

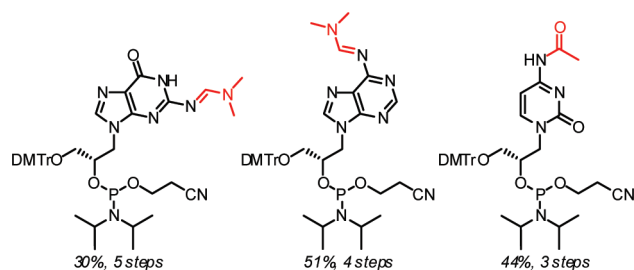
Improved Phosphoramidite Building Blocks for the Synthesis of the Simplified Nucleic Acid GNA

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An improved synthesis of glycol nucleic acids is reported using new phosphoramidite building blocks in which the exocyclic amino groups of adenine and guanine are protected as *N*-dimethylformamidines, whereas the amino group of cytosine is protected via an acetamide. Besides a more rapid synthesis with higher yields, these phosphoramidites allow the use of a quicker deprotection procedure in the subsequent solid-phase synthesis of GNA oligonucleotides.

Glycol nucleic acid (GNA) constitutes a minimal solution for a phosphodiester-containing nucleic acid backbone. The propylene glycol nucleotide building blocks contain just three carbon atoms and one stereocenter and are connected by phosphodiester bonds (Figure 1). Surprisingly, GNA forms antiparallel Watson–Crick duplexes that significantly exceed the stabilities of analogous duplexes of DNA and RNA.^{1–4} The simplicity of the GNA backbone renders it an interesting scaffold for future nanoscale architectures.⁵ However, this requires a straightforward chemical or enzymatic synthesis of this artificial nucleic acid. Here we report our progress toward improved GNA phosphoramidite building blocks for automated solid phase synthesis of GNA oligonucleotides.

The traditional amide protection scheme^{6,7} employed for the original solid-phase synthesis of GNA suffers from the formation

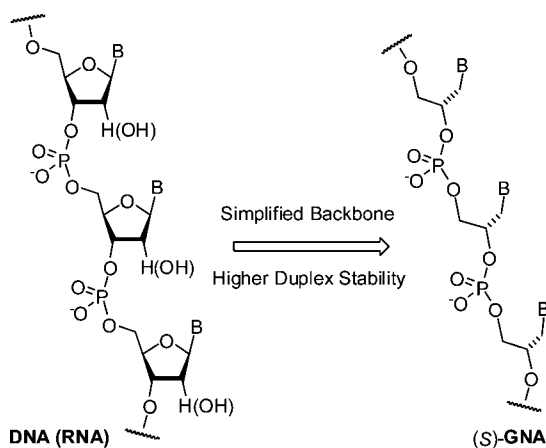


FIGURE 1. Comparison of the constitution of DNA with the (*S*)-enantiomer of GNA.

of side products, long workup times, and suboptimal yields.⁸ Furthermore, as previously reported, the **G** phosphoramidite (Figure 2) is slightly unstable, cannot be purified by chromatography, and slowly decomposes at $-20\text{ }^{\circ}\text{C}$. To address these issues we set out to modify the protection groups on the exocyclic amino groups of the GNA building blocks. For adenine and guanine we chose the *N*-dimethylformamidine group^{9–11} and an acetamide for cytosine (Figure 2). Both of these protection groups have been reported to be compatible with quicker deprotection conditions after oligonucleotide synthesis.¹²

Accordingly, using methods similar to those previously described,⁸ *O*⁶-benzylguanine (**1**)¹³ was used in the ring-opening of (*S*)-glycidyl 4,4'-dimethoxytrityl ether **2**⁸ in DMF to afford compound **3** in 47% yield (Scheme 1). The benzyl group was subsequently removed using catalytic hydrogenation to produce compound **4** in 97% yield. The *N*²-dimethylformamidine derivative was synthesized by heating a mixture of compound **4** and dimethylformamide dimethylacetal in DMF at $60\text{ }^{\circ}\text{C}$ for 1 h, affording compound **5** in 86% yield. Conversion to the phosphoramidite **G**^{*} was then accomplished in 76% yield by the reaction with 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite and substoichiometric amounts of 4,5-dicyanoimidazole (DCI)¹⁴ in CH_2Cl_2 . Gratifyingly, the new phosphoramidite **G**^{*} was stable to flash chromatography over silica gel unlike

(7) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.

