Inverse Phosphotriester DNA Synthesis Using Photochemically-Removable Dimethoxybenzoin Phosphate Protecting Groups

Michael C. Pirrung,*[,1] Lara Fallon, David C. Lever, and Steven W. Shuey

Department of Chemistry, P. M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708-0346

Received August 31, 1995 (Revised Manuscript Received December 13, 1995[⊗]*)*

A method has been developed to prepare short DNA sequences using light to deprotect a nucleoside 3′-phosphotriester, generating a phosphodiester useful for coupling with a free 5′-OH-nucleotide. The dimethoxybenzoin group is used as the photochemically-removable protecting group for the 3′-phosphate. Cyanoethyl is most effective as the second protecting group on the phosphodiester. Because the method is directed at the preparation and use of the DNA sequences while still bound to the support, allyl and allyloxycarbonyl protecting groups are used for the nitrogenous bases since, based on the work of Hayakawa and Noyori, they can be removed without cleaving the DNA from the support. Two simple trinucleotides have been prepared in solution using this method. It has been demonstrated that the photochemical deprotection conditions do not lead to the formation of cyclobutane dimers from adjacent T residues.

Introduction

The collection of the complete sequence of human DNA (Human Genome Project)[2] and its use once it is available will require powerful new techniques in genetic analysis. One of the most widely-discussed technologies for this purpose is based on "DNA chips", miniaturized arrays of diverse DNA sequences that can be used to detect complementary sequences in unknown nucleic acid samples. The preparation of DNA arrays can be accomplished by robotic immobilization of pre-synthesized oligonucleotides[3,4] or by *in situ* synthesis.[5] The latter provides an advantage in preparing arrays of thousands of oligonucleotides because the principles of combinatorial chemistry[6] can be used to prepare sequences in parallel in relatively few chemical steps. Miniaturization of DNA arrays furthermore makes the manipulation of complete libraries of thousands or millions of sequences physically convenient and minimizes dilution of the DNA sample, providing a high concentration and therefore more effective binding kinetics. The primary means to prepare miniaturized arrays of polymeric molecules is light-directed synthesis.[7] Development of light-based versions[8,9] of the traditional phosphoramidite DNA synthesis method[10] that can be used for the preparation of DNA arrays has recently been reported.

The key to adapting a solid-phase synthesis method to the light-directed format is a photochemically-removable protecting group that is bound to the surface. When this work was begun, effective photochemically-removable alcohol protecting groups that could be used to replace a conventional dimethoxytrityl (DMTr) 5′-protecting group were not well-known, though we have subsequently developed several.[11] We instead considered an approach involving two significant deviations from standard practice in oligonucleotide synthesis. By attaching the 5′-hydroxyl of the DNA chain to the solid support, a photoremovable phosphate protecting group[12] could be used for a 3′-phosphate in the phosphotriester[13] coupling method (Figure 1). This format requires activation of a 3′-phosphodiester bound to the support, but still permits the reaction of an incoming 5′-hydroxyl with a 3′-activated phosphate and has been reported.[14] This inverse 5′ → 3′ synthesis permits a photoremovable group for the phosphate rather than the hydroxyl to be used. The tethering of the DNA to the support through the 5′ end also offers the opportunity for enzymatic reactions at free 3′ hydroxyls of the resulting short oligonucleotides.

One concern with the use of oligonucleotide libraries for genetic analysis is the low melting temperatures of short oligonucleotides and possible mishybridization because of the short recognition sequence and end-fraying effects.[15] Lehrach has addressed these issues through

⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1996.

(1) Fellow of the John Simon Guggenheim Memorial Foundation, 1994−95.

(2) Yager, T. D.; Zewert, T. E.; Hood, L. E. *Acc. Chem. Res.* **1994**, *27*, 94−100.

(3) Livshits, M. A.; Ivanov, I. B.; Mirzabekov, A. D.; Florentiev, V. A. *Mol. Biol. (Moscow)* **1992**, *26*, 1298−1313. Pevzner, P. A.; Lysov, Yu. P.; Khrapko, K. R.; Belyavskii, A. V.; Florent'ev, V. L.; Mirzabekov, A. D. *J. Biomol. Struct. Dyn.* **1991**, *9*, 399−410. Mirzabekov, A. D. *Bioorg. Khim.* **1992**, *18*, 1361−74. Eggers, M. D.; Hogan, M. E.; Reich, R. K.; Lamture, J. B.; Beattie, K. L.; Hollis, M. A.; Ehrlich, D. J.; Kosicki, B. B.; Shumaker, J. M.; et al. *Proc. SPIE-Int. Soc. Opt. Eng.* **1993**, 1891 (Proceedings of Advances in DNA Sequencing Technology, 1993), 113−26.

(4) Shumaker, J. M.; Metspalu, A.; Caskey, C. T. *Hum. Mutat.* **1996**, in press.

(5) Southern, E. M.; Maskos, U. *Nucleic Acids Res.* **1993**, *21*, 4663−9. Southern, E. M.; Maskos, U. *Nucleic Acids Res.* **1993**, *21*, 2269−70. Southern, E. M.; Maskos, U. *Nucleic Acids Res.* **1993**, *21*, 2267−8. Southern, E. M.; Maskos, U. *Nucleic Acids Res.* **1992**, *20*, 1675−8. Southern, E. M.; Maskos, U. *Nucleic Acids Res.* **1992**, *20*, 1679−84. Matson, R. S.; Rampal, J. B.; Coassin, P. J. *Anal. Biochem.* **1994**, *217*, 306−10.

(6) Pirrung, M. C. *Chemtracts: Org. Chem.* **1995**, *8*, 3−8.

(7) Pirrung, M. C.; Read, J. L.; Fodor, S. P. A; Stryer, L. US Patent, 5,143,854, Sept 1, 1992. Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Liu, A. T.; Solas, D. *Science* **1991**, *251*, 767.

(8) Pirrung, M. C.; Bradley, J.-C. *J. Org. Chem.* **1995**, *60*, 6270.

(9) Pease, A. C.; Solas, D.; Sullivan, E. J.; Cronin, M. T.; Holmes, C. P.; Fodor, S. P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5022−5026.

(10) Caruthers, M. H. *Science* **1985**, *230*, 281.

(11) Pirrung, M. C.; Lee, Y. R. *J. Org. Chem.* **1993**, *58*, 6961. Pirrung, M. C.; Bradley, J.-C. *J. Org. Chem.* **1995**, *59*, 1116.

(12) Pirrung, M. C.; Shuey, S. W. *J. Org. Chem.* **1994**, *59*, 3890 and references cited therein.

(13) Gait, M. J. *Oligonucleotide Synthesis*; IRL Press: Washington, DC, 1984.

(14) Pochet, S.; Huynh-Dinh, T.; Igolen, J. *Tetrahedron* **1987**, *43*, 3481−90. Gorn, V. V.; Zarytova, V. F.; Yarmolinskaya, E. V. *Bioorg. Khim.* **1983**, *9*, 1511−7.

0022-3263/96/1961-2129$12.00/0 © 1996 American Chemical Society

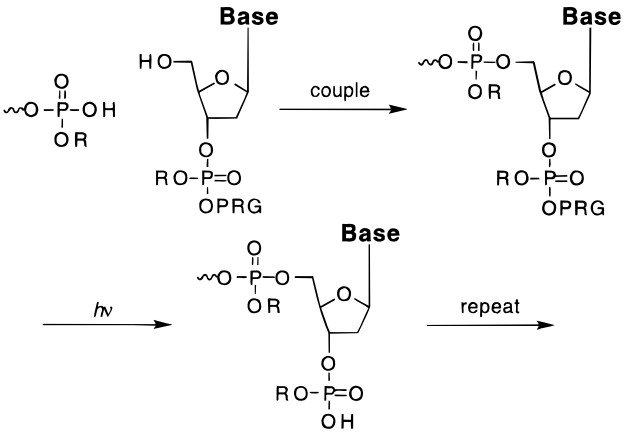


Figure 1.

