Preparation of 2′-O-(β-Cyanoethyl phosphoramidites) of 3′-Deoxycytidine and 3′-Deoxyguanosine and Their Use for Solid-Phase Synthesis of Oligodeoxynucleotides Containing 2′,5′-Phosphodiester Linkages

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Convenient, preparative scale synthetic routes to 2′-O-(β-cyanoethyl *N,N*-diisopropylphosphoramidites) of 3′-deoxycytidine (**1**) and 3′-deoxyguanosine (**2**) are described. The 3′-deoxycytidine nucleoside **5** was constructed by a modified Hilbert–Johnson reaction in which *N*-(4-isobutyryl)-cytosine (**4**) was ribosylated with anomeric acetate **3**. Nucleoside **5** was converted to 5′-O-(dimethoxytrityl)-4-*N*-isobutyryl-3′-deoxycytidine (**7**) and phosphitylated to provide phosphoramidite **1**. Access to derivatives of 3′-deoxyguanosine was provided by selective removal of the 3′-hydroxyl of guanosine (**10**). Thus, the 3′-O-thiocarbamate of 5′-O-(dimethoxytrityl)-2-*N*-(dimethylformamidyl)-2′-O-(triisopropylsilyl)guanosine (**12**) was reduced with tributyltin hydride and converted to phosphoramidite **2**. In results to be reported elsewhere, phosphoramidites **1** and **2** were used to prepare oligodeoxynucleotides containing novel 2′,5′-phosphodiester linkages using automated solid-phase DNA synthesis methods with average stepwise coupling yields of >97%.

Introduction

The recent widespread use of modified oligonucleotides as diagnostic biochemical probes[1] and as tools for the control of gene expression by antisense and antigene strategies[2] has fostered the development of synthetic methods for the preparation of nucleic acids containing a wide variety of modified bases and backbone structures.[3] We have been interested in the properties of DNA that is connected via 2′,5′-phosphodiester linkages instead of the natural 3′,5′-bonds. We have previously described the synthesis of phosphoramidites of 3′-deoxyadenosine and 3′-deoxy-5-methyluridine[4] and used these monomer units to prepare oligodeoxynucleotides containing 2′,5′-phosphodiester linkages using solid-phase synthesis methods.[5] Studies of this isomeric DNA showed that complementary 2′,5′-linked DNA strands associate weakly only under high salt conditions.[5] Other workers have observed similar behavior with 2′,5′-linked oligodeoxynucleotides[6] and 2′,5′-linked oligoribonucleotides.[7] Our biophysical and molecular modeling studies of the hybridization properties of homopolymers of 2′,5′-linked 3′-deoxyadenosine (3′-dA_{16}) and 2′,5′-linked 3′-deoxy-5-methyluridine (3′-dT_{16}) showed that a triplex structure, involving one purine strand and two pyrimidine strands, is preferred over a duplex structure.[8] The observed low stability of 2′,5′-linked DNA duplexes may have provided selective pressure against this isomeric nucleic acid for use as a genetic material.

To explore the effect of G–C base pairs on the stability of 2′,5′-linked DNA association and to further characterize the nature of the interactions in this series of novel DNA molecules, we became interested in preparing oligonucleotides containing 3′-deoxyguanosine and 3′-deoxycytidine. The 3′-deoxy derivatives of cytidine and guanosine are both known;[9] however, protected phosphoramidites of these nucleosides were not available. We now report convenient preparative scale syntheses of 2′-O-(β-cyanoethyl *N,N*-diisopropylphosphoramidites) of 3′-deoxycytidine (**1**) and 3′-deoxyguanosine (**2**) and outline the successful use of these compounds to assemble oligonucleotides containing 2′,5′-linkages using automated DNA synthesis methods.

Results and Discussion

The phosphoramidites **1** and **2** were designed for use in automated DNA synthesis machines. Thus, the 5′-hydroxyl was protected as the dimethoxytrityl ether and the 2′-hydroxyl was activated as a β-cyanoethyl *N,N*-diisopropylphosphoramidite. The exocyclic amines of the cytosine and guanine bases were protected as the isobutyrylamide and dimethylformamidine derivatives, respectively, to provide compatibility with the Applied Biosystems FOD base protection scheme.[10] The synthe-

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(1) (a) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925–1963. (b) Goodchild, J. *Bioconj. Chem.* **1990**, *1*, 165–187.

(2) For recent reviews, see: (a) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923–1937. (b) Hélène, C.; Toulmé, J.-J. *Biochimica et Biophysica Acta* **1990**, *1049*, 99–125.

(3) For recent reviews, see: (a) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123–6194. (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223–2311. (c) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544–584.

(4) Rizzo, C. J.; Dougherty, J. P.; Breslow, R. *Tetrahedron Lett.* **1992**, *33*, 4129–4132. In references 4, 5, and 8 we referred to the oligonucleotides as having a 2′,5″ link, not a 2′,5′ link. The latter naming implies linkage within the same sugar, i.e. an internal link, but it seems unlikely that we can reverse the common naming system (in which "cyclic" is used to indicate a link within the same sugar). Thus here we revert to the 2′,5′ system.

(5) Dougherty, J. P.; Rizzo, C. J.; Breslow, R. *J. Am. Chem. Soc.* **1992**, *114*, 6254–6255.

(6) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.* **1992**, *114*, 6255–6256.

(7) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685–1690.

(8) Jin, R.; Chapman, W. H., Jr.; Srinivasan, A. R.; Olson, W. K.; Breslow, R.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10568–10572.

(9) (a) For a synthesis of 3′-deoxycytidine, see: Lin, T.-S.; Yang, J.-H.; Liu, M.-C; Shen, Z.-Y.; Cheng, Y.-C.; Prusoff, W. H.; Birnbaum, G. I.; Giziewicz, J.; Ghazzouli, I.; Brankovan, V.; Feng, J.-S.; Hsiung, G.-D. *J. Med. Chem.* **1991**, *34*, 693–701. (b) For a preparation of 3′-deoxyguanosine, see: Jenkins, S. R.; Holly, F. W.; Walton, E. *J. Org. Chem.* **1965**, *30*, 2851–2852.