(8) Zhang, L.; Peritz, A. E.; Carroll, P. J.; Meggers, E. *Synthesis* **2006**, 645–653.

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

(10) Vu, H.; McCollum, C.; Jacobsen, K.; Theisen, P.; Vinayak, R.; Spiess, E.; Andrus, A. *Tetrahedron Lett.* **1990**, *31*, 7269–7272.

(11) Vinayak, R.; Anderson, P.; McCollum, C.; Hampel, A. *Nucleic Acids Res.* **1992**, *20*, 1265–1269.

(12) For DNA, the *N*²-isobutyryl amide protection group of the guanosine nucleoside takes approximately 10 h in aqueous ammonia at $55\text{ }^{\circ}\text{C}$ to be removed. See, for example: (a) Koster, H.; Kulikowski, K.; Liese, T.; Heikens, W.; Kohli, V. *Tetrahedron* **1981**, *37*, 363–369. (b) Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397–416.

(13) Lembicz, N. K.; Grant, S.; Clegg, W.; Griffin, R. J.; Heath, S. L.; Golding, B. T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 185–186.

(14) Rosenbohm, C.; Christensen, S. M.; Sørensen, M. D.; Pedersen, D. S.; Larsen, L.-E.; Wengel, J.; Koch, T. *Org. Biomol. Chem.* **2003**, *1*, 655–663.

(1) Zhang, L.; Peritz, A.; Meggers, E. *J. Am. Chem. Soc.* **2005**, *127*, 4174–4175.

(2) Schlegel, M. K.; Peritz, A. E.; Kittigowittana, K.; Zhang, L.; Meggers, E. *ChemBioChem* **2007**, *8*, 927–932.

(3) Schlegel, M. K.; Essen, L.-O.; Meggers, E. *J. Am. Chem. Soc.* **2008**, *130*, 8158–8159.

(4) Schlegel, M. K.; Xie, X.; Zhang, L.; Meggers, E. *Angew. Chem., Int. Ed.* **2009**, *48*, 960–963.

(5) Zhang, R. S.; McCollum, E. O.; Chaput, J. C. *J. Am. Chem. Soc.* **2008**, *130*, 5846–5847.

(6) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821–3827.

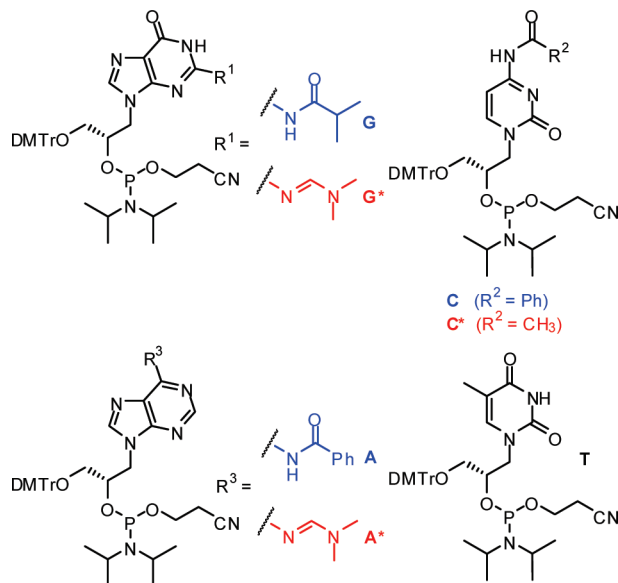