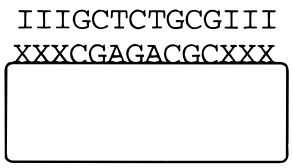


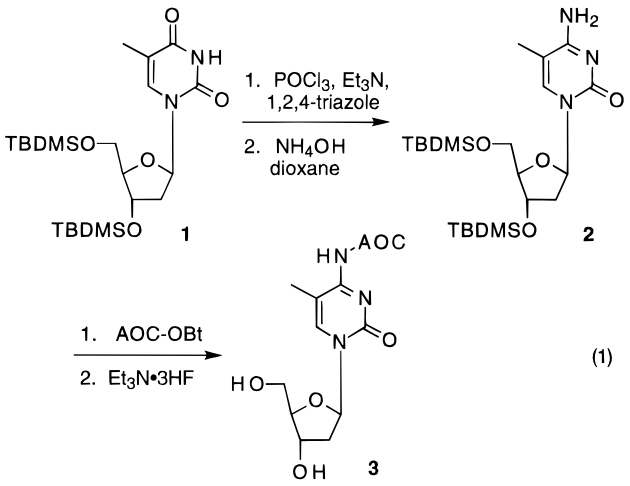
Figure 2.

the use of 5-methylcytidine (5MC), which increases discrimination between target and nontarget DNA[16] in probes used to map the *Herpes simplex* genome by hybridization.[17] The use of inosine as a "universal base"[18] flanking the analyzing sequence of the probe can increase hybrid stability[19] without increasing the recognition sequence, the length of the probes, and therefore the length of the synthesis. It also places mismatches that would otherwise be located at the end of a hybridization probe in a duplex region where the thermodynamic cost will be greater (Figure 2).

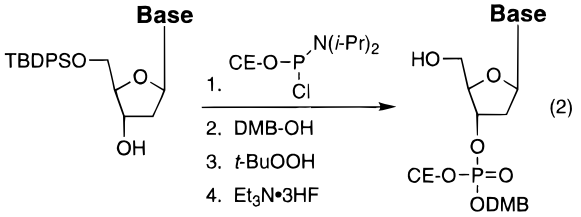
This report focuses on an inverse 5′ → 3′ photochemical phosphotriester DNA synthesis method using our previously-developed dimethoxybenzoinphosphate protecting group, including the four natural bases and the modified bases inosine and 5-methylcytidine. It also exploits the allyloxycarbonyl protecting group that has previously been developed by Hayakawa and Noyori[20] that can be removed without cleaving the DNA from the support on which it is prepared.

Results

The first need was a source of nucleoside monomers deoxyadenosine, deoxycytidine, and 5-methyldeoxycytidine with allyloxycarbonyl (AOC) protection on their exocyclic amino groups. Allyl ether protection of O6 (required to prevent its activation by the condensing agent) and AOC protection of the exocyclic amino group of deoxyguanine are also required (Chart 1). While procedures to prepare these or very similar compounds are available in the literature, difficulties were encountered in reproducing some of these methods. Modification of the routes to include in most cases the protection of the 5′ alcohol with *tert*-butyldiphenylsilyl (TBDPS) eventually provided pathways that were reproducible and provided 5−10 g quantities of material. These routes relied on the deprotection of the 5′-O-TBDPS group (and other silyl groups) using our recently-described triethylamine trihydrofluoride procedure.[21] New protocols for the preparation of protected A, G, and C are provided in the Experimental Section. The synthesis of 5-methyldeoxycytidine is based on a synthetic route to 4-substituted thymidine nucleosides through formation of a 4-triazolothymidine intermediate (eq 1).[22] *tert*-Butyldimethylsilyl (TBDMS)-protected thymidine (**1**) is obtained in 96% yield and converted to the 4-triazolo derivative by addition to a suspension of 1,2,4-triazole, triethylamine, and phosphorus oxychloride in acetonitrile. Conversion to the desired bis-TBDMS-protected 5-methyl-2′-deoxycytidine **2** can be effected in 94% yield (chromatographed) by stirring with 1:3 ammonium hydroxide/1,4-dioxane for 4 h. Protection of the amino function can be accomplished in 90% yield using the easily-prepared hydroxybenzotriazole active carbonate AOC-OBt. Desilylation gives **3**, which can be selectively protected at the 5′ alcohol with a TBDPS group.

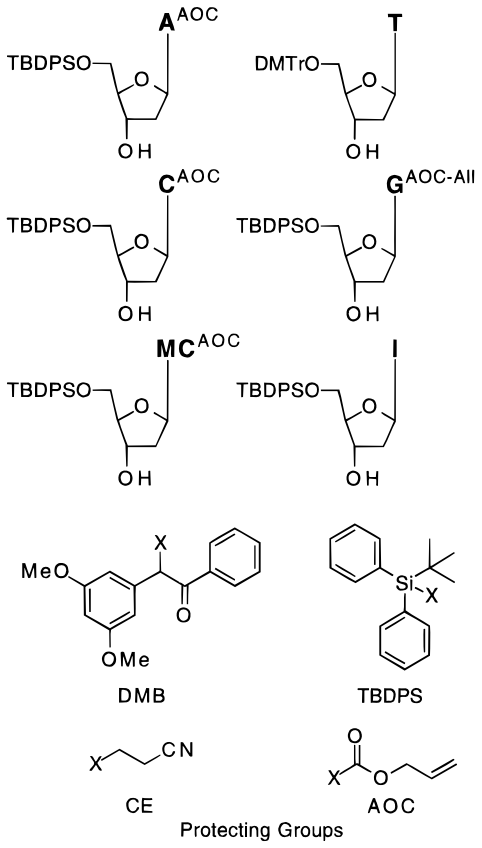


The preparation of the 3′-*O*-(cyanoethyl dimethoxybenzoin phosphate) esters of five of these specially-protected nucleosides can be accomplished by conversion first to the 3′-*O*-(cyanoethyl phosphoramidite) and then condensation with dimethoxybenzoin (eq 2). As expected, these reactions are exquisitely sensitive to moisture and



(15) Wood, W. I.; Gitscher, L. A.; Lasky, L. A.; Lawn, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1585−1588.
(16) Bugg, C. E.; Thomas, J. M. *Biopolymers* **1971**, *10*, 175−219.
(17) Hoheisel, J. D.; Craig, A. G.; Lehrach, H. *J. Biol. Chem.* **1990**, *265*, 16656−60.
(18) Crick, F. H. C. *J. Mol. Biol.* **1966**, *19*, 548−555. Ohtsuka, E.; Matsuki, S.; Ikehara, M.; Takahasi, Y.; Matsubara, K. *J. Biol. Chem.* **1985**, *260*, 2605−2608.
(19) Martin, F. H.; Castro, M. M.; Aboul-ela, F.; Tinoco, I. *Nucl. Acids Res.* **1985**, *24*, 8927−8938.
(20) Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2400−2. Hayakawa, Y.; Kato, H.; Noyori, R. *Tetrahedron Lett.* **1985**, *26*, 6505−8. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691−1696.
(21) Pirrung, M. C.; Shuey, S. W.; Lever, D. C.; Fallon, L. *Bioorg. Med. Chem. Lett.* **1994,** *4*, 1345.
(22) Reese, C. B.; Skone, P. A. *J. Chem Soc. Perkin Trans. 1* **1984**, 1263−1271.

Chart 1



require azeotropic drying of the tetrazole, phosphoramidite, and benzoin. The sixth 3′-*O*-(cyanoethyl dimethoxybenzoin phosphate) ester, of T, is prepared from the condensation of the commercially-available T-phosphoramidite as was described in our earlier report on dimethoxybenzoin phosphates. The choice was made to use the racemic 3′,5′-dimethoxybenzoin based on the difficulty in obtaining the optically-active material on very large scale. Racemic 3′,5′-dimethoxybenzoin can be prepared by a fairly simple, two-step process and is crystalline, which facilitates its purification. The preparation is also amenable to scale up, and 25−30 g batches of this material have been made repeatedly in our laboratory. The only disadvantage in using racemic benzoin is the formation of four diastereomers of the desired mononucleotides instead of two (the phosphorus center is also stereogenic and under no stereochemical control). Since ^{1}H NMR is virtually useless for characterization and purity evaluation of these compounds even when only two diastereomers are present, the benefits of using chiral benzoin do not outweigh the disadvantages presented by the difficulty of its preparation.

