Improved Utility of Photolabile Solid Phase Synthesis Supports for the Synthesis of Oligonucleotides Containing 3′-Hydroxyl Termini

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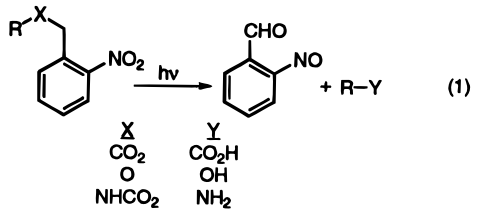
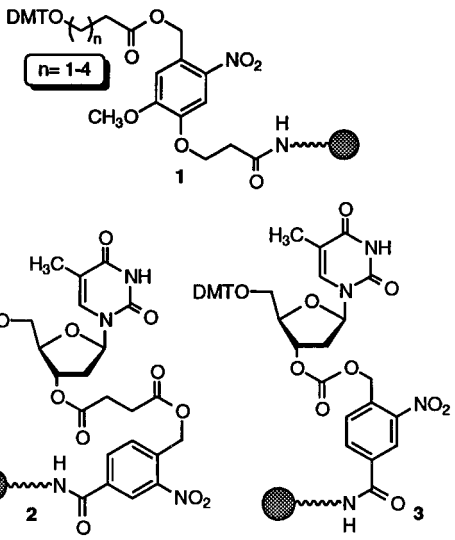
Oligonucleotides are synthesized on, and cleaved from, a solid phase support (**6**) using the *o*-nitrobenzyl intramolecular photochemical redox reaction. The yields of isolated oligonucleotides relative to yields obtained using conventional hydrolytic cleavage vary between 67% and 82.5%. Synthesis of oligonucleotides using phosphoramidites that do not contain *N*-benzoyl protecting groups enables one to photolytically cleave the biopolymers in good yields using a commonly available UV irradiation source. Tritium labeling indicates that less than 3% thymidine·thymidine photodimers are formed during photolytic cleavage of polythymidylates from **6** using a transilluminator. No UV-induced damage is detected via HPLC analysis of enzymatically digested oligonucleotides that were obtained following photolytic cleavage from **6**.

The *o*-nitrobenzyl photoredox process (eq 1) has been employed in a variety of synthetic strategies and as a tool for probing biological processes.[1] We have utilized this venerable reaction to construct solid phase oligonucleotide synthesis supports that enable one to cleave the biopolymers from their solid supports at room temperature under neutral conditions. The supports are

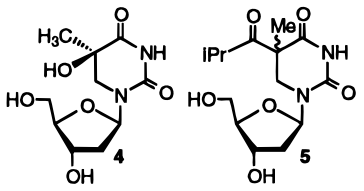


compatible with commercially available oligonucleotide synthesis reagents, and do not require altering of standard automated synthesis cycles. Elimination of NH_4OH from the cleavage process enables one to release protected oligonucleotides containing a single functional group at their 3′ termini that is suitable for conjugation (e.g., **1**).[2] Other supports (**2**, **3**) yield biopolymers containing 3′-hydroxy termini and are useful for site-specific incorporation of alkaline labile nucleosides (e.g., **4**, **5**) in oligonucleotides, which are in turn amenable to enzymatic manipulation.[3,4]

Despite the general utility of *o*-nitrobenzyl supports **2** and **3**, there is need for improvement. Carbonate support **3** produces moderate yields (61%) of oligonucleotides after 9 h of band pass (λ_{max} = 400 nm) photolysis. Seven percent of the contiguous thymidines present are transformed into thymine·thymine photodimers under these photolysis conditions.[3] Photolabile supports that are more efficient are needed in order to reduce this type of damage. It is also highly desirable to carry out the



photolytic cleavage of the biopolymers from the solid phase supports using light sources, such as transilluminators, which are routinely available in chemistry and biology laboratories. Good yields of polythymidylates are obtained from **1** using a transilluminator which emits at



365 nm. However, similar treatment of heteropolymers[5] containing the most commonly used commercially available phosphoramidite protecting groups yields less than 15% of isolable, deprotected oligonucleotides. Similar results were obtained using a Rayonet photoreactor equipped with lamps exhibiting a maximum output at 350 nm. We now report a photolabile solid phase synthesis support (**6**) that releases oligonucleotides con-

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(1) (a) Pillai, V. N. R. In *Organic Photochemistry*, Vol. 9; Padwa, A., Ed.; Marcel Dekker: New York, 1987. (b) Corrie, J. E. T.; Trentham, D. R. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley: New York, 1993; Vol. 2, pp 243−305.

(2) Yoo, D. J.; Greenberg, M. M. *J. Org. Chem.* **1995**, *60*, 3358.