(10) (a) Huynh, V.; McCollum, C.; Jacobson, K.; Theisen, P.; Vinayak, R.; Spiess, E.; Andrus, A. *Tetrahedron Lett.* **1990**, *31*, 7269–7272. (b) Vu, H.; McCollum, C.; Lotys, C.; Andrus, A. *Nucleic Acids Symp. Ser.* **1990**, *22*, 63–64.

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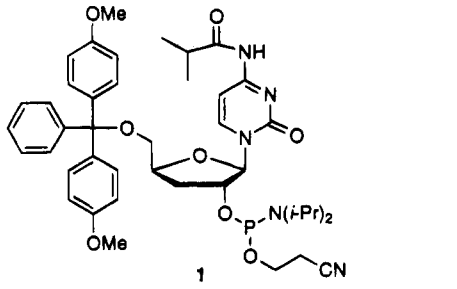

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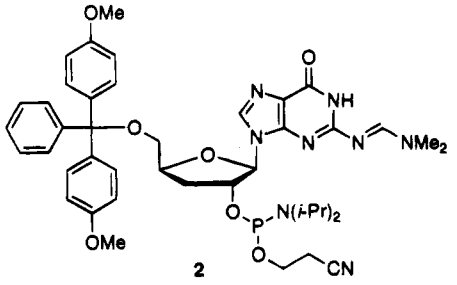


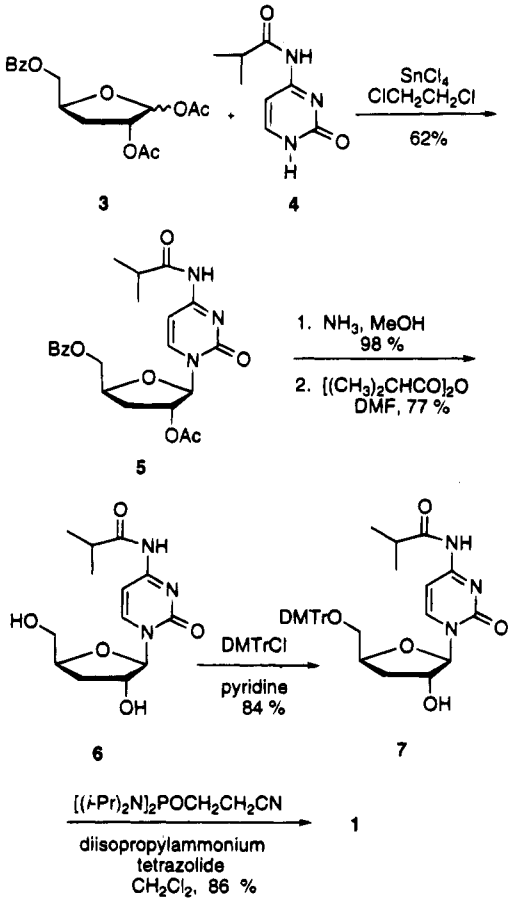
2

ses reported here can be used to prepare gram quantities of the desired phosphoramidites. Slightly higher yields are obtained on a smaller scale.

Synthesis of 3′-Deoxycytidine 2′-*O*-Phosphoramidite 1. The approach to phosphoramidite **1**, outlined in Scheme 1, is modeled after the route developed for the synthesis of 3′-deoxy-5-methyluridine.[4] The method involves installation of the nucleobase on the 3′-deoxy anomeric acetate **3**, followed by protecting group manipulation and phosphitylation. The starting sugar **3** was prepared as described[4] in four steps from 1,2-*O*-isopropylidene-D-xylofuranose. The cytosine moiety was installed on acetate **3** by the application of a ribosylation procedure developed by Sugiura and co-workers.[11] This method does not employ the labile trimethylsilyl-protected bases typically used in these Hilbert–Johnson glycosylations,[12] yet gives selective formation of cytidine derivatives of the correct anomeric and regiochemical configuration due to neighboring-group participation of the 2′-acetate (*vide infra*). 4-*N*-Isobutyrylcytosine (**4**) was prepared as described[11] and reacted in excess with the sugar **3** using tin(IV) chloride as a Lewis acid catalyst to provide the cytidine diester **5** in 62% yield. The α-anomer was formed in less than 10% yield and was separable from the desired β-anomer **5** by column chromatography.

All attempts to selectively remove the benzoyl and acetyl groups of **5** in the presence of the labile isobutyramide were unsuccessful. Thus, compound **5** was converted to 3′-deoxycytidine by overnight treatment with ammonia-saturated methanol, and the exocyclic amino group was selectively reprotected by reaction with isobutyric anhydride in DMF[13] to give the desired 4-(*N*-isobutyryl)-3′-deoxycytidine (**6**). The intermediate 3′-deoxycytidine was fully characterized and shown to be identical in all respects (^{1}H-NMR, ^{13}C-NMR, IR, melting point, HRMS) to authentic samples of 3′-deoxycytidine

Scheme 1



purchased from Sigma and prepared by independent synthesis.[14]

After protection of the 5′-hydroxyl of compound **6** as the dimethoxytrityl ether using dimethoxytrityl chloride in pyridine, the resulting nucleoside **7** was phosphitylated using conditions developed by Caruthers and co-workers for the preparation of phosphoramidites of 2′-deoxynucleosides.[15] Thus, nucleoside **7** was treated with 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (115 mol %) and diisopropylammonium tetrazolide (50 mol %) in dichloromethane to give the desired phosphoramidite **1** in 86% yield after chromatography. The final product was fully characterized by ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, and high-resolution mass spectrometry (HRMS). Presently, the sequence is being performed using a benzoyl group for protection of the exocyclic amine to parallel the latest series of base protecting groups.[16]

(11) Sugiura, Y.; Furuya, S.; Furukawa, Y. *Chem. Pharm. Bull.* **1988**, *36*, 3253–3256.

(12) (a) Nishimura, T.; Shimizu, B.; Iwai, I. *Chem. Pharm. Bull.* **1964**, *12*, 1471. (b) Niedballa, U.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 461. (c) Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654–3660 and following papers. (d) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234–1255. (e) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279–1286.