Though they were not ultimately useful in coupling reactions, 3′-*O*-(allyl dimethoxybenzoin phosphate) esters of these six nucleosides were also prepared (eq 3). This

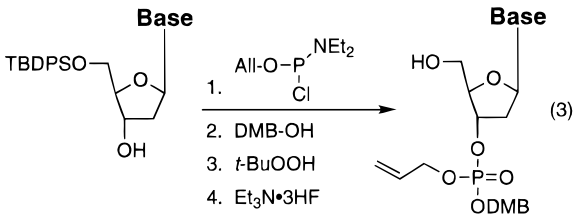


Table 1.

base	T	C	A	G	MeC	I
allyl/AOC	71	67	61	60	65	58
CE/AOC	74	65	68	89	50	40

work required an (allyloxy)chlorophosphine reagent that is not available commercially. (Allyloxy)dichlorophosphine could be prepared by slow addition of freshly-distilled allyl alcohol to neat, freshly-distilled PCl_3. Yields for this reaction were never very high, but it can be successfully conducted on a 1.0 mol scale, so that adequate quantities of the compound are available. Preparation of a chlorophosphoramidite analogous to the commercially-available cyanoethoxy(*N*,*N*-diisopropylamino)chlorophosphine proved more difficult. Allyloxy(*N*,*N*-diisopropylamino)chlorophosphine could be prepared by the addition of 2 equiv of diisopropylamine to (allyloxy)dichlorophosphine, but filtration of the amine hydrochloride was inevitably accompanied by large amounts of decomposition, and the product also could not be distilled. The removal of the amine hydrochloride could be obviated by the use of 1 equiv of (trimethylsilyl)-diisopropylamine instead of the free amine. The TMSCl thus produced could be removed by vacuum distillation, yielding material which, although not very pure, was nevertheless usable in the preparation of allyl nucleoside diisopropyl phosphoramidites. Yields were lower than in the preparation of the corresponding cyanoethyl derivatives. Another solution to this problem was to switch to the corresponding *N*,*N*-diethylamino derivatives. (Allyloxy)(*N*,*N*-diethylamino)chlorophosphine proved distillable under high vacuum and could be isolated as a clear, colorless liquid, pure by ^{1}H and ^{31}P NMR, and used to derivatize nucleosides. The resulting allyl diethyl phosphoramidites cannot be chromatographed, however, as they are sufficiently more reactive than the corresponding diisopropyl phosphoramidites in that they are hydrolyzed despite attempts to prevent it by adding triethylamine to the solvent or using triethylamine-pretreated silica gel. These phosphoramidites could be partially purified by precipitation from petroleum ether, however, and if the 5′ protecting group were dimethoxytrityl, the resulting product would be a foam. If the 5′ protecting group were TBDPS, the phosphoramidites would form thick gums rather than foams. These gums were generally used in subsequent coupling reactions without any purification, in the same flask in which they were prepared, since attempts to precipitate these materials inevitably led to large losses. The yields in the conversion of the cyanoethyl and allyl phosphoramidites to the 3′-*O*-(dimethoxybenzoin phosphate) derivatives are collected in Table 1.

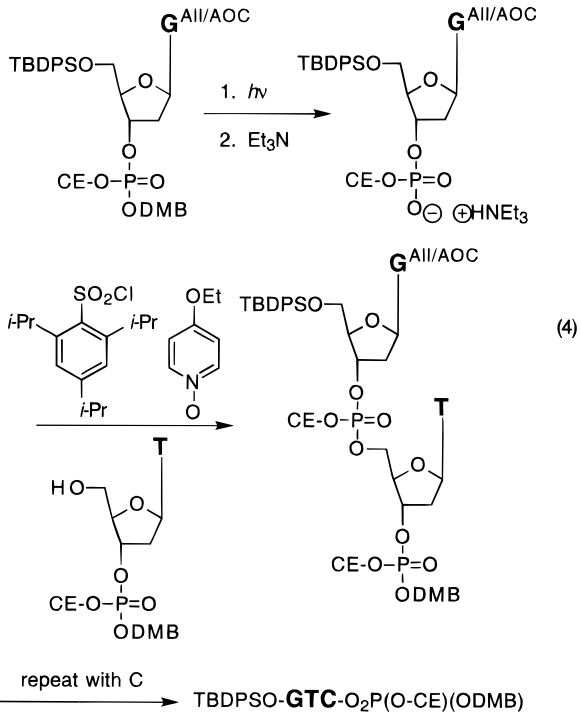
Four of these allyl/AOC-protected benzoin nucleotides were characterized by UV spectroscopy, and the half-lives for their photochemical deprotection (Rayonet photochemical reactor, 350 nm phosphor lamps) to the corresponding phosphate salts were measured. Table 2 lists the UV maxima, extinction coefficients, and half-lives (at the given concentration) for deprotection of the four monomers with a free 5′ hydroxyl group, as well as the 5′ acetylated thymidine and deoxycytidine derivatives. The values for the half-lives of deprotection are very similar to those found in our earlier work. The two deoxycytidine derivatives show the expected longer wavelength UV absorption, but the absorption at 360 nm (the wavelength for the deprotection step) is much less than with the benzoyl-protected deoxycytidine studied earlier

Table 2.

compound	concn (mM)	half-life, s	UV λ_{max} (nm)	extinction coeff
thymidine	4.65	109	276	20400
cytidine	3.50	105	263	29100
			316	10100
adenosine	3.17	95	278	25600
guanine	2.68	81	274	25400
5′-acetyl thymidine	2.66	82	279	17700
5′-acetyl cytidine	2.75	71	262	19700
			316	6960

and very close to zero. It is not thought that the longer wavelength absorption affects the rate of deprotection. The observed half-lives support this belief, since the values for the deoxycytidine derivatives are in line with the remaining nucleotides.

With these protected mononucleotides in hand, their coupling in solution to form short, protected oligonucleotides was investigated. In place of a 5′-link to the solid support, a silyl group was used which was stable to the conditions of deprotection and coupling. Dimethoxytrityl is not stable, since the phosphodiester is acidic enough to slowly remove it. The dimethoxybenzoin group was removed by irradiation and the phosphodiester converted to its triethylammonium salt (eq 4). The first coupling



(4)

reagent investigated was 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole/*N*-methylimidazole.[23] While it generally accomplishes phosphotriester couplings with 2-chlorophenyl esters in high yield, coupling is slow with cyanoethyl diesters.[24] In one instance, we prepared the T-T dinucleotide with cyanoethyl internucleotide protection (*vide infra*) in >95% yield by using a 10-fold molar excess of the phosphate salt over the alcohol, treating this coupling like a conventional 3′ → 5′ solid-phase experiment. (The excess of the phosphate salt could be removed by base washing of the reaction mixture.) We then turned to the improved phosphotriester coupling conditions developed by Efimov utilizing ethoxypyridine *N*-oxide and triisopropylbenzenesulfonyl chloride.[25] These reactions were generally successful with cyanoethyl diesters, but do not couple the extremely recalcitrant allyl diesters. In the initial example, the purified yield of G-T is 75%. Repetition of an irradiation/coupling cycle with the protected deoxycytidine mononucleotide gives the protected G-T-C trinucleotide in 69% yield.

To ensure that the photochemical deprotection reaction conditions do not result in the degradation of the resulting DNA strand by formation of T-T cyclobutane dimers, a T-T-T trinucleotide was prepared (eq 5), with 68%

(5)

coupling yield in the first step and 53% coupling yield in the second step. The resulting trinucleotide was degraded in acid and analyzed for the presence of dimeric heterocycle using the method of Greenberg.[26] None was seen.