(3) Greenberg, M. M.; Gilmore, J. L. *J. Org. Chem.* **1994**, *59*, 746.

(4) (a) Matray, T. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1994**, *116*, 6931. (b) Barvian, M. R.; Greenberg, M. M. *J. Am. Chem. Soc.* **1995**, *117*, 8291.

(5) A heteropolymer is defined as an oligonucleotide containing thymidine and other nucleosides.

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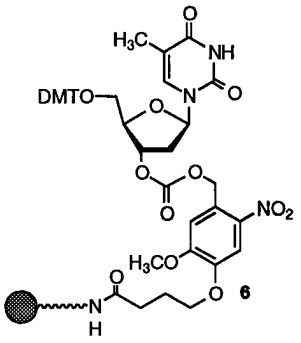
taining 3′-hydroxy termini, which is more effective than **2** and **3**. Using **6** as a vehicle, we also report: (1) an improved synthesis of the veratrole derivative, which is the reactive chromophore in **6** and other photolabile solid phase synthesis supports[2] and (2) a strategy that enables one to synthesize heteropolymers from **6** and other photolabile solid phase synthesis supports, using convenient sources of light, such as a transilluminator.

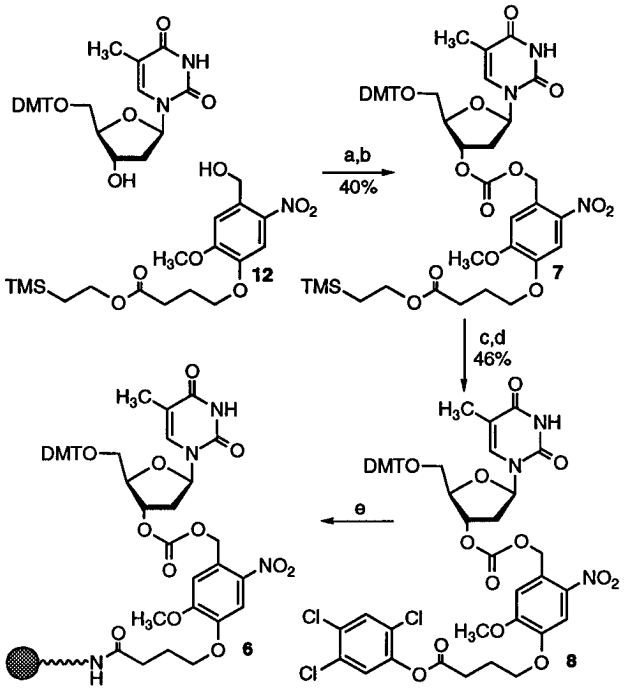


Design and Synthesis of 6. Solid phase support **6** contains a carbonate linker between the *o*-nitrobenzyl chromophore and the nucleoside component. This feature was incorporated in **6**, because prior work with **2** established that photolabile supports containing carbonate linkages between the 3′-terminal nucleotide and the photolabile moiety are readily synthesizable.[3] Similarly, the increased photochemical efficiency observed with **1** attributable to alkoxyl substitution of the *o*-nitrobenzyl group led us to incorporate this feature in **6** as well.[2] Finally, we found that the three-carbon linker between the *o*-nitrobenzyl group and the solid support (such as is found in **1**) is prone to elimination during synthesis of the respective activated esters (e.g., **8**) that are used to load the solid phase supports. This limits the range of reactions which can be carried out in the presence of this side chain. Consequently, **6** was designed so as to contain a four-carbon linker between the aromatic chromophore and the solid phase support.

We routinely prepare modified solid phase oligonucleotide synthesis supports by carrying out the loading of the support by an activated ester as the last step in the process. This strategy minimizes the introduction of impurities to the support (Scheme 1). Because activated esters (e.g., 2,4,5-trichlorophenyl, **8**) are unstable to the conditions needed to effect coupling of the chloroformate of **12** to the nucleoside, the choice of the carboxylate protecting group in **12** was crucial. It was necessary that the ester be cleavable under conditions which would not cleave the dimethoxytrityl group (nonacidic) or hydrolyze the carbonate (nonnucleophilic). The (trimethylsilyl)-ethyl protecting group was chosen, because it could be removed under anhydrous conditions using CsF.