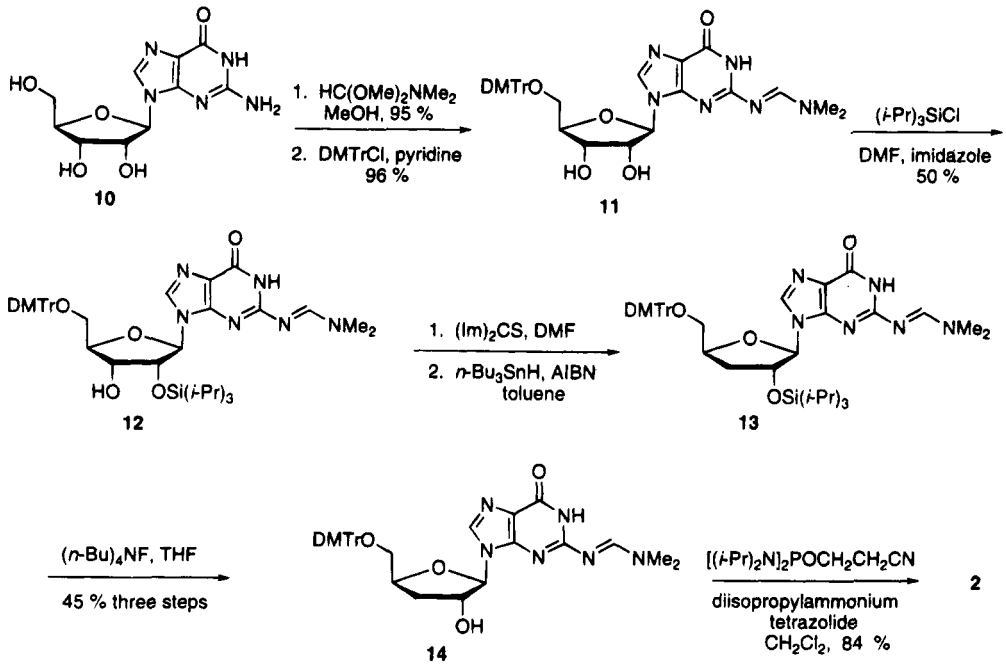
(13) Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E. *Nucleosides Nucleotides* **1989**, *8*, 179–183.

(14) An authentic sample of 3′-deoxycytidine was prepared by the method of Ogilvie (see reference 18). Thus, 5′-*O*-dimethoxytrityl-*N*-4-isobutyrylcytidine was prepared and protected as the 2′-*O*-*tert*-butyldimethylsilyl ether, converted to the 3′-*O*-thionocarbamate and deoxygenated with tributyltin hydride. The resulting 3′-deoxynucleoside was deprotected with TBAF, and deblocked by treatment with concentrated aqueous ammonia followed by 80% acetic acid to give 3′-deoxycytidine. In a parallel set of experiments, the isomeric 3′-*O*-*tert*-butyldimethylsilyl ether was deoxygenated at the 2′-position and subsequently deprotected to produce the natural 2′-deoxycytidine, identical in all respects to a sample purchased from Sigma (^{1}H-NMR, ^{13}C-NMR, IR, HRMS, melting point).

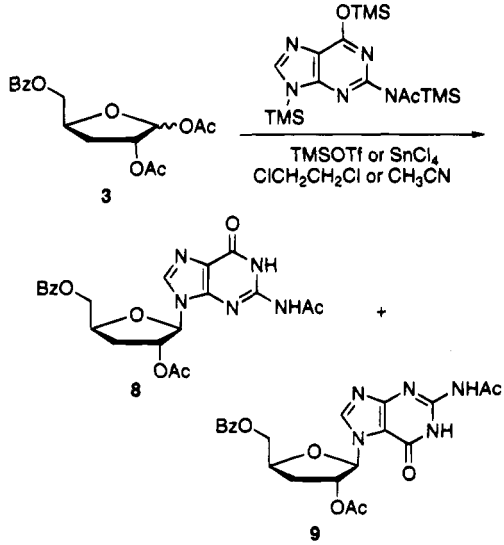
(15) Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J.-Y. *Methods Enzymol.* **1987**, *154*, 287–313.

(16) The latest protecting group scheme utilizes benzoyl for A and C and dimethylformamidine for G: Andrus, A.; Theisen, P. D.; McCollum, C. *Nucleic Acids Symp. Ser.* **1993**, *29*, 5–6.

Scheme 2



Synthesis of 3'-Deoxyguanosine 2'-*O*-Phosphoramidite 2. We initially intended to prepare the phosphoramidite of 3'-deoxyguanosine **2** by a route analogous to that used for synthesis of compound **1**; however, attempts to ribosylate **3** selectively using trimethylsilyl derivatives of guanine provided mixtures of the desired *N*-9-β 3'-deoxyguanosine derivative **8** and the isomeric *N*-7-β guanosine derivative **9** under all conditions tried (**8**:**9** ratio, 1:1 to 4:1).[17]



Our approach to phosphoramidite **2** was then modeled after the methodology developed by Ogilvie and coworkers for the synthesis of 3'-deoxynucleosides.[18] The standard procedures were modified slightly to accommodate the different protecting scheme, namely, the exocyclic dimethylformamidine group and the triisopropylsilyl group. As illustrated in Scheme 2, the method involves selective protection and deoxygenation of the starting ribonucleoside, followed by conversion to the phosphoramidite. Guanosine **10** was converted to the known[19] diprotected nucleoside **11** in high yield by treatment with dimethylformamide dimethyl acetal in methanol followed by reaction with dimethoxytrityl chloride in pyridine.[10a,20] Subsequently, **11** was transformed into the 2'-*O*-(triisopropylsilyl) ether **12** by reaction with triisopropylsilyl chloride and imidazole in DMF.[21] The 3'-*O*-(triisopropylsilyl) isomer was separated from the desired compound **12** by column chromatography.[22]