Conclusion

Monomers and coupling chemistry have been provided for the photochemical preparation of DNA using phosphotriester chemistry. The two syntheses described reach the limit of oligomers that can be prepared using solution-phase synthesis because higher oligomers and intermediates leading to them demonstrate much-reduced solubility. This work completes a first step toward the development of a novel chemical combinatorial DNA synthesis methodology; solid-phase synthesis of single sequences will lay the groundwork for preparation of permutational libraries[27] of oligonucleotides on solid phase. This method will permit the synthesis of short oligonucleotide libraries attached to the support through their 5′-ends, which will leave their 3′-ends free for further manipulations.

Experimental Section

General. All starting materials were purchased from Aldrich Chemical Co. except 5′-*O*-(dimethoxytrityl) nucleosides, which were from Cruachem Inc. and were used without further purification. Dichloromethane, acetonitrile, benzene, and pyridine were freshly-distilled from calcium hydride. THF and diethyl ether were distilled from sodium/benzophenone ketyl. Triethylamine and diisopropylamine were distilled from sodium and stored under argon. All reactions were carried out under an atmosphere of argon in glassware which was oven-dried and/or flame-dried. Chromatography was performed using EM Reagents 0.042−0.063 mm grade silica gel (Kieselgel 60). NMR spectra were recorded at 121 MHz for phosphorus and 300 MHz for proton. Phosphorus shifts are reported referenced to 85% phosphoric acid, and proton shifts relative to internal $CHCl_3$. Elemental analyses were conducted by Atlantic Microlabs, Inc. High resolution mass spectrometry was performed at the University of North Carolina and the University of South Carolina.

***N*⁴-(Allyloxycarbonyl)-2′-deoxycytidine.** To a slurry of 14.3 g of 2′-deoxycytidine (twice azeotroped from 50 mL of pyridine) in 160 mL of pyridine, cooled in an ice bath, was

(23) Chandrasegaran, S.; Murakami, A.; Kan, L.-s. *J. Org. Chem.* **1984**, *49*, 4951−57. Zarytova, V. F.; Knorre, D. G. *Nucleic Acids Res.* **1984**, *12*, 2091−2110.

(24) 2-Chlorophenyl esters could not be used because of their lability. They undergo an intramolecular cyclization from the benzoin carbonyl group to form a cyclic phosphate that leads to hydrolysis of the triester.

(25) Efimov, A.; Chakhmakhcheva, O. G. *Chem. Scr.* **1985**, 26.

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

(27) Pirrung, M. C. *Chemtracts: Org. Chem.* **1993**, *6*, 88−91. Pirrung, M. C. *Chemtracts: Org. Chem.* **1995**, *8*, 5−12.

added 40 mL of TMSCl over 10 min After 15 min the cold bath was removed and stirring was continued for 1.5 h. Allyl 1-benzotriazolyl carbonate (Aldrich) (27.5 g) was added portionwise, and the residue was washed in with 40 mL of pyridine. After stirring overnight the mixture was poured into 300 mL of saturated $NaHCO_3$ and stirred for 2 h. Water (500 mL) was added, and the mixture was extracted with CH_2Cl_2 (150 mL) followed by 1:1 CH_2Cl_2/pyridine (4 × 250 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated, finally under high vacuum, to give a cream-colored, greasy solid, which was recrystallized from EtOAc to give 10.8 g (55%) of the product as a white powder. This material showed spectroscopic properties identical to those reported by Noyori. ^{1}H NMR ($CDCl_3$): δ 2.04 (m, 1H), 2.29 (m, 1H), 3.61 (m, 2H), 4.24 (m, 1H), 5.05 (m, 1H), 5.22-5.39 (m, 2H), 5.98 (m, 1H), 6.17 (t, 1H, J = 6.0), 7.05 (d, 1H, J = 8), 8.32 (d, 1H, J = 8). Anal. Calcd for $C_{13}H_{17}N_3O_6$: C, 50.16; H, 5.51; N, 13.50. Found: C, 50.16; H, 5.56; N, 13.50.

5′-*O*-(*tert*-Butyldiphenylsilyl)-*N*^{4}-(allyloxycarbonyl)-2′-deoxycytidine. To a solution of the above yellow grease in 150 mL of pyridine was added 11.0 mL of TBDPSCl via syringe over 10 min The reaction was stirred for 3 d, pyridine was removed under vacuum, and the residue was triturated with toluene to remove silanol. Flash chromatography on silica gel with a step gradient from 2:98−5:95−10:90 EtOH/CH_2Cl_2 gave 8.52 g (55% based on 2′-deoxycytidine) of product as a white foam. In addition ∼10 g (40%) of 3′,5′-bis-silylated material was obtained as a yellow grease. ^{1}H NMR ($CDCl_3$): δ 1.07 (9H, s), 2.19 (1H, m), 2.58 (1H, m), 3.84−4.05 (2H, m), 4.08 (1H, m), 4.49 (1H, br), 4.67 (2H, d, J = 5), 5.25−5.4 (2H, m), 5.92 (1H, m), 6.30 (1H, t, J = 6.5), 7.35−7.45 (6H, m), 7.6−7.75 (4H, m), 8.25 (1H, br). Anal. Calcd for $C_{29}H_{35}N_3O_6Si$: C, 63.36; H, 6.42; N, 7.64. Found: C, 63.31; H, 6.38; N, 7.70.

3′,5′-Bis(*tert*-Butyldimethylsilyl)-2′-deoxy-5-methylcytidine (2). To a solution of 10 g of 2′-deoxythymidine in DMF (80 mL) were added 12.37 g of imidazole and 13.69 g of *tert*-butyldimethylsilyl chloride. After stirring overnight, the solution was poured into ether (350 mL) and washed with saturated $NaHCO_3$ and brine and was dried ($MgSO_4$). The solution was concentrated to give 18.83 g of product as a white solid (96%). This material was used without further purification.

To a solution of 1,2,4-triazole (55.63 g) and triethylamine (120 mL) in CH_3CN (100 mL), cooled in an ice bath, was added dropwise phosphorus oxychloride (16.2 mL). The slurry was stirred for 30 min, and a solution of protected 2′-deoxythymidine (10 g) in CH_3CN was added over 15 min at 0 °C. After stirring overnight at room temperature, the mixture was poured into CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, and dried ($NaSO_4$). The solution was concentrated to give the triazolyl intermediate as an orange solid. The orange solid was immediately treated with 1:3 v/v NH_4OH/dioxane (400 mL) and was stirred at room temperature for 4 h. The mixture was poured into ether, washed with saturated $NaHCO_3$ and brine, and dried ($MgSO_4$). Flash chromatography on silica gel eluting with 5:95 EtOH:CH_2Cl_2 gave 9.4 g of the product as a white solid (97% based on protected thymidine). ^{1}H NMR ($CDCl_3$): δ 0.035, 0.083 (12H, 2 × s), 0.857, 0.895 (18H, 2 × s), 1.89 (3H, s), 1.95 (1H, m), 2.34 (1H, m), 3.68−3.89 (2H, m), 3.91 (1H, m), 4.34 (1H, m), 6.29 (1H, t, J = 6.3), 7.5 (1H, s)). Anal. Calcd for $C_{22}H_{43}N_3O_4Si_2$: C, 56.25; H, 9.23; N, 8.94. Found: C, 56.18; H, 9.25; N, 8.90.

***N*^{4}-(Allyloxycarbonyl)-2′-deoxy-5-methylcytidine.** To a solution of 4 g of **2** in pyridine (50 mL), cooled in an ice bath, was added allyl 1-benzotriazolyl carbonate (3.7 g). After stirring overnight, the pyridine was removed under vacuum and the resulting oil was taken up in CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, and dried ($NaSO_4$). The solution was concentrated to give the product as a yellow oil which could be used directly in the next reaction.

To a solution of the oil (5.3 g) in THF (50 mL) was added 6.9 mL of triethylamine trihydrofluoride. After stirring overnight, the solution was concentrated. Flash chromatography on silica gel eluting with 10:90 EtOH:CH_2Cl_2 gave 2.55 g of the product as a white solid (92% based upon **2**). ^{1}H NMR (acetone): δ 1.96 (s, 3H), 2.26 (1H, m), 2.37 (1H, m), 3.82−3.95 (2H, m), 3.96 (1H, m), 4.57 (1H, m), 4.63 (2H, d, J = 3), 5.20−5.38 (2H, m), 5.97 (1H, m), 6.15 (1H, t, J = 6.3). Anal. Calcd for $C_{14}H_{19}N_3O_6\cdot H_2O$: C, 48.98; H, 6.17; N, 12.24. Found: C, 49.24; H, 6.69; N, 12.30.