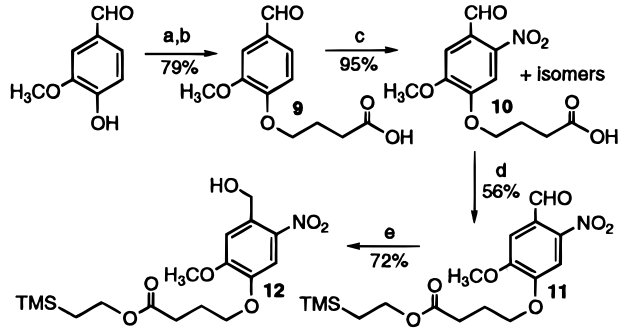
The requisite alcohol (**12**) was obtained in a simple, albeit circuitous, manner (Scheme 2). Saponification and subsequent esterification of the carboxylate was necessary, because attempted alkylation of the phenol with 4-chlorobutyric acid yielded the respective benzoate of 4-hydroxybutyric acid. Presumably, this product arises from lactonization of the acid in situ, followed by nucleophilic attack of the phenolate under the mildly basic reaction conditions. Moreover, the (trimethylsilyl)ethyl ester was unstable to electrophilic aromatic nitration reaction conditions. Despite the necessary protecting group manipulations, **12** was obtainable in multigram quantities.

Scheme 1[a]



[a] Key: (a) phosgene (1 M) in toluene, THF, 25 °C; (b) NaH, THF, 25 °C; (c) CsF, DMF, 35 °C; (d) 2,4,5-trichlorophenol, DCC, CH_2Cl_2, 25 °C; (e) LCAA-CPG, DMF.

Scheme 2[a]



[a] Key: (a) methyl 4-chlorobutyrate, K_2CO_3, *n*-$Bu_4N^+I^-$, CH_3CN, reflux; (b) LiOH, dioxane, 25 °C; (c) fuming HNO_3, AcOH, 25°C; (d) 2-(trimethylsilyl)ethanol, DCC, CH_2Cl_2, 25°C; (e) $NaBH_4$, EtOH, 0 °C.

Photochemical Release of Oligonucleotides from 6. Photolytic cleavage yields of an eicosameric polythymidylate (T_{20}) were optimized separately using the 400 nm band pass filtered output of a high-pressure Hg/Xe lamp and a transilluminator (λ_{max} = 365 nm). Photolytically cleaved oligonucleotides were subsequently deprotected with NH_4OH and isolated by denaturing gel electrophoresis. Isolated yields in all experiments are expressed as percent yields relative to those obtained for the oligonucleotide without irradiation, but with identical NH_4OH cleavage. The yields presented are averages of those obtained over three separate experiments and vary by less than 15% of the average value. Maximum yields of T_{20} (67%) were obtained in one-fourth the time (1.5 h) needed to obtain comparable yields (within experimental error) of the identical oligonucleotide from **2** using the same light source (Figure 1). Prolonged irradiation of oligonucleotides synthesized on **6** results in diminution of isolated yields of biopolymers. This is attributed to formation of alklaine labile lesions in the biopolymer.

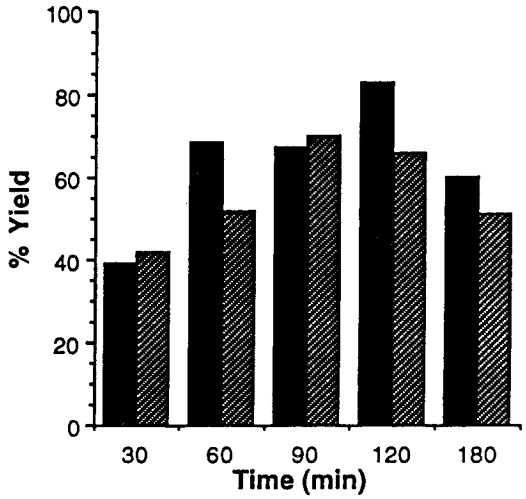


Figure 1. Isolated yields of T_{20} as a function of irradiation time. Irradiation source: transilluminator (365 nm), solid bar; Hg/Xe (400 nm), striped bar. Photocleavage was carried out on detritylated material, prior to NH_4OH deprotection. Photolytically cleaved oligonucleotide was subsequently deprotected with NH_4OH and isolated by denaturing gel electrophoresis. Isolated yields are expressed relative to yield of oligonucleotide obtained without irradiation, but with identical NH_4OH cleavage.

These lesions give rise to shorter oligonucleotides via strand breaks upon ammonolysis. The shorter fragments are separated from the intact material upon purification. Extrapolation of previous experiments using tritiated oligonucleotides indicates that these photolysis conditions will produce less than 2% thymidine photodimers.[3] While a slightly longer (2 h) photolysis time was needed to optimize yields of T_{20} using the transilluminator, the yields (82.5%) were higher than those obtained using the Hg/Xe lamp. The isolated yields of T_{20} and required photolysis times from **6** are similar to those measured for a 3′-alkyl carboxylic acid containing polythymidylates from **1**.[2]