The 2'-*O*-(triisopropylsilyl) nucleoside **12** was then converted to the 3'-*O*-thiocarbamate by treatment with *N*,*N*'-(thiocarbonyl)diimidazole. The long reaction time for complete conversion to the thiocarbamate resulted in a small proportion of silyl group migration to the 3'-position; however, the minor amount of isomeric product could be easily separated later in the sequence. The unstable thiocarbamate was deoxygenated directly under radical conditions with tributyltin hydride and 2,2'-azobisisobutyronitrile (AIBN) in refluxing toluene to afford the 2'-*O*-silyl-protected 3'-deoxyguanosine derivative **13**. This intermediate was purified and fully characterized; however, **13** could be desilylated directly with tetrabutylammonium fluoride in THF to give, after column chromatography, the desired (dimethoxytrityl)-

(17) A variety of conditions have been developed to prepare purine nucleosides by ribosylation of silylated purine bases, including: (a) Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 2195–2202. (b) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1984**, *27*, 175–181. (c) Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* **1984**, *3*, 33–44. Utilization of silylated purine prepared prior to use, as in (a), consistently gives 1:1 mixtures of **8**:**9**. *In situ* preparation of the silylated bases with *N*,*O*-bis(trimethylsilyl)acetamide followed by addition of Lewis acid and sugar, as in (b) and (c), gives better **8**:**9** ratios (4:1); however, the yields of the desired *N*-β-9 isomer **8** are at present only 20–50%.

(18) Ogilvie, K. K.; Hakimelahi, G. H.; Proba, Z. A.; Usman, N. *Tetrahedron Lett.* **1983**, *24*, 865–868.

(19) Zemlicka, J.; Holy, A. *Coll. Czech. Chem. Commun.* **1967**, *32*, 3159–3168.

(20) McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040–2048.

(21) Usman, N.; Ogilvie, K. K.; Jiang, M.-Y.; Cedergen, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 7845–7854.

(22) The ^{1}H-NMR spectra of the isomeric 2'-*O*-TIPS and 3'-*O*-TIPS derivatives were unambiguously assigned by selective proton decoupling experiments.

dimethylformamidine-protected 3′-deoxynucleoside **14**. The fidelity of the deoxygenation sequence was confirmed by deprotection of **14** to the nucleoside. Detritylation of **14** (80% acetic acid, room temperature, 30 min) followed by deblocking of the exocyclic amino group (concd aqueous ammonia, 55 °C, 2 h) affords 3′-deoxyguanosine, identical in all respects (^{1}H-NMR, ^{13}C-NMR, melting point, HRMS) to an authentic sample purchased from Sigma.[23] The synthesis was completed by phosphitylation of **14** using the method of Caruthers and co-workers[15] to afford the 3′-deoxyguanosine 2′-*O*-phosphoramidite **2**. All spectral data (^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, HRMS) are consistent with the structure of phosphoramidite **2**. The phosphoramidites **1** and **2** can be stored for several months under argon in a desiccator at −20 °C with no apparent degradation.

Synthesis of 2′,5′-Linked Oligonucleotides. We have used the phosphoramidites **1** and **2** to prepare oligonucleotides containing 2′,5′-phosphodiester linkages ranging from 10 to 15 bases in length. Oligonucleotides were synthesized on a Model 394 Applied Biosystems DNA synthesizer using standard cycles and 2′-deoxynucleoside controlled-pore glass (CPG) columns (0.2 and 1.0 μmol). Stepwise coupling yields were >97% based on the trityl cation assay. The resulting oligonucleotides were deprotected under FOD conditions[10] and analyzed and purified by denaturing polyacrylamide gel electrophoresis (PAGE). Oligonucleotide synthesis and characterization will be reported in detail later as we describe the properties of these interesting oligonucleotides.

Summary. We have developed preparative routes to phosphoramidites of 3′-deoxycytidine (**1**) and 3′-deoxyguanosine (**2**) and have successfully used these monomers to synthesize oligonucleotides containing 2′,5′-linkages. Studies of this isomeric DNA are ongoing and will be reported in due course.

Experimental Section

General. High-resolution FAB mass spectroscopy (HRMS) was performed using a glycerol matrix. Thin layer chromatography (TLC) was performed on EM Science Kieselgel 60 F-254 (1 mm) plates. EM Science silica 60 (0.040−0.063 mm, 230−400 mesh ASTM) silica gel was used for flash column chromatography. Columns using methanol/chloroform eluents were slurry packed after 1−2 h equilibration of the silica and washed with methanol/chloroform until the opaque solvent front had eluted from the column.[24] All reactions were run under argon atmosphere with oven-dried (140 °C) glassware. Dichloromethane, acetonitrile, and benzene were distilled from calcium hydride prior to use. Tetrahydrofuran (THF) was dried over 4 Å molecular sieves and distilled from sodium with benzophenone as an indicator. Other anhydrous solvents were purchased from Aldrich. Dimethylformamide was degassed *in vacuo* prior to use. Chemicals and reagents were supplied by Aldrich and used without further purification unless otherwise noted. Tetrazole and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite were supplied by Sigma. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Oligonucleotide synthesis was carried out using Applied Biosystems equipment and reagents.