5′-*O*-(*tert*-Butyldiphenylsilyl)-*N*^{4}-(allyloxycarbonyl)-2′-deoxy-5-methylcytidine. To a solution of *N*^{4}-AOC-2′-deoxy-5-methylcytidine (0.746 g) in 20 mL of pyridine was added *tert*-butyldiphenylsilyl chloride (1.01 mL). After stirring overnight, the pyridine was removed under vacuum and the resulting oil was taken up in CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, and dried ($NaSO_4$). Flash chromatography on silica gel with a step gradient from 3:97−5:95 EtOH:CH_2Cl_2 gave the 1.23 g of the product as a white foam (95%). ^{1}H NMR ($CDCl_3$): δ 1.07 (9H, s), 1.69 (3H, s), 2.22 (1H, m), 2.43 (1H, m), 3.83−3.94 (2H, m), 3.99 (1H, s), 4.53 (1H, m), 4.64 (2H, d, J = 5.7), 5.26−5.38 (2H, m), 5.97 (1H, m), 6.32 (1H, t, J = 6.0), 7.36−7.49 (6H, m), 7.64 (4H, d, J = 7.5). Anal. Calcd for $C_{30}H_{37}N_3O_6Si\cdot{}^1/_2H_2O$: C, 62.91; H, 6.69; N, 7.33. Found: C, 63.10; H, 6.68; N, 7.08.

***N*^{6}-(Allyloxycarbonyl)-2′-deoxyadenosine.** To a solution of 7.0 g of 2′-deoxyadenosine (twice azeotroped from 40 mL of pyridine) in 100 mL of pyridine was added 10.0 g of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. After stirring overnight the solvent was removed under reduced pressure and the residue dissolved in 300 mL of CH_2Cl_2 and washed with saturated $NaHCO_3$ (2 × 150 mL) and brine (150 mL). The resulting solution was dried (Na_2SO_4) and concentrated to give a white foam that was used without purification.

To a solution of 4.0 g of 1*H*-tetrazole in 100 mL of dry THF, cooled in an ice bath, was added 8.75 mL of triethylamine, followed by 6.66 mL of allyl chloroformate dropwise over 30 min. After 15 min the cooling bath was removed and the reaction was stirred for 2 h. The resulting suspension was filtered through a glass frit, washed with 60 and 30 mL portions of THF, and concentrated to a volume of ∼25−30 mL. This solution was added rapidly to a solution of the above white foam in 100 mL of dry THF, and the reaction was heated at 70 °C overnight. The solvent was removed under reduced pressure, and the residue was dissolved in 300 mL of CH_2Cl_2 and washed with 200 mL portions of saturated $NaHCO_3$ and brine. The resulting solution was dried (Na_2SO_4) and concentrated to give a thick yellow oil, which was used directly.

To a solution of this oil in 300 mL of THF was added 18.2 mL of triethylamine trihydrofluoride, and the resulting solution was stirred for 2 d. The solvent was removed under reduced pressure, and the resulting brown tarry material was applied directly to a silica gel column and eluted with a step gradient of 5:95−10:90−35:65 EtOH/CH_2Cl_2 to give 6.59 g (71%) of the product as a white foam. This material showed spectroscopic properties identical to those reported by Noyori. ^{1}H NMR ($CDCl_3$): δ 2.33 (1H, m), 2.75 (1H, m), 3.49−3.69 (2H, m), 3.91 (1H, m), 4.42 (1H, m), 4.65 (2H, d, J = 5.7), 5.26−5.42 (2H, m), 5.9 (1H, m), 6.45 (1H, t, J = 6.3), 8.61 (1H, s), 8.64 (1H, s). HRMS (FAB, MH^+): Calcd for $C_{14}H_{18}N_5O_5$: 336.3291 Found: 336.1308.

5′-*O*-(*tert*-Butyldiphenylsilyl)-*N*^{6}-(alloxycarbonyl)-2′-deoxyadenosine. To a solution of 3.0 g of *N*^{6}-(allyloxycarbonyl)-2′-deoxyadenosine in 50 mL of pyridine was added 3.5 mL of TBDPSCl dropwise over 5 min. After stirring overnight the solvent was removed under reduced pressure and the residue was dissolved in 250 mL of CH_2Cl_2 and washed with 150 mL portions of saturated $NaHCO_3$ and brine. The resulting solution was dried (Na_2SO_4) and concentrated, and the residue chromatographed on silica gel with a step gradient of 5:95−10:90−20:80 EtOH/CH_2Cl_2 to give 3.9 g (76%) of product as a white foam. ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H), 2.55 (1H, m), 2.78 (1H, m), 3.81−3.96 (2H, m), 4.12 (1H, m), 4.75 (3H, br d), 5.21−5.42 (2H, m), 5.95 (1H, m), 6.48 (1H, t, J = 6.0), 7.3−7.45 (6H, m), 7.59−7.66 (4H, m), 8.15 (1H, s), 8.69 (1H, s). Anal. Calcd for $C_{30}H_{35}N_5O_5Si$: C, 62.80; H, 6.15; N, 12.21. Found: C, 62.86; H, 6.22; N, 12.22.

5′-*O*-(*tert*-Butyldiphenylsilyl)-*N*^{2}-(allyloxycarbonyl)-*O*^{6}-allyl-2′-deoxyguanosine. Application of Noyori's procedure for protection of bis(*tert*-butyldimethylsilyl)deoxygua-

nine[28] on a 15 g scale gave 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-N^2-(allyloxycarbonyl)-O^6-allyl-2′-deoxyguanine in 40% yield after several column chromatographies. To a solution of 5.2 g of this compound in 100 mL of THF was added 5.5 mL of triethylamine trihydrofluoride. The mixture was stirred overnight, solvent was removed under reduced pressure, and the residue was chromatographed on silica gel with 1:9 MeOH/$CHCl_3$ to give 2.9 g (88%) of slightly impure diol, which was used directly. To a solution of this material in 60 mL of pyridine was added 2.83 mL of TBDPSCl via syringe over 5 min. After stirring overnight, the solvent was removed under reduced pressure, and the residue was dissolved in 150 mL of EtOAc and washed with saturated $NaHCO_3$ (3 × 50 mL), saturated $CuSO_4$ (3 × 50 mL), and brine (75 mL). The resulting solution was dried (Na_2SO_4) and concentrated, and the residue was chromatographed on silica gel with 3:97 EtOH/CH_2Cl_2 to give 3.2 g (70%) of product as a white foam (61% for both steps). ^{1}H NMR: δ 1.03 (9H, m), 2.5−2.75 (2H, m), 3.79−3.92 (2H, m), 4.12 (m, 1H), 4.58 (2H, m), 4.8 (1H, br s), 5.05 (2H, m), 5.18−5.5 (4H, m), 5.9−6.0 (1H, m), 6.04−6.2 (1H, m), 6.6 (1H, m), 7.2−7.4 (6H, m), 7.6 (4H, m), 8.02 (1H, s). Anal. Calcd for $C_{33}H_{39}N_5O_6Si$: C, 62.93; H, 6.24; N, 11.12. Found: C, 62.86; H, 6.25; N, 11.11.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-deoxyinosine. To a solution of 1.0 g of 2′-deoxyinosine (Sigma) in 40 mL of pyridine was added TBDPSCl (1.24 mL), and the suspension was stirred overnight. Monitoring by showed TLC unreacted starting material, so another 0.5 mL of TBDPSCl was added and the solution was stirred for 4 h. The pyridine was removed under vacuum, and the resulting oil was taken up in CH_2Cl_2 and washed with saturated $NaHCO_3$, causing the product to precipitate as a white solid which was collected and washed to give 1.67 g of the desired compound (86%). ^{1}H NMR (d_6-DMSO): δ 0.95 (9H, s), 2.34 (1H, m), 2.72 (1H, m), 3.69−3.89 (2H, m), 3.96 (1H, m), 4.49 (1H, m), 6.33 (1H, t, J = 6.6), 7.33−7.45 (6H, m), 7.58 (4H, t, J = 5.4), 7.98 (1H, s), 8.21 (1H, s). Anal. Calcd for $C_{26}H_{30}N_4O_4Si\cdot{}^1/_2H_2O$: C, 62.50; H, 6.25; N, 11.21. Found: C, 62.88; H, 6.25; N, 11.14.