As expected, photolysis with a transilluminator of a protected eicosameric heteropolymer prepared on **6** yielded negligible amounts (≤15%) of product (**13**) following ammonolysis and gel electrophoresis. The recurring observation that irradiation sources which emit maximally in the 350−365 nm region could not be used to prepare heteropolymers from **6**, or other *o*-nitrobenzyl-based supports, posed a significant limitation to the application of this methodology. Considering the widespread use of the *o*-nitrobenzyl photoredox reaction, we suspected that the problem lay in the properties of the protected oligonucleotides and not the actual photochemical reaction. Two observations suggested that the source of low yields of **13** upon irradiation at 365 nm were due to photochemistry associated with the protecting groups on the nucleobases: (1) isolated yields of polythymidylates (which do not contain nucleobase protecting groups) are not strongly dependent upon wavelength and (2) oligonucleotides whose nucleobases are deprotected are unaffected by irradiation at 350−365 nm.[3,4]

5'-d(TAC GCA ATC CTA GAT CTA AT)
13

The most commonly utilized commercially available nucleobase protecting groups contain benzoyl groups on deoxyadenosine (dA^{Bz}) and deoxycytidine (dC^{Bz}). The exocyclic amine of deoxyguanosine is protected by an isobutyryl group (dG^{iBu}). We anticipated that the benzamide groups were the most likely to be photoactive via either direct absorption of the irradiation or energy transfer within the protected oligonucleotide.

5'-d(TGG TCG CGC GTC CGT CTG CT)
14

In order to test this hypothesis, an eicosamer (**14**) containing T, dG, and dC in which the exocyclic amine of dC was protected as its acetate (dC^{Ac}) was prepared on **6**. Standard isobutyryl-protected dG was utilized. Fully deprotected **14** was obtained in 80.5% yield, following photolytic cleavage with a transilluminator for 2 h. Further confirmation of the detrimental effect of the benzamide protecting groups was ascertained via isolation of **13** in 67% yield using dC^{Ac} and *N*-phenoxyacetyl-protected deoxyadenosine (dA^{Pac}).[6] A comparable yield (68.5%) of **13** was obtained using all commercially available "fast deprotecting" phosphoramidites.[6,7]

Integrity of the Photolytically Cleaved Oligonucleotides. Enzymatic digestion of oligonucleotides released from **1** and **3**, following band pass filtered photolysis, did not reveal the presence of any damaged nucleosides.[2,3] Oligonucleotide **13**, prepared using fast-deprotecting phosphoramidites and thymidine, was digested using snake venom phosphodiesterase, and the nucleotides released were dephosphorylated with calf alkaline phosphatase (Figure 2).[6,8] The area ratio of nucleosides obtained from the photolytically (transilluminator, 2 h) cleaved oligonucleotide was identical (within experimental error) to that measured from the sample subjected directly to ammonolysis. The broad featureless peak present in both samples (t_R ~22.5 min) is found in a blank injection as well (data not shown).

Thymidine photodimer formation is a prevalent type of damage induced via UV irradiation. Extrapolation from previous experiments utilizing band pass filtered photolysis (λ_{max} = 400 nm) suggests that less than 2% of thymidine·thymidine photodimers will be formed upon cleavage from **6** under these conditions.[3] There is no previous data involving the transilluminator from which to extrapolate. Consequently, thymidine photodimer formation was assayed for by HPLC analysis of formic acid cleaved material, as previously described.[3,9] Detection sensitivity of the released thymine·thymine dimers was enhanced by incorporating tritiated thymidine (2.55 Ci/mol) at the 11th thymidine (5′ numbering) in T_{20}. After accounting for background tritium, less than 3% of thymine·thymine dimers (detection limits) are formed during a 2 h photolysis.

Summary. Photolabile solid phase oligonucleotide synthesis support **6** enables one to synthesize biopolymers containing 3′-hydroxy termini in good yields, under conditions which induce minimal amounts of UV photodamage. The yields obtained using **6** are superior than those obtained from **3**. Comparable yields of identical oligonucleotides are obtained from **2** and **6**. However,

(6) Schulhof, J. C.; Molko, D.; Teoule, R. *Tetrahedron Lett.* **1987**, *28*, 51.
(7) Similar observations and suggested solutions were reported while this manuscript was under review. See: Pirrung, M. C.; Bradley, J.-C. *J. Org. Chem.* **1995**, *60*, 6270.
(8) Eadie, J. S.; McBride, L. J.; Efcavitch, J. W.; Hoff, L. B.; Cathcart, R. *Anal. Biochem.* **1987**, *165*, 442.
(9) (a) Cadet, J.; Voituriez, L.; Hruska, F. E.; Kan, L.-S.; de Leeuw, F. A. A. M.; Altona, C. *Can. J. Chem.* **1985**, *63*, 2861. (b) Cadet, J.; Voituriez, L.; Hahn, B.-S.; Wang, S. Y. *J. Chromatogr.* **1980**, *195*, 139.