2′-*O*-Acetyl-5′-*O*-benzoyl-4-*N*-isobutyryl-3′-deoxycytidine (5). *N*-4-Isobutyrylcytosine (**4**)[11] (0.480 g, 2.64 mmol) and 1,2-*O*-diacetyl-5-*O*-benzoyl-3-deoxyribose (**3**)[4] (0.500 g, 1.55 mmol) were dissolved in 20 mL of anhydrous 1,2-dichloroethane under argon atmosphere. Tin(IV) chloride (0.250 mL, 0.557 g, 2.14 mmol) was added dropwise to the stirring solution. After 20 h at 27 °C, 5% sodium bicarbonate (40 mL) and chloroform (60 mL) were added. After being stirred for an additional 20 min, the solution was filtered through a Celite pad and the resulting layers were separated. The aqueous phase was washed with two 20 mL portions of chloroform, and the combined organics were dried over sodium sulfate and concentrated *in vacuo*. The residue was applied to a silica gel column (4 cm diameter, 75 g of silica) which was slurry packed with 1% methanol in chloroform. The compound was eluted with a 1−3% methanol/chloroform gradient to afford 0.409 g (62%) of **5** as a white solid: mp 193−194 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.08 (d, J = 7.4 Hz, 1H), 8.04 (d, J = 7.3 Hz, 2H), 7.64 (t, J = 7.3 Hz, 1H), 7.51 (t, J = 7.6 Hz, 2H), 7.34 (d, J = 7.3 Hz, 1H), 5.97 (s, 1H), 5.39 (m, 1H), 4.65 (m, 3H), 2.56 (m, 1H), 2.18 (m, 2H), 2.15 (s, 3H), 1.23 (d, J = 6.8 Hz, 6H); ^{13}C-NMR ($CDCl_3$) δ 176.50, 169.64, 166.13, 162.59, 154.30, 143.96, 133.62, 129.52, 129.23, 128.64, 96.30, 92.15, 79.00, 92.15, 64.21, 36.70, 32.00, 20.92, 18.98; HRMS calcd for $C_{22}H_{26}N_3O_7$ (M + 1) 444.1771, found 444.1783.

4-(*N*-Isobutyryl)-3′-deoxycytidine (6). 3′-Deoxycytidine. Compound **5** (0.409 g, 0.921 mmol) was dissolved in 20 mL of 7 N ammonia in methanol and stirred at 25 °C for 12 h. The clear solution was concentrated *in vacuo*, and the residual gum was dissolved in water (30 mL) and diethyl ether (20 mL). The resulting aqueous phase was extracted with an additional 20 mL of ether, and the combined organics were back-extracted with 20 mL of water. The aqueous portions were combined and lyophilized to afford 0.209 g (98%) of 3′-deoxycytidine as a hygroscopic white powder: mp 235−237 °C (lit.[9a] mp 230−232 °C); ^{1}H-NMR (400 MHz, CD_3OD) δ 8.16 (d, J = 7.5 Hz, 1H), 5.84 (d, J = 7.5 Hz, 1H), 5.72 (s, 1H), 4.45 (m, 1H), 4.28 (m, 1H), 3.94 (m, 1H), 3.68 (m, 1H), 2.00 (m, 1H), 1.81 (m, 1H); ^{13}C-NMR (CD_3OD) δ 167.79, 158.44, 142.61, 95.21, 94.96, 83.11, 77.63, 63.02, 33.55; HRMS calcd for $C_9H_{14}N_3O_4$ (M + 1) 228.0984, found 228.0995. Anal. Found (Calcd for $C_9H_{13}N_3O_4$): C 47.39, 47.35 (47.57); H 5.55, 5.42 (5.77); N 18.77, 18.91 (18.49).

3′-Deoxycytidine (0.209 g, 0.902 mmol) from the previous reaction was dissolved in anhydrous dimethylformamide (5 mL) and isobutyric anhydride (0.16 mL, 0.15 g, 0.94 mmol) was added dropwise under argon. The reaction was stirred at 25 °C for 18 h and was concentrated *in vacuo* to provide a white solid. The solid was triturated with diethyl ether (3 mL) to give pure **6** (210 mg, 77%): mp 110−112 °C; ^{1}H-NMR (400 MHz, CD_3OD) δ 8.65 (d, J = 7.5 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 5.77 (s, 1H), 4.52 (m, 1H), 4.34 (m, 1H), 4.01 (m, 1H), 3.72 (m, 1H), 2.68 (m, 1H), 2.00 (m, 1H), 1.83 (m, 1H), 1.17 (d, J = 7.0 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 179.77, 164.56, 158.09, 146.29, 97.50, 95.63, 83.91, 77.67, 62.73, 37.26, 33.16, 19.37; HRMS calcd for $C_{13}H_{20}N_3O_5$ (M + 1) 298.1403, found 298.1325.

4-(*N*-Isobutyryl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-deoxycytidine (7). Nucleoside **6** (0.187 g, 0.629 mmol) was dried by three evaporations from anyhydrous pyridine (5 mL each) and dried *in vacuo* (0.1 Torr) overnight. The resulting foam was dissolved in anhydrous pyridine (6.5 mL), and 4,4′-dimethoxytrityl chloride (0.235 g, 0.673 mmol) was added. The solution was stirred under argon at 25 °C for 24 h with periodic additions of dimethoxytrityl chloride to drive the reaction to completion. The reaction was quenched with saturated sodium bicarbonate (10 mL) and concentrated, and the residue was partitioned between dichloromethane (25 mL) and sodium bicarbonate (25 mL). The resulting aqueous portion was back-extracted with dichloromethane (25 mL), and the combined organics were dried with sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash silica chromatography (column 3 cm diameter, 75 g silica) using a 2% methanol/chloroform eluent. The fractions containing product were combined and concentrated in the presence of a few drops of triethylamine (to prevent detritylation) to afford **7** as an off-white foam (317 mg, 84%): mp 95−100 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.32 (d, J = 7.5 Hz, 1H), 7.5−7.2 (m, 9H), 7.02 (d, J = 7.5 Hz, 1H), 6.89 (m, 4H), 5.69 (s, 1H), 5.68 (m, 1H), 4.48 (m, 1H), 4.20 (m, 1H), 3.75 (s, 6H),

(23) In addition, the 3′-*O*-triisopropylsilyl ether isomer of **12** was separately converted to the 2′-*O*-thioimidazolide, deoxygenated, and deprotected to afford authentic 2′-deoxyguanosine identical in all respects to a sample purchased from Sigma (^{1}H-NMR, ^{13}C-NMR, IR, HRMS, melting point).

(24) Dado, G. P.; Desper, J. M.; Holmgren, S. K.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4834−4843.

3.36 (m, 2H), 2.69 (m, 1H), 2.06 (m, 1H), 1.74 (m, 1H), 1.05 (m, 6H); ^{13}C-NMR (CD_3OD) δ 179.51, 164.50, 160.23, 146.05, 146.66, 137.10, 136.87, 131.34, 131.24, 129.51, 128.95, 128.12, 114.30, 97.88, 95.47, 82.68, 77.83, 64.27, 47.07, 43.24, 33.54, 19.36; HRMS calcd for $C_{34}H_{38}N_3O_7$ 600.2710, found 600.2728.