General Procedure for the Preparation of Phosphate Triester Building Blocks with Cyanoethyl Internucleotide Phosphate Protection and Allyloxycarbonyl and Allyl Base Protection. To a solution of the appropriately protected nucleoside (1.0 equiv) in dichloromethane (∼30 mL/gram of substrate) was added diisopropylethylamine (2.0 equiv), followed by a solution of cyanoethyl(*N*,*N*-diisopropylamino)chlorophosphine (1.6 equiv) in dichloromethane (∼10 mL/g) via cannula. After stirring for 1.5 h a portion of MeOH (∼1.5 mL) was added to quench excess phosphitylating reagent and stirring was continued for 15 min. The reaction mixture was then poured into 300 mL of EtOAc with 15 mL of Et_3N added (for 1−2 g scale reactions) and washed with 10% Na_2CO_3 (2 × 100 mL) and brine (2 × 100 mL). The resulting solution was dried (Na_2SO_4) and concentrated to give the phosphoramidite in nearly quantitative yield, which was checked for purity by ^{31}P NMR and used directly.

A mixture of the above phosphoramidite (1.0 equiv) and 3′,5′-dimethoxybenzoin (2.0 equiv) was thrice azeotroped from 40−50 mL of benzene. To the solid residue thus obtained was added rapidly via syringe 5.0 equiv of a freshly-prepared solution of 1*H*-tetrazole in acetonitrile (25 mg/mL) and the reaction swirled to effect full solution and let stand for 1−2 h. (The tetrazole solution was prepared by thrice azeotroping the required amount of 1*H*-tetrazole from benzene and dissolving in freshly distilled acetonitrile under Ar.) A stir bar and 1.8 equiv of a 3.0 M solution of *tert*-butyl hydroperoxide in isooctane were added, and the reaction mixture was stirred for 30−45 min and poured into 250 mL of EtOAc (for 1.5−3 g scale reactions) and washed with 150 mL portions of saturated $NaHCO_3$, 10% Na_2CO_3, and brine (twice). The resulting solution was dried (Na_2SO_4) and concentrated to give a yellow foam, which was checked by ^{31}P NMR and used in the next step without further purification.

The thymidine derivative was deprotected with 3% dichloroacetic acid in CH_2Cl_2. The remaining compounds were treated with 10.0 equiv of triethylamine trihydrofluoride in THF (∼50 mL/g of substrate) overnight, the solvent was removed under reduced pressure, and the residue was chromatographed directly on silica gel using ∼500 mL of 1:1 EtOAc/CH_2Cl_2 followed by a step gradient of 2:98−5:95−10:90 EtOH/CH_2Cl_2 to give the expected phosphate triesters in yields ranging from 50−75%.

AOC-Nucleoside-5′-TBDPS-3′-cyanoethyl-DMB-phosphates. 5′-*O*-TBDPS-O^6-allyl-*N*-(allyloxycarbonyl)deoxyguanine (1.0 g, 1.59 mmol) was azeotroped 3× from 5 mL of benzene and then dissolved in 10 mL of CH_2Cl_2. Diisopropylethylamine (0.60 mL, 3.32 mmol) was added, and the reaction mixture was cooled to 0 °C. Cyanoethoxy-(*N*,*N*-diisopropylamino)chlorophosphine (0.533 mL, 2.39 mmol) was added by syringe, and the reaction mixture was stirred under nitrogen for 1 h. It was poured into 50 mL of cold 5% bicarbonate solution and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4, and the solvent was removed to give the crude product. The products were checked by ^{31}P and ^{1}H NMR and taken on to the next step without further purification.

The phosphoramidite thus prepared and dimethoxybenzoin (0.866 g, 3.18 mmol) were charged to a flask and azeotroped 3× from benzene. A stock solution of rigorously dried tetrazole (17.84 mL, 6.36 mmol) was added by syringe, and the mixture was stirred for 1 h. TLC (98:2 CH_2Cl_2:EtOH) showed the reaction to be complete. The solvent was removed and the residue taken up in CH_2Cl_2. Washing with 5% bicarbonate and brine followed by drying and removal of the solvent left a clear yellow residue which after chromatography on silica with 98:2 CH_2Cl_2:EtOH gave 1.41g (89%) of the title compound as a white foam. The NMR was consistent with a mixture of four diastereomers: ^{31}P NMR showed only four lines; ^{1}H NMR is complex but shows the correct diagnostic resonances. Yields for the other compounds are shown in Table 1. Thymidine was done using DMTr as the 5′ protecting group. The deprotection of the 5′-O-TBDPS-nucleotides was performed using at least a 2-fold excess of $Et_3N\cdot3HF$, with stirring for 3−12 h followed by basic aqueous workup and chromatography using 95:5 CH_2Cl_2:EtOH.

N^4-(Allyloxycarbonyl)-2′-deoxycytidine-3′-*O*-(cyanoethyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR δ 2.34 (1H, m), 2.62 (2H, m), 2.85 (1H, m), 3.76 (6H, s), 3.80−4.20 (4H, m), 4.40 (1H, m), 4.70 (2H, m), 5.1 (1H, m), 5.26−5.44 (2H, m), 5.9−6.10 (1H, m), 6.20 (1H, m), 6.45 (1H, m), 6.60 (2H, m), 7.39−7.60 (3H, m), 7.90 (1H, m). Anal. Calcd for $C_{32}H_{35}N_4O_{12}P$: C, 55.01; H, 5.05; N, 8.02. Found: C, 55.36; H, 5.25; N, 7.88.

N^4-(Allyloxycarbonyl)-2′-deoxy-5-methylcytidine-3′-*O*-(cyanoethyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR ($CDCl_3$): δ 1.99 (3H, br d), 2.38 (1H, m), 2.62 (2H, m), 2.88 (1H, m), 3.76 (6H, s), 3.9−4.12 (4H, m), 4.30 (1H, m), 4.66 (2H, br d), 5.22−5.41 (2H, m), 5.9−6.1 (1H, m), 6.44 (1H, br s), 6.59 (2H, m), 7.39−7.46 (2H, m), 7.51−7.59 (1H, m), 7.89 (2H, br d). Anal. Calcd for $C_{33}H_{37}N_4O_{12}P$: C, 55.62; H, 5.23; N, 7.86. Found: C, 55.76; H, 5.28; N, 7.78.

N^6-(Allyloxycarbonyl)-2′-deoxyadenosine-3′-*O*-(cyanoethyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR: δ 2.45 (1H, m), 2.62 (2H, m), 2.9 (1H, m), 3.76 (6H, m), 3.95−4.15 (4H, m), 4.45 (1H, m), 4.77 (2H, m), 5.28−5.65 (3H, m), 6.0 (1H, m), 6.2 (1H, m), 6.35−6.50 (2H, m), 6.60 (2H, m), 7.38−7.6 (3H, m), 7.90 (2H, m), 8.7 (1H, s). Anal. Calcd for $C_{33}H_{37}N_6O_{12}P$: C, 53.51; H, 5.04; N, 11.35. Found: C, 53.76; H, 5.10; N, 11.30.

N^2-)Allyloxycarbonyl)-O^6-allyl-2′-deoxyguanine-3′-*O*-(cyanoethyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR: δ 2.6 (3H, m), 2.89 (1H, m), 3.75 (6H, m), 3.9−4.25 (3H, m), 4.45 (2H, m), 4.68 (2H, m), 5.09 (2H, m), 5.2−5.6 (5H, m), 5.95 (1H, m), 6.12 (2H, m), 6.29 (1H, m), 6.43 (1H, m), 6.60 (2H, m), 7.39−7.6 (3H, m), 7.89 (3H, m). Anal. HRMS (FAB, MH^+) Calcd for $C_{36}H_{39}N_6O_{12}P$: 778.7081. Found: 779.2442.