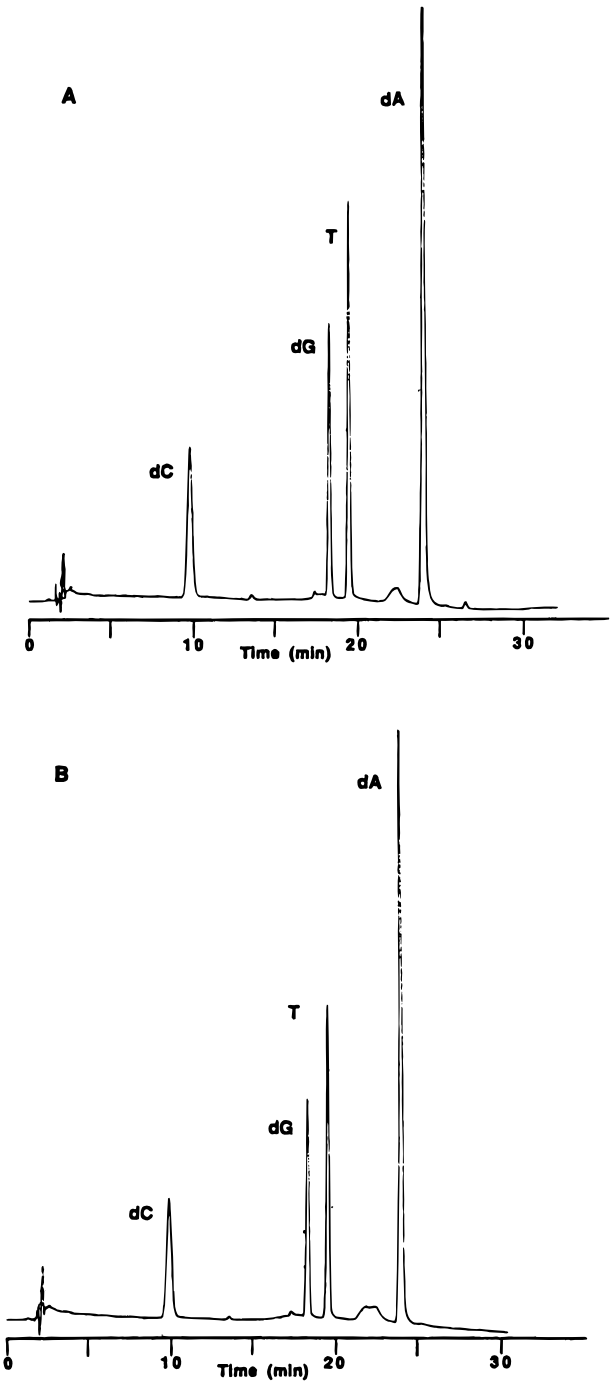


Figure 2. Base composition analysis of **13** via enzymatic digestion. (A) Oligonucleotide cleaved using concentrated NH_4OH. (B) Oligonucleotide cleaved photochemically.

significantly shorter irradiation times are required when using **6**. Utilization of commercially available phosphoramidites which do not contain *N*-benzoyl protecting groups allows one to prepare oligonucleotides containing all four common naturally occurring nucleosides, using a UV irradiation source which is commonly available in chemistry and biology laboratories. Finally, the utility of **6** for the incorporation of alkaline labile nucleosides has been demonstrated via the synthesis of oligonucleotides containing **4** and **5**.[10]

(10) (a) Greenberg, M. M.; Barvian, M. R.; Cook, G. P.; Matray, T. J.; Venkatesan, H. *J. Am. Chem. Soc.* (submitted). (b) Matray, T. J.; Greenberg, M. M. (unpublished results).

Experimental Section

General Methods. ^1H NMR spectra were recorded at 300, 270, or 200 MHz. HPLC chromatography was carried out on a Rainin Microsorb-MV C-18 (5 μm) column. Photolyses were carried using a Oriel 1000 W high-pressure Hg/Xe lamp or a UVP dual wavelength transilluminator. The transilluminator contained 4 × 8 W bulbs. The band pass filter was from Oriel (λ_{max} = 400 nm, no. 59820). [*methyl*-^3H]Thymidine was purchased from Amersham. Tritiated thymidine phosphoramidite was prepared as described previously (2.54 Ci/mol).[3] Enzymatic digests and polyacrylamide gel electrophoresis were carried out as previously described.[2,3]

All reactions were run under a nitrogen atmosphere in oven-dried glassware, unless specified otherwise. Acetonitrile, CH_2Cl_2, and DMF were freshly distilled from CaH_2. THF was freshly distilled from Na/benzophenone ketyl. Long chain alkylamine controlled pore glass support (CPG) was purchased from Sigma. Snake venom phosphodiesterase was obtained from Boehringer-Mannheim. Calf intestine alkaline phosphatase was from New England Biolabs.