4-(*N*-Isobutyryl)-5'-*O*-(4,4'-dimethoxytrityl)-3'-deoxycytidine 2'-*O*-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (1). Protected nucleoside **7** (0.421 g, 0.70 mmol) was dried by consecutive evaporation from anhydrous pyridine (10 mL), toluene (10 mL), and THF (8 mL) followed by overnight drying at 0.05 Torr. The nucleoside was transferred to a dried flask containing diisopropylammonium tetrazolide[15] (0.063 g, 0.37 mmol, previously dried overnight at 0.05 Torr) as a solution in distilled CH_2Cl_2 (3.6 mL). To the stirred solution was added 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (0.26 mL, 0.25 g, 0.82 mmol) dropwise over a 1 min period. The reaction was stirred under argon at 25 °C for 7 h and transferred to a separatory funnel containing 2% sodium carbonate (15 mL) and CH_2Cl_2 (15 mL). After extraction, the organic portion was washed with 2% sodium carbonate (15 mL), followed by brine (15 mL). The combined aqueous portions were back-extracted with two 15 mL portions of CH_2Cl_2, and the resulting organics were pooled, dried over sodium sulfate at 0 °C for 20 min, and concentrated to an off-white foam. Pure phosphoramidite was obtained by silica gel chromatography (2 cm diameter column, 20 g silica, slurry packed) eluting under argon pressure with 45/45/10 ethyl acetate/hexane/triethylamine which was degassed with argon (two diastereomers, R_f 0.44 and 0.39, 95/5 ethyl acetate/triethylamine). The phosphoramidite **1** was obtained as a white foam (485 mg, 86%): ^{1}H-NMR (400 MHz, CD_3CN) δ 8.7 (s, 1H), 8.35 (d, J = 8.5 Hz, 1H), 7.32 (m, 9H), 7.02 (d, J = 8.5 Hz, 1H), 6.89 (m, 4H), 5.85 (s, 0.6H), 5.84 (s, 0.4H), 4.53 (m, 2H), 4.87 (m, 2H), 3.77 (s, 6H), 3.63 (m, 2H), 3.50 (m, 1H), 3.51 (m, 1H), 2.64 (m, 3H), 2.10 (m, 1H), 1.91 (m, 1H) 1.15 (m, 18H); ^{13}C-NMR (CD_3CN) δ 178.39, 163.58, 159.82, 155.99, 145.76, 145.56, 136.99 and 136.78 (diast), 131.11 and 130.96 (diast), 129.22 and 128.99 (diast), 128.04, 114.23, 96.24, 93.79 and 93.72 (diast), 93.58, 87.70, 82.05 and 81.99 (diast), 79.59 and 79.32 (diast), 64.18, 60.02 and 59.77 (diast), 59.45, 55.85, 44.40 and 44.26 (diast), 44.36 and 44.18 (diast), 36.86, 33.19 and 32.94 (diast), 24.90 and 24.82 (diast), 24.74, 21.00, 19.36; ^{31}P-NMR (CD_3CN, 85% H_3PO_4 external reference) δ 156.80, 155.62; ^{31}P-NMR (CD_2Cl_2, 85% H_3PO_4 external reference) δ 154.42, 152.72; HRMS calcd for $C_{43}H_{55}N_5O_8P$ (M + 1) 800.3788, found 800.3796.

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*-((dimethylamino)methylene)guanosine (11).[19] Guanosine **10** (50.0 g, 0.17 mol) was dissolved in 500 mL of anhydrous methanol, and 84.0 mL (75.0 g, 0.63 mol) of *N,N*-dimethylformamide dimethyl acetal was added under argon. The suspension was stirred for 96 h at 27 °C. The resulting white precipitate was removed by filtration, washed with cold methanol (100 mL), and dried under under reduced pressure (1 Torr) to afford 57.9 g of 2-*N*-(dimethylformamidyl)guanosine (95% yield): mp 249–250 °C dec (lit.[19] mp 247–248 °C); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H), 8.53 (s, 1H), 8.03 (s, 1H), 5.77 (d, J = 6.7 Hz, 1H), 5.41 (d, J = 6.1 Hz, 1H), 5.19 (d, J = 6.1 Hz, 1H), 5.02 (t, J = 6.0 Hz, 1H), 4.45 (m, 1H), 4.09 (m, 1H), 3.87 (m, 1H), 3.54 (m, 2H), 3.15 (s, 3H), 3.02 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ 157.88, 157.51, 157.21, 149.94, 137.10, 136.89, 119.75, 86.74, 85.38, 73.74, 70.43, 61.47, 40.60, 34.60; HRMS calcd for $C_{13}H_{19}N_6O_5$ (M + 1) 339.1417, found 339.1410. Anal. Found (Calcd for $C_{13}H_{18}N_6O_5$): C 46.00 (46.15); H 5.36 (5.36); N 24.43 (24.84).