2′-Deoxyinosine-3′-*O*-(cyanoethyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR: δ 2.62 (2H, m), 2.90 (1H, m), 3.38 (1H, m), 3.75 (6H, m), 3.92 (1H, m), 4.05−4.50 (4H, m), 5.45 (1H, m), 6.12 (1H, m), 6.30 (1H, m), 6.41 (1H, m), 6.61 (2H, m), 7.30−7.55 (3H, m), 7.8−8.0 (3H, m), 8.15 (1H, m).

(28) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799−3807.

Anal. HRMS (FAB, MH+) Calcd for $C_{29}H_{30}N_5O_{10}P$: 639.6450. Found: 640.1807.

2′-Thymidine-3′-*O*-(cyanoethyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR: δ 1.95 (3H, m), 2.4 (1H, m), 2.65 (2H, m), 2.95 (1H, m), 3.78 (6H, m), 3.98−4.2 (4H, m), 4.30 (1H, m), 4.50 (1H, m), 5.4 (1H, m), 6.1 (1H, m), 6.28 (1H, m), 6.5 (1H, m), 6.65 (2H, m), 7.4−7.65 (3H, m), 7.95 (2H, m), 8.5 (1H, br s). Anal. Calcd for $C_{16}H_{32}N_3O_{11}P\cdot{}^1/_2H_2O$: C, 54.54; H, 5.21; N, 6.58. Found: C, 54.44; H, 5.21; N, 6.58.

(Allyloxy)dichlorophosphine. To 87 mL of freshly distilled phosphorus trichloride, cooled in an ice bath, was added 68 mL of freshly distilled allyl alcohol dropwise, with stirring, over 8 h. After stirring overnight, house vacuum was applied for 2 h to remove most of the HCl, and then Ar was bubbled through the solution to remove the last vestiges of HCl. The product was obtained by distillation through a short path still at 0.1 torr into a dry ice-cooled receiver. In this way the explosive decomposition reported to result from distillation at higher pressures was avoided. Great care still must be taken to discontinue the distillation as soon as the temperature begins to rise, or as soon as the pot residue turns orange, in order to avoid a vigorous exotherm. The yield is 95 g (60%) of colorless liquid, with properties consistent with those reported in the literature.[29] ^{31}P NMR: δ 178.5

(Allyloxy)(*N*,*N*-diethylamino)chlorophosphine. To 32 g of (allyloxy)dichlorophosphine dissolved in 150 mL of dry Et_2O was added dropwise 42 mL of diethylamine, with vigorous stirring, over 3−4 h. The resulting slurry was filtered through a Schlenck funnel under Ar and washed with 50 mL of additional Et_2O. Solvent was removed by distillation under house vacuum, and the residue was distilled at 0.04 torr (bp 50−58 °C) to give 20.55 g (52%) of the product as a colorless liquid. ^{31}P NMR ($CDCl_3$): δ 181.6. ^{1}H NMR ($CDCl_3$): δ 1.15 (6H, m), 3.25−3.40 (4H, m), 4.35−4.45 (2H, m), 5.18−5.38 (2H, m), 5.9−6.05 (1H, m).

General Procedure for the Preparation of Phosphate Triester Building Blocks with Allyl Internucleotide Phosphate Protection and Allyloxycarbonyl Base Protection. To a solution of protected nucleoside (1.0 equiv) in dichloromethane (∼30 mL/g) was added diisopropylethylamine (2.0 equiv), followed by a solution of (allyloxy)(*N*,*N*-diethylamino)chlorophosphine (1.6 equiv) in dichloromethane (∼10 mL/g) via cannula. After stirring for 1.5 h a portion of MeOH (∼1.5 mL) was added to quench excess phosphitylating reagent and stirring was continued for 15 min. The reaction mixture was then poured into 300 mL of EtOAc with 15 mL of Et_3N added (for 1−2 g scale reactions) and washed with 10% Na_2CO_3 (2 × 100 mL) and brine (2 × 100 mL). The resulting solution was dried (Na_2SO_4) and concentrated to give in nearly quantitative yield the phosphoramidites, which were checked for purity by ^{31}P NMR and used directly.

A mixture of the above phosphoramidite (1.0 equiv) and 3′,5′-dimethoxybenzoin (2.0 equiv) was thrice azeotroped from 40−50 mL of benzene. To the solid residue thus obtained was added rapidly via syringe 5.0 eq of a freshly prepared solution of 1*H*-tetrazole in acetonitrile (25 mg/mL), and the reaction swirled to effect full solution and allowed to stand for 1−2 h. A stir bar and 1.8 equiv of a 3.0 M solution of *tert*-butylhydroperoxide in isooctane were added, and the reaction mixture was stirred for 30−45 min. It was poured into 250 mL of EtOAc (for 1.5−3 g scale reactions) and washed with 150 mL portions of saturated $NaHCO_3$, 10% Na_2CO_3, and brine (twice). The resulting solution was dried (Na_2SO_4) and concentrated to give a yellow foam, which was checked by ^{31}P NMR and used in the next step without further purification.

The thymidine derivative was deprotected with 3% dichloroacetic acid in CH_2Cl_2 as reported below for the compounds with acyl base protection. The remaining compounds were treated with 10.0 equiv of triethylamine trihydrofluoride in THF (∼50 mL/g of substrate) overnight, the solvent was removed under reduced pressure, and the residue was chromatographed directly on silica gel using ∼500 mL of 1:1 EtOAc/CH_2Cl_2 followed by a step gradient of 2:98−5:95−10:90 EtOH/CH_2Cl_2 to give the expected phosphate triesters in yields ranging from 50−75%.

***N*^{4}-(Allyloxycarbonyl)-2′-deoxycytidine-3′-*O*-(allyl 3″,5″-dimethoxybenzoin phosphate).** ^{1}H NMR ($CDCl_3$): δ 2.25 (m, 1H), 2.45 (m, 1H), 3.69 (s, 6H), 3.77−4.4 (m, 4H), 4.62 (m, 4H), 5.1−5.4 (m, 4H), 5.75 (m, 1H), 5.89 (m, 1H), 6.1 (m, 1H), 6.2 (m, 1H), 6.37 (m, 1H), 6.54 (m, 2H), 7.35 (m, 2H), 7.48 (m, 1H), 7.86 (m, 2H), 8.35 (m, 2H). HRMS (FAB, MH$^+$) Calcd for $C_{32}H_{36}N_3O_{12}P$: 685.6288. Found: 686.2101.

***N*^{4}-(Allyloxycarbonyl)-2′-deoxy-5-methylcytidine-3′-*O*-(allyl 3″,5″-dimethoxybenzoin phosphate).** ^{1}H NMR ($CDCl_3$): δ 1.98 (m, 3H), 2.37 (m, 1H), 2.48 (m, 1H), 3.74 (m, 6H), 3.85−4.08 (m, 4H), 4.22-4.45 (m, 4H), 5.2−5.4 (m, 4H), 5.72−5.88 (m, 2H), 5.9−6.1 (m, 1H), 6.21 (m, 1H), 6.41 (m, 1H), 6.55−6.6 (m, 2H), 7.40 (m, 2H), 7.54 (m, 1H), 7.74 (s, 1H), 7.89 (m, 2H). HRMS (FAB, MH$^+$) Calcd for $C_{33}H_{38}N_3O_{12}P$: 699.6532. Found: 700.2267.

