.80 g (99%) of 9b. ¹H NMR (CDCl₃) δ 7.68 (s, 1H), 7.42-7.10 (m, 9H), 6.91 (s, 1H), 6.77 (d, 4H, J = 9 Hz), 5.46 (s, 2H), 4.09 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.75 (s, 6H), 3.68 (s, 3H), 3.15 (m, 2H), 3.02 (t, 2H, J = 7 Hz), 2.53 (t, 2H, J = 7 Hz), 2.14 (m, 2H), 1.68-1.31 (m, 6H); IR (film) 2937, 1733, 1608, 1580, 1510, 1464, 1444, 1328, 1278, 1249, 1218, 1174, 1066, 1034, 828 cm⁻¹; Anal. calcd. for C₄₀H₄₆O₁₁N₂: C, 65.74; H, 6.34; N, 3.83. Found: C, 65.90; H, 6.47; N, 3.82.

Preparation of 9c. The reaction of **8c** (0.49 g, 1.10 mmol) and dimethoxytritylchloride as described for preparation of **9a** yielded 0.33 g (41%) of **9c**. ¹H NMR (CDCl₃) δ 7.68 (s, 1H), 7.44-7.10 (m, 9H), 6.97 (s, 1H), 6.79 (d, 4H, J = 9 Hz), 5.45 (s, 2H), 4.08 (t, 2H, J = 6 Hz), 3.90 (s, 3H), 3.75 (s, 6H), 3.67 (s, 3H), 3.15 (m, 2H), 3.02 (t, 2H, J = 7 Hz), 2.52 (t, 2H, J = 7 Hz), 2.13 (m, 2H), 1.72-1.19 (m, 8H); IR (film) 2936, 2862, 1732, 1608, 1581, 1520, 1464, 1445, 1278, 1250, 1218, 1175, 1067, 1034, 829 cm⁻¹; Anal. calcd. for C₄₁H₄₈O₁₁N₂: C, 66.12; H, 6.50; N, 3.76. Found: C, 66.22; H, 6.63; N, 3.78.

Preparation of 10a. To **9a** (0.58 g, 0.80 mmol) was added dioxane (3.7 mL) and a mixture of H₂O (1 mL), MeOH (1 mL), and LiOH (0.055 g, 1.34 mmol). The reaction was allowed to stir at room temperature for 2 h. The solvents were removed in vacuo and the resulting solid was dissolved in H₂O (30 mL). The aqueous solution was acidified to pH 6.5 with H₂SO₄ (1 M), extracted with EtOAc (3 x 15 mL) and dried with MgSO₄. Flash chromatography (EtOAc:Hexanes; 1:1) yielded 0.48 g (85%) of **10a** as foam. ¹H NMR (CDCl₃) δ 7.68 (s, 1H), 7.42 - 7.12 (m, 9H), 6.95 (s, 1H), 6.81 (d, 4H, J = 9 Hz), 5.48 (s, 2H), 4.12 (t, 2H, J = 6 Hz), 3.88 (s,

3H), 3.77 (s, 6H), 3.20 (m, 2H), 3.09 (m, 2H), 2.61 (t, 2H, J = 7 Hz), 2.18 (m, 2H), 1.63 (m, 4H); IR (thin film) 3334, 2934, 2861, 1716, 1608, 581, 1509, 1464, 1328, 1279, 1249, 1218, 1175, 1065, 1033, 829, 755, 702 cm⁻¹.

Preparation of 10b. The reaction of **9b** (0.48 g, 0.66 mmol) with LiOH (0.055 g, 1.34 mmol) as described for preparation of **10a** yielded 0.22 g (48%) of **10b** as foam. ¹H NMR (CDCl₃) δ 7.68 (s, 1H), 7.42 - 7.14 (m, 9H), 6.95 (s, 1H), 6.80 (d, 7H, J = 9 Hz), 5.45 (s, 2H), 4.10 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.77 (s, 6H), 3.19 (m, 2H), 3.05 (t, 2H, J = 7 Hz), 2.55 (t, 2H, J = 7 Hz), 2.14 (m, 2H), 1.68 - 1.30 (m, 6H); IR (thin film) 335, 2935, 1717, 1521, 1508, 1457, 1328, 1280, 1250, 1218, 1176, 1065, 1033, 829, 754 cm⁻¹.

Preparation of 10c. The reaction of 9c (0.30 g, 0.40 mmol) with LiOH (0.033 g, 0.80 mmol) as described for preparation of 10a yielded 0.27 g (89%) of 10c as foam. ¹H NMR (CDCl₃) δ 7.68 (s, H), 7.43 - 7.18 (m, 9H), 6.98 (s, H), 6.81 (d, 4H, J = 9 Hz), 5.47 (s, 2H), 4.10 (t, 2H, J = 6 Hz), 3.90 (s, 3H), 3.78 (s, 6H), 3.18 (m, 2H), 3.02 (t, 2H, J = 7 Hz), 2.58 (t, 2H, J = 7 Hz), 2.16 (m, 2H), 1.68 - 1.26 (m, 8H); IR (thin film) 2935, 1713, 1608, 1580, 1510, 1327, 1278, 1249, 1066, 1034, 829, 668 cm⁻¹.