Oligonucleotides were synthesized as previously described.[3] "Fast deprotecting" phosphoramidites were from Pharmacia.[6] *N*-Acetyldeoxycytidine and all other phosphoramidites were from Glen Research.

General Procedure for Photolytic Cleavage of Oligonucleotides from Solid Phase Supports Using a Transilluminator. Resin (~1.0 mg) was stirred in a Pyrex tube containing 3 mL of a 9:1 mixture of CH_3CN:H_2O. The mixture was sparged with N_2 for ~20 min before photolysis. The volume above the solution was continuously purged with N_2 during photolysis. The resin was filtered through a 0.45 μm filter upon completion of the photolysis. The tube and filter were washed with H_2O (3 × 1 mL) and CH_3CN (3 × 1 mL). The filtrates were concentrated, combined, and subjected to the appropriate deprotection and/or purification method.

Preparation of 9. Methyl 4-chlorobutyrate (5.4 g, 39.5 mmol) was refluxed in CH_3CN (80 mL) with vanillin (5 g, 33 mmol), K_2CO_3 (10.9 g, 79 mmol), and *n*-$Bu_4N^+I^-$ (2.43 g, 6.5 mmol) for 22 h. The reaction mixture was cooled and diluted with ether (200 mL) and H_2O (50 mL). The aqueous layer was extracted with ether (2 × 100 mL). The combined organics were washed with brine (50 mL) and dried over $MgSO_4$. After removing excess methyl 4-chlorobutyrate under vacuum, the crude product was recrystallized from EtOAc to yield 6.6 g (80%) of alkylated vanillin. The product was carried on to the next step in the synthetic procedure without further purification. Mp: 67−68.5 °C. ^1H NMR ($CDCl_3$): δ 9.76 (s, 1H), 7.36 (m, 2H), 6.94 (d, 1H, J = 8 Hz), 4.1 (t, 2H, J = 6 Hz), 3.86 (s, 1H), 3.64 (s, 1H), 2.52 (t, 2H, J = 7 Hz), 2.2 (tt, 2H, J = 7,7 Hz). ^{13}C NMR ($CDCl_3$): δ 190.6, 173.1, 153.6, 149.6, 129.9, 126.4, 111.4, 109.1, 67.6, 55.7, 51.4, 30.1, 24. IR (film): 3079, 2951, 2882, 1732, 1682, 1586, 1510, 1467, 1425, 1397, 1267, 1196, 1031, 867, 731, 654 cm^{-1}.

LiOH (1 M, 28.5 mL) was added to a solution of the above alkylated vanillin (3 g, 11.9 mmol) in dioxane (90 mL). After 8 h of stirring at room temperature, the reaction mixture was acidified to pH 3 with 10% HCl. Additional H_2O (40 mL) was added and the aqueous layer extracted with EtOAc (3 × 150 mL). The combined organics were washed with brine (50 mL) and dried over $MgSO_4$. Removal of solvent yielded 2.8 g (99%) of **9**, which was used without further purification. Mp: 155 °C. ^1H NMR (CD_3OD): δ 9.62 (s, 1H), 7.51 (d, 1H, J = 8 Hz), 7.42 (s, 1H), 7.12 (d, 1H, J = 8 Hz), 4.16 (t, 2H, J = 7 Hz), 3.88 (s, 1H), 2.37 (t, 2H, J = 7 Hz), 2.14 (tt, 2H, J = 7, 7 Hz). ^{13}C NMR (CD_3OD): δ 182.7, 156.3, 151.7, 132.1, 128.5, 124.7, 113.8, 111.6, 70.8, 57.2, 35.8, 27.7. IR (film): 3584, 3520, 2957, 2922, 1712, 1694, 1673, 1585, 1556, 1361, 1315, 1273, 1261, 1231, 1158, 1132, 1092, 933, 681, 654 cm^{-1}.

Preparation of 11. A mixture of fuming nitric acid (7.5 mL) and glacial acetic acid (30 mL) was added dropwise to crude **9** (3 g, 12.76 mmol). After stirring for 6 h, the reaction mixture was poured into ice water (50 mL) and extracted with EtOAc (3 × 150 mL). The combined organics were washed with a saturated solution of $NaHCO_3$ (**caution**) until all effervescence ceased. The aqueous layer was acidified to pH

3 with 10% HCl (**caution)** and extracted with EtOAc (3 × 150 mL). The combined organics were washed with brine (75 mL) and dried over $MgSO_4$. Removal of solvent yielded 3.3 g (95%) of **10** and its regioisomers in a ratio of 4:1:1.