***N*^{6}-(Allyloxycarbonyl)-2′-deoxyadenosine-3′-*O*-(allyl 3″,5″-dimethoxybenzoin phosphate).** ^{1}H NMR ($CDCl_3$): δ 2.35 (m, 1H), 2.6 (m, 1H), 3.81 (s, 6H), 3.76−4.7 (m, 4H), 4.75 (m, 4H), 5.1−5.47 (m, 4H), 5.8−6.03 (m, 2H), 6.12 (m, 1H), 6.29−6.4 (m, 2H), 6.58 (m, 2H), 7.35 (m, 2H), 7.48 (m, 1H), 7.87 (m, 2H), 8.08 (s, 1H), 8.68 (m, 1H). HRMS (FAB, MH$^+$) Calcd for $C_{30}H_{36}N_5O_{11}P$: 709.6671. Found 710.2217.

***N*^{2}-(Allyloxycarbonyl)-*O*^{6}-allyl-2′-deoxyguanine-3′-*O*-(allyl 3″,5″-dimethoxybenzoin phosphate).** ^{1}H NMR ($CDCl_3$): δ 2.3 (m, 1H), 2.6 (m, 1H), 3.68 (s, 6H), 3.7−4.45 (m, 6H), 4.62 (m, 4H), 5.05 (m, 2H), 5.1−5.5 (m, 6H), 5.7−6.15 (m, 3H), 6.2 (m, 1H), 6.36 (m, 1H), 6.57 (m, 2H), 7.3−7.5 (m, 3H), 7.78−7.92 (m, 3H). HRMS (FAB, MH$^+$) Calcd for $C_{36}H_{40}N_5O_{12}P$: 765.7203. Found: 766.2520.

2′-Deoxyinosine-3′-*O*-(allyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR ($CDCl_3$): δ 2.42 (m, 1H), 2.8 (m, 1H), 3.74 (s, 6H), 4.45 (m, 3H), 4.7 (m, 2H), 5.1−5.35 (m, 2H), 5.45 (m, 1H), 5.75−6.05 (m, 1H), 6.1 (m, 1H), 6.3 (m, 1H), 6.42 (m, 1H), 6.63 (m, 2H), 7.4 (m, 2H), 7.52 (m, 1H), 7.91 (m, 2H), 8.24 (m, 2H). HRMS (FAB, MH$^+$) Calcd for $C_{29}H_{31}N_4O_{10}P$: 626.5651. Found: 627.1848.

Thymidine-3′-*O*-(allyl 3″,5″-dimethoxybenzoin phosphate). ^{1}H NMR ($CDCl_3$): δ 2.04 (s, 3H), 2.32(m, 1H), 2.50 (m, 1H), 3.72 (s, 6H), 3.8−4.4 (m, 4H), 4.64 (m, 1H), 4.9 (m, 1H), 5.15−5.4 (m, 2H), 5.7−5.92 (m, 1H), 6.19 (m, 1H), 6.4 (s, 1H), 6.58 (m, 2H), 7.37 (m, 2H), 7.52 (m, 1H), 7.9 (m, 2H), 9.0 (br, 1H). HRMS (FAB, MH$^+$) Calcd for $C_{29}H_{33}N_2O_{11}P$: 616.5667. Found: 617.1911.

Coupling Reactions To Prepare Cyanoethyl Phosphotriesters. G-T Dinucleotide. 5′-*O*-TBDPS-AOC-*O*^{6}-allyl dG-3′-CE DMB phosphate (300 mg, 0.295 mmol) was dissolved in 100 mL of benzene with 1 mL of pyridine. The solution was degassed by bubbling with Ar and irradiated for 30 min in a Rayonet photochemical reactor using 350 nm phosphor lamps. The solvent was removed. Column chromatography on triethylamine-treated silica gel using 90:9:1 CH_2Cl_2:EtOH:Et_3N gave the desired deprotected product plus a great deal of $Et_3N\cdot$HCl, which was removed by precipitation from ethyl acetate. ^{31}P NMR showed one clean singlet, and this material was taken to the next step. The dG salt, 5′-OH T-DMB-CE phosphate (0.278 g, 0.443 mmol) and 4-ethoxypyridine *N*-oxide (0.205 g, 1.48 mmol) were thrice azeotroped from pyridine and taken up in 12 mL of dichloroethane. Triisopropylbenzenesulfonyl chloride was added (0.183 g, 0.590 mmol), and the mixture was stirred under Ar overnight. TLC revealed three spots, the 5′-sulfonate of the T monomer, the desired product, and excess T monomer. The solvent was removed. Column chromatography on silica gel using 95:5 CH_2Cl_2:EtOH gave 305 mg (75%) of a white foam. Its spectra were consistent with the title compound. HRMS (FAB, MH$^+$) Calcd for $C_{65}H_{73}N_9O_{19}P_2Si$: 1374.3800. Found: 1374.4346.

G-T-C Trinucleotide. The above dinucleotide (240 mg, 0.175 mmol) was dissolved in 100 mL of benzene and 1 mL of pyridine. The solution was degassed by bubbling with Ar and irradiated for 30 min in a Rayonet photochemical reactor using 350 nm phosphor lamps. Triethylamine (1 mL) was added, the solvent was removed, and this material was taken directly to the next step without purification. This salt, 5′-OH-dC-DMB-CE-phosphate (0.35 mmol) and 4-ethoxypyridine *N*-oxide

(29) *J. Gen. Chem. (USSR)* **1936**, *6*, 1198−1202.

were azeotroped thrice from pyridine and then taken up in 10 mL of dichloroethane. Triisopropylbenzenesulfonyl chloride (106 mg, 0.350 mmol) was added and the mixture stirred overnight. Column chromatography on silica gel using 95:5 CH_2Cl_2:EtOH gave 219 mg (69%) of a white foam. HRMS (FAB, MH^+) Calcd for $C_{65}H_{77}N_{13}O_{24}P_3Si$: 1545.4091. Found: 1546.4309.

T-T-T Trinucleotide. The procedure for the G-T dinucleotide was applied to 5′-TBDPS T-DMB-CE-phosphate and 5′-OH-T-DMB-CE-phosphate to give 262 mg (68%) of the T-T dinucleotide. This compound (120 mg) was dissolved in 40 mL of benzene and 1 mL of pyridine and degassed by bubbling with Ar. The solution was irradiated for 40 min as described above, followed by the addition of 1 mL of Et_3N. The solution was concentrated and 70 mg of the desired salt was isolated by flash chromatography on Et_3N-treated silica gel, eluting with a step gradient of 11:89−20:80−50:50 EtOH:CH_2Cl_2. The salt, 5′-OH-T-DMB-CE (62 mg), and 4-ethoxypyridine *N*-oxide (45 mg) were azeotroped thrice from pyridine and then dissolved in 10 mL of dichloroethane. Triisopropylbenzenesulfonyl chloride (40 mg) was added, and the reaction mixture was stirred overnight. The solution was concentrated. Flash chromatography on silica gel using a step gradient of 4:96−6:94−10:90 EtOH:CH_2Cl_2 gave 50 mg of the desired trinucleotide (53% based upon dinucleotide). ^{31}P NMR ($CDCl_3$): δ −3.89 to −2.51. HRMS (FAB, MH^+) Calcd for $C_{71}H_{82}N_9O_{25}P_3Si$: 1582.4878. Found. 1581.4332.

Acknowledgment. Financial support by a Human Genome Distinguished Postdoctoral Fellowship (to D.C.L.), sponsored by the U. S. Department of Energy, Office of Health and Environmental Research, and administered by the Oak Ridge Institute for Science and Education, and the Department of Energy Human Genome Program (DE-FG05-92ER61388) is greatly appreciated.

Supporting Information Available: NMR spectra of compounds characterized by HRMS: 3′-*O*-(CE-DMB-phosphate)-2′-deoxy-I, 3′-*O*-(allyl-DMB-phosphate)-*N*-AOC-2′-deoxy-C, 3′-*O*-(allyl-DMB-phosphate)-*N*-AOC-2′-deoxy-5-MC, 3′-*O*-(allyl-DMB-phosphate)-*N*-AOC-2′-deoxy-A, 3′-*O*-(allyl-DMB-phosphate)-*O*-allyl-*N*-AOC-2′-deoxy-G, 3′-*O*-(allyl-DMB-phosphate)-2′-deoxy-I, 3′-*O*-(allyl-DMB-phosphate)-thymidine (9 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.

JO951593C