Preparation of 11a. To 10a (0.20 g, 0.28 mmol) in CH₂Cl₂ (2.7 mL), was added DCC (0.46 g, 2.22 mmol), and 2,4,5-trichlorophenol (0.44 g, 2.23 mmol). The mixture was allowed to stir at room temperature overnight. The mixture was filtered, and solid DCU was washed with dry Et₂O (5 x 10 mL). Flash chromatography (EtOAc:Hexanes; 1:2) yielded 0.20 g, 0.23 mmol of 11a (82%) as a foam. ¹H NMR (CDCl₃) 7.70 (s, 1H), 7.53 (s, 1H), 7.41 - 7.17 (m, 10H), 6.96 (s, 1H), 6.79 (d, 4H, J = 9 Hz), 5.47 (s, 2H), 4.16 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.76 (s, 6H), 3.20 (m, 2H), 3.06 (m, 2H), 2.85 (t, 2H, J = 7 Hz), 2.30 (m, 2H), 1.61 (m, 4H); IR (thin film) 3394, 2935, 1771, 1717, 1608, 1577, 1508, 1456, 1278, 1249, 1218, 1119, 10 81, 1066, 1034, 828 cm⁻¹; ¹³C NMR (CDCl₃) δ 170.0, 158.4, 155.8, 153.9, 147.2, 145.8, 145.2, 139.7, 131.5, 131.0, 130.6, 130.0, 128.7, 128.1, 127.7, 126.7, 126.1, 125.3, 113.0, 110.5, 109.8, 85.9, 68.1, 62.9, 56.3, 55.2, 41.1, 30.4, 24.9, 24.2; HRMS FAB (M*) calcd 880.1932, found 880.1999.

Preparation of 11b. The reaction of **10b** (0.21 g, 0.30 mmol) with DCC (0.49 g, 2.38 mmol) and 2,4,5-trichlorophenol (0.47 g, 2.38 mmol) as described for preparation of **10a** yielded 0.13 g (50%) of **11b** as foam. ¹H NMR (CDCl₃) δ 7.69 (s, 1H), 7.53 (s, 1H), 7.42 - 7.17 (m, 10H), 6.97 (s, 1H), 6.80 (d, 4H, J = 9 Hz), 5.47 (s, 2H), 4.16 (t, 2H, J = 6 Hz), 3.89 (s, 3H), 3.76 (s, 6H), 3.18 (m, 2H), 3.03 (t, 2H, J = 7 Hz), 2.85 (t, 2H, J = 7 Hz), 2.30 (m, 2H), 1.63 - 1.23 (m, 6H); IR (thin film) 3399, 2936, 2862, 1771, 1726, 1608, 1580, 1510, 1462, 1278, 1249, 1218, 1175, 1082, 1034, 828, 736, 703 cm⁻¹; ¹³C NMR (CDCl₃) δ 169.9,

158.2, 155.8, 153.8, 147.1, 145.7, 145.2, 139.7, 136.5, 131.4, 131.0, 130.5, 129.9, 128.3, 128.1, 127.6, 126.5, 126.0, 125.3, 112.9, 110.5, 109.7, 85.7, 68.0, 63.0, 56.2, 55.1, 30.3, 29.6, 24.1, 23.4; HRMS FAB (M⁺) calcd 894.2089, found 894.2135.

Preparation of 11c. The reaction of **10c** (0.27 g, 0.38 mmol) with DCC (0.62 g, 3.00 mmol) and 2,4,5-trichlorophenol (0.59 g, 3.00 mmol) as described for preparation of **10a** yielded 0.23 g (67%) of **11c** as foam. ¹H NMR (CDCl₃) δ 7.70 (s, 1H), 7.53 (s, 1H), 7.42 - 7.17 (m, 10H), 6.98 (s, 1H), 6.80 (d, 4H, J = 9 Hz), 5.48 (s, 1H), 4.17 (t, 2H, J = 6 Hz), 3.92 (s, 3H), 3.77 (s, 6H), 3.18 (m, 2H), 3.02 (t, 2H, J = 7 Hz), 2.86 (t, 2H, J = 7 Hz), 2.31 (m, 2H), 1.61 - 1.25 (m, 8H); IR (thin film) 2934, 1772, 1507, 1456, 1278, 1249, 1082, 668 cm⁻¹; ¹³C NMR (CDCl₃) δ 169.9, 158.2, 155.8, 153.8, 147.1, 145.7, 145.3, 139.7, 136.6, 131.4, 131.0, 130.7, 129.9, 128.1, 127.6, 126.5, 126.0, 125.3, 112.9, 109.7, 85.6, 68.0, 63.1, 56.2, 55.1, 41.1, 30.3, 29.9, 26.5, 25.9, 24.1; HRMS FAB (M⁺) calcd 908.2245, found 908.2244.

General Procedure for Photolytic Cleavage of Oligonucleotides. Oligonucleotide bound to support (~1.0 mg, ~50 nmoles) was added to a Pyrex tube containing a magnetic stir bar and 3 mL of CH_3CN/H_2O (9:1 by volume). The tube was fitted with a rubber septum and sparged with N₂ for 20 min. Photolysis was carried out using the appropriate irradiation source and any necessary filters for the prescribed period under dynamic N₂ (following sparging, the needle is raised well above the surface of the solvent, in order to avoid evaporation during photolysis) with stirring. Following photolysis, the solution was filtered through a 0.45 µm membrane filter. The photolysis tube was washed with CH₃CN (3 x 1 mL), followed by H₂O (3 x 1 mL). Each washing was filtered through the membrane. The washings were combined and concentrated in vacuo, after which they were subjected to NH₄OH deprotection. When using a transilluminator, the photolysis tube was maintained at a distance of 7 cm from the lamp surface. A cylinder of aluminum foil surrounded the photolysis of apparatus.

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