2-*N*-(Dimethylformamidyl)guanosine from the previous reaction (31.1 g, 91.9 mmol) was dried by three evaporations from anhydrous pyridine (100 mL). Anhydrous pyridine (500 mL) was added, and the stirring solution was chilled in a cool water bath (5–10 °C). 4,4'-Dimethoxytrityl chloride (31.1 g, 91.9 mmol) was added, and the system was purged with argon and stirred at 25 °C. After 26 h of reaction, saturated sodium bicarbonate (30 mL) was added and the solution was concentrated. The residue was dissolved in chloroform (300 mL) and saturated sodium bicarbonate (200 mL), and the layers were separated. The aqueous portion was extracted twice with chloroform (300 mL), and the combined organics were washed with brine (200 mL). After being dried with sodium sulfate, the chloroform solution was concentrated to a white solid, which was redissolved in 100 mL of chloroform and 30 mL of ethanol and added dropwise to vigorously stirred diethyl ether (2.5 L). The resulting white precipitate was filtered, crushed with a mortar and pestle, and dried *in vacuo* (1 Torr), giving 56.6 g (96%) of **11** as a white powder: mp 228–231 °C (lit.[19] mp 225–230 °C); TLC R_f = 0.25 (10% MeOH/$CHCl_3$); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.49 (s, 1H), 7.93 (s, 1H), 7.35–7.10 (m, 9H), 6.84–6.80 (m, 4H), 5.85 (d, J = 4.6 Hz, 1H), 5.55 (d, J = 5.4 Hz, 1H), 5.23 (d, J = 5.4 Hz, 1H), 4.50 (m, 1H), 4.21 (m, 1H), 4.00 (m, 1H), 3.71 (s, 6H), 3.16 (m, 2H), 3.09 (s, 3H), 3.01 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ 157.49, 157.29, 157.06, 156.70, 149.44, 144.29, 136.05, 134.96, 129.10, 127.23, 127.13, 126.11, 119.23, 112.59, 86.53, 84.94, 82.40, 73.00, 63.45, 54.47, 40.01, 34.13; HRMS calcd for $C_{34}H_{37}N_6O_7$ (M + 1) 641.2724, found 641.2716. Anal. Found (Calcd for $C_{34}H_{36}N_6O_7$): C 63.69, 63.71 (63.74); H 5.57, 5.61 (5.66); N 12.86, 12.92 (13.12).

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*-((dimethylamino)methylene)-2'-*O*-(triisopropylsilyl)guanosine (12). The protected nucleoside **11** (15.8 g, 24.7 mmol) was dried by two consecutive evaporations from 25 mL of anhydrous pyridine followed by drying *in vacuo* (1 Torr) overnight. The dried nucleoside was dissolved in 80 mL of DMF, and imidazole (4.32 g, 63.5 mmol) was added. Under argon, triisopropylsilyl chloride (10.9 mL, 9.82 g, 50.9 mmol) was added dropwise over a 10 min period, and the solution was stirred under argon at 25 °C for 27 h. The reaction was quenched with 5% aqueous sodium bicarbonate (20 mL) and concentrated *in vacuo*. The residue was redissolved in dichloromethane (300 mL), washed with water (3 × 200 mL) and brine (200 mL), dried over sodium sulfate, and concentrated. The resulting solid was dissolved in toluene (50 mL) and concentrated to dryness. The residue was applied to a flash column (10 cm diameter, 600 g silica, slurry packed), and the products were eluted with 90/5/5 $CHCl_3/CH_2Cl_2/Et_3N$ (desired 2'-*O*-TIPS compound **12**, R_f =0.20; isomeric 3'-*O*-TIPS compound, R_f = 0.10). The desired fractions were pooled to yield compound **12** as a white foam-glass: 9.58 g (50%); mp 70–75 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.41 (s, 1H), 7.95 (s, 1H), 7.35 (m, 2H), 7.24 (m, 7H), 6.83 (m, 4H), 5.91 (d, J = 4.9 Hz, 1H), 5.20 (d, J = 6.1 Hz, 1H), 4.77 (t, J = 4.8 Hz, 1H), 4.21 (m, 1H), 4.04 (m, 1H), 3.72 (s, 6H), 3.22 (m, 2H), 3.04 (s, 3H), 3.02 (s, 3H), 1.02–0.87 (m, 21H); ^{13}C-NMR (DMSO-d_6) δ 158.06, 157.50, 157.18, 149.82, 144.77, 136.56, 135.39, 129.68, 129.63, 127.76, 127.63, 126.69, 113.12, 87.47, 85.56, 83.36, 75.57, 70.44, 63.69, 55.00, 40.60, 34.63, 17.82, 11.61; HRMS calcd for $C_{43}H_{57}N_6O_7Si$ (M + 1) 797.4058, found 797.4045. The isomeric 3'-*O*-TIPS compound was obtained in 27% yield.

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*-((dimethylamino)methylene)-2'-*O*-(triisopropylsilyl)-3'-deoxyguanosine (13). The silyl-protected nucleoside **12** (5.03 g, 6.31 mmol) was dissolved in 57 mL of anhydrous DMF under argon. *N,N'*-(Thiocarbonyl)diimidazole (3.52 g, 19.8 mmol) was added with stirring. The reaction was stirred at 24 °C for 100 h, ethyl acetate (400 mL) and water (250 mL) were added, and the layers were separated. The aqueous portion was back-extracted with ethyl acetate (250 mL), and the combined organics were washed with water (2 × 200 mL) and brine (200 mL), dried with sodium sulfate, concentrated, and dried at reduced pressure (1 Torr). The compound was carried on to the next reaction without further purification: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.55 (s, 1H), 8.37 (s, 1H), 7.95 (s, 1H), 7.85 (s, 1H), 7.4–6.7 (m, 14H), 6.13 (m, 1H), 6.08 (d, J = 6.1 Hz, 1H), 5.50 (m, 1H), 4.40 (m, 1H), 4.21 (m, 1H), 3.72 (s, 6H), 3.42 (m, 2H), 2.96 (s, 3H), 2.80 (s, 3H), 1.00–0.60 (m, 21H); HRMS calcd for $C_{47}H_{59}N_5O_7SSi$ (M + 1) 907.3996, found 907.4028.

A solution of the thiocarbamate from the previous reaction in anhydrous toluene (150 mL) was degassed with argon for 45 min. In a second flask, 2,2'-azobis(isobutyronitrile) (AIBN, 550 mg, 3.35 mmol), tributyltin hydride (5.1 mL, 5.5 g, 19 mmol), and anhydrous toluene (35 mL) were combined and degassed with argon for 30 min. The tin hydride solution was added dropwise to the refluxing substrate solution under argon

over a 30 min period. After a total of 6 h, the solution was cooled to room temperature and concentrated. The resulting brown oil was typically desilylated directly without further purification; however, the compound could be purified by silica gel chromatography by isocratic elution with 50/50 acetone/chloroform to afford **13** as an off-white foam: mp 89–94 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 8.47 (s, 1H), 7.93 (s, 1H), 7.35–7.18 (m, 9H), 6.81 (m, 4H), 5.89 (d, J = 2.0 Hz, 1H), 4.83 (m, 1H), 4.42 (m, 1H), 3.07 (s, 3H), 3.03 (s, 3H), 2.49 (m, 3H), 1.95 (m, 1H), 1.01 (m, 21H); ^{13}C-NMR (DMSO-d_6) δ 157.99, 157.58, 157.12, 149.39, 144.77, 136.23, 135.46, 129.61, 127.70, 127.61, 119.64, 113.06, 90.33, 85.46, 78.56, 76.39, 65.01, 54.94, 45.66, 35.71, 34.57, 17.62, 17.53, 11.35; HRMS calcd for $C_{43}H_{57}N_6O_6Si$ (M + 1) 781.4109, found 781.4135.