2-(Trimethylsilyl)ethanol (1.65 g, 14 mmol) was added to crude **10** (3.3 g, 11.7 mmol) in CH_2Cl_2 (65 mL) and EtOAc (10 mL). A solution of DCC (2.9 g, 14 mmol) in CH_2Cl_2 (5 mL) was added dropwise, and the reaction mixture was stirred for 5 h at room temperature. The mixture was filtered to remove DCU and poured into H_2O (75 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organics were washed with 10% HCl (25 mL) and brine (50 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 1:4) yielded 2.7 g (56%) of **11**. The product was carried on to the next step in the synthetic procedure without further purification. ^{1}H NMR ($CDCl_3$): δ 10.41 (s, 1H), 7.58 (s, 1H), 7.38 (s, 1H), 4.2−4.1 (m, 4H), 3.97 (s, 3H), 2.53−2.48 (m, 2H), 2.23−2.14 (m, 2H), 0.99−0.92 (m, 2H), 0.01 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 187.5, 172.6, 153.3, 151.6, 143.6, 125.3, 109.7, 107.9, 68.5, 62.7, 56.4, 30.3, 23.9, 17.2, −1.7. IR (film): 2953, 1731, 1689, 1573, 1520, 1469, 1403, 1335, 1283, 1223, 1060, 837, 737 cm^{-1}.

Preparation of 12. $NaBH_4$ (0.3 g, 7.8 mmol) was added to **11** (2.5 g, 6.5 mmol) in EtOH (54 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 h. After quenching with a saturated solution of NH_4Cl (2 mL), EtOH was removed in vacuo. H_2O (50 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organics were washed with brine (50 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 2:3) yielded 1.8 g (72%) of **12**. Mp: 84 °C. ^{1}H NMR ($CDCl_3$): δ 7.67 (s, 1H), 7.14 (s, 1H), 4.94 (d, 2H, J = 6 Hz), 4.2−4.08 (m, 4H), 3.95 (s, 3H), 2.75 (t, 1H, J = 7 Hz), 2.53 (t, 2H, J = 7 Hz), 2.18−2.11 (m, 2H), 1.0−0.93 (m, 2H), 0.02 (s, 9H). ^{13}C ($CDCl_3$): δ 173, 154.3, 147.1, 139.5, 132.5, 111, 109.6, 68.34, 62.7, 62.6, 56.3, 30.7, 24.3, 17.3, −1.6. IR (film): 3533, 3101, 2953, 1731, 1615, 1577, 1519, 1466, 1435, 1386, 1325, 1273, 1215, 1170, 939, 756, 664 cm^{-1}. Anal. Calcd for $C_{17}H_{27}NO_7$-Si: C, 52.97; H, 7.06; N, 3.63. Found: C, 53.17; H, 7.06; N, 3.69.

Preparation of 7. Phosgene in toluene (1.2 mL, 1.9 M solution) was added to **12** (0.15 g, 0.39 mmol) in THF (2 mL). After the reaction mixture was stirred for 4 h, N_2 was bubbled through the solution for 1 h to remove excess phosgene. After removal of the solvent *in vacuo*, an aliquot of the crude product was analyzed by IR and ^{1}H NMR. IR showed two carbonyl stretches at 1778 and 1730 cm^{-1}. ^{1}H NMR showed a shift of the benzylic protons from 4.94 to 5.82 ppm. This reaction was carried out on this scale in two separate flasks. The above analytical methods indicated that each reaction was quantitative. Significant reductions in yield of the subsequent coupling reaction were observed when the chloroformate formation was carried out on a larger scale. The sodium alkoxide of (dimethoxytrityl)thymidine (0.353 g, 0.65 mmol) in THF (2.3 mL) prepared from NaH was added to the chloroformate (0.35 g, 0.76 mmol) in THF (2 mL), and the reaction mixture was stirred for 2 h at 25 °C. The reaction was diluted with EtOAc (40 mL), and water (10 mL) was added. The aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organics were washed with brine (20 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes:MeOH, 50:48:2) yielded 0.45 g (72%) of **7**. The product was carried on to the next step in the synthetic procedure without further purification. Mp: 89 °C. ^{1}H NMR ($CDCl_3$): δ 8.67 (s, 1H), 7.72 (s, 1H), 7.6 (s, 1H), 7.37−7.22 (m, 9H), 7.02 (s, 1H), 6.84 (d, 4H, J = 8 Hz), 6.49 (dd, 1H, J = 5, 9 Hz), 5.55 (s, 2H), 5.39 (d, 1H, J = 5 Hz), 4.22−4.09 (m, 5H), 3.96 (s, 3H), 3.77 (s, 6H), 3.49 (m, 2H), 2.55−2.14 (m, 6H), 1.66 (s, 3H), 0.99−0.94 (m, 2H), 0.02 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.8, 163.3, 158.6, 154, 153.7, 150.1, 147.5, 143.9, 139.3, 135.1, 134.9, 129.89, 127.89, 127.87, 127.1, 125.9, 113.16, 111.56, 109.8, 109.4, 87.13, 84.12, 83.64, 79.18, 68.18, 66.6, 63.6, 62.6, 56.4, 55.1, 37.7, 30.5, 24.1, 17.15, 11.4, −1.7. IR (film): 3064, 2932, 1751, 1690, 1608, 1580, 1559, 1522, 1509, 1463, 1329, 1278, 1219, 1154, 1081, 956, 910, 754, 730, 702, 647 cm^{-1}.