5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-((dimethylamino)methylene)-3′-deoxyguanosine (14). To a solution of **13** obtained from the previous reaction in distilled THF (100 mL) was added tetrabutylammonium fluoride (TBAF, 12 mL of a 1 M solution in THF, 12 mmol) by syringe. The solution was stirred under argon at 22 °C for 5 h, diluted with water (50 mL), and stirred an additional 15 min. The layers were separated, and the organic phase was washed with two 40 mL portions of water. The organic phase was dried with sodium sulfate and concentrated *in vacuo*. The residue was applied to a flash column (80 g silica, slurry packed in 5% MeOH/$CHCl_3$) and eluted with 5–7% MeOH/$CHCl_3$ (desired 3′-deoxy isomer R_f = 0.30; 2′-deoxy isomer R_f = 0.55). The desired fractions were combined, concentrated, and taken up into 3 mL of chloroform/ethanol (5:1). The product was precipitated by dropwise addition of the concentrated solution to stirring diethyl ether (500 mL). Filtration and drying *in vacuo* (1 Torr) provides 1.44 g (45% from **12**) of **14**: mp 130–132 °C dec; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.55 (s, 1H), 7.88 (s, 1H), 7.35–7.10 (m, 9H), 6.79 (m, 4H), 5.87 (s, 1H), 5.70 (d, J = 1.1 Hz), 4.60 (m, 1H), 4.45 (m, 1H), 3.71 (s, 6H), 3.12 (m, 2H), 3.12 (s, 3H), 3.02 (s, 3H), 2.24 (m, 1H), 1.97 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ 157.99, 157.58, 157.51, 157.12, 149.39, 144.77, 136.12, 135.46, 129.61, 127.70, 127.61, 126.59, 113.04, 85.34, 78.83, 74.49, 65.32, 54.94, 40.59, 35.71, 34.57; HRMS calcd for $C_{34}H_{37}N_6O_6$ (M + 1) 625.2775, found 625.2785.

5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-((dimethylamino)methylene)-3′-deoxyguanosine 2′-*O*-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (2). The diprotected nucleoside **14** (0.616 g, 0.99 mmol) was dried by consecutive evaporation from anhydrous pyridine (10 mL), toluene (8 mL), and THF (7 mL) followed by overnight drying (0.1 Torr). The nucleoside was transferred to a dried flask containing diisopropylammonium tetrazolide[15] (0.091 g, 0.53 mmol, previously dried overnight at 0.05 Torr) as a solution in 5 mL of distilled CH_2Cl_2. With stirring under argon, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.37 mL, 0.35 g, 1.15 mmol) was added dropwise. The reaction was stirred under argon at 24 °C for 6 h and transferred to a separatory funnel containing 2% sodium carbonate (25 mL) and CH_2Cl_2 (25 mL). The layers were separated, and the organic portion was washed with 2% sodium carbonate (25 mL) and brine (25 mL). The combined aqueous portions were back extracted with two 25 mL portions of CH_2Cl_2, and the resulting organics were pooled, dried over sodium sulfate at 0 °C for 1.5 h, filtered (vacuum, washed with distilled CH_2Cl_2), and concentrated to an off-white foam. The phosphoramidite was purified by silica gel chromatography (3 cm diameter column, 18 g silica, slurry packed) eluting under argon pressure with 47/43/10 ethyl acetate/dichloromethane/triethylamine which was degassed with argon (two diastereomers, R_f 0.2–0.3, 45/45/10 ethylacetate/dichloromethane/triethylamine). Concentration of the fractions yielded the desired phosphoramidite **2** (908 mg, 84%) as a white foam: ^{1}H-NMR (400 MHz, CD_3CN) δ 9.23 (s, 1H), 8.60 (2s (diast), 1H), 7.68 (s, 1H, H-8), 7.2–7.4 (m, 9H), 6.79 (m, 4H), 6.02 (2s (diast), 1H), 4.91 (m, 1H), 4.50 (m, 1H), 3.76 (m, 2H), 3.75 (s, 6H), 3.59 (m, 2H), 3.08 (s, 3H), 3.04 (s, 3H), 3.42 (m, 1H), 2.61 (m, 1H), 2.57 (m, 1H), 2.35 (m, 1H), 2.10 (m, 1H) 1.20 (m, 12H); ^{13}C-NMR (CD_3CN) δ 178.0, 161.10, 159.63, 159.30, 159.24, 146.09, 137.16, 137.11, 136.87, 131.00, 129.00, 128.80, 127.81, 114.01, 91.51 and 91.27 (diast), 80.78 and 80.62 (diast), 78.59 and 78.25 (diast), 78.36 and 78.01 (diast), 66.01, 59.66 (m), 55.88, 44.15 and 44.05 (diast), 41.71, 35.26 and 35.16 (diast), 24.81 and 24.70 (diast), 23.19 and 23.15 (diast), 21.07, 20.97 and 20.89 (diast); ^{31}P-NMR (CD_3CN, 85% H_3PO_4 external reference, 20 °C) δ 156.39, 156.04; ^{31}P-NMR (CD_2Cl_2, 85% H_3PO_4 external reference, 20 °C) δ 153.33, 153.05; HRMS calcd for $C_{43}H_{54}N_8O_7P$ (M + 1) 825.3853, found 825.3860.

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Supplementary Material Available: Spectral data (^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, and HRMS) are available for all numbered compounds and synthetic intermediates (27 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.