Preparation of 8. CsF (0.18 g, 1.2 mmol), which was dried over P_2O_5, was added to **7** (0.23 g, 0.24 mmol) in DMF (1.6 mL). After stirring at 35 °C for 36 h, EtOAc (40 mL) was added. Water (10 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organics were washed with brine (20 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes:MeOH, 50:40:10) yielded 0.12 g (59%) of the carboxylic acid, which was carried on in the synthetic sequence without further purification. ^{1}H NMR ($CDCl_3$): δ 9.37 (s, 1H), 7.72 (s, 1H), 7.61 (s, 1H), 7.46−7.22 (m, 9H), 7.03 (s, 1H), 6.83 (d, 4H, J = 8 Hz), 6.45 (dd, 1H, J = 5, 9 Hz), 5.54 (s, 2H), 5.38 (d, 1H, J = 6 Hz), 4.23 (s, 1H), 4.14−4.06 (m, 2H), 3.93 (s, 3H), 3.76 (s, 6H), 3.52 (m, 2H), 2.61−2.12 (m, 6H), 1.36 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 179, 164.24, 158.8, 154.22, 153.93, 150.55, 147.66, 144.1, 139.5, 135.49, 135.08, 135.13, 130.05, 128.07, 127.24, 126.15, 113.33, 111.68, 110.1, 109.69, 87.3, 84.37, 83.83, 79.3, 68.23, 66.72, 63.72, 56.55, 55.22, 37.97, 30.21, 24.04, 11.55. IR (film): 3178, 3006, 2933, 1748, 1699, 1652, 1608, 1580, 1558, 1522, 1509, 1445, 1371, 1252, 980, 829, 791, 648 cm^{-1}.

2,4,5-Trichlorophenol (53 mg, 0.27 mmol) was added to the above carboxylic acid (0.15 g, 0.18 mmol) in CH_2Cl_2 (1 mL). DCC (55 mg, 0.27 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise to the mixture. After stirring for 12 h at 25 °C, the reaction mixture was diluted with CH_2Cl_2 (40 mL) and H_2O (10 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL). The combined organics were washed with brine (20 mL) and dried over $MgSO_4$. Flash chromatography (EtOAc:hexanes, 3:2) yielded 0.14 g (78%) of **8**. Mp: 97 °C. ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H), 7.74 (s, 1H), 7.59 (s, 1H), 7.53 (s, 1H), 7.37−7.23 (m, 10H), 7.05 (s, 1H), 6.83 (d, 4H, J = 8 Hz), 6.49 (dd, 1H, J = 5, 9 Hz), 5.56 (s, 2H), 5.39 (d, 1H, J = 5 Hz), 4.22−4.17 (m, 3H), 3.97 (s, 3H), 3.53 (s, 6H), 3.53−3.43 (m, 2H), 2.88 (t, 2H, J = 7 Hz), 2.58−2.21 (m, 4H), 1.36 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 207.95, 169.17, 163.20, 158.63, 154.04, 153.763, 150.55, 147.34, 145.53, 143.91, 139.24, 135.1, 134.84, 131.31, 130.86, 130.47, 129.86, 127.89, 127.11, 126.25, 125.88, 125.12, 113.16, 111.58, 109.83, 109.51, 87.14, 84.11, 83.65, 79.25, 67.85, 66.56, 63.57, 56.45, 55.08, 37.72, 30.19, 23.97, 11.41. IR (film): 3064, 2932, 1751, 1690, 1608, 1580, 1559, 1522, 1509, 1463, 1329, 1278, 1219, 1154, 1081, 956, 910, 754, 730, 702, 647 cm^{-1}. HRMS FAB (M^+): calcd 1033.1994, found 1033.2004.

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Supporting Information Available: ^{1}H NMR spectra of compounds **7**, **8**, **9**, and **11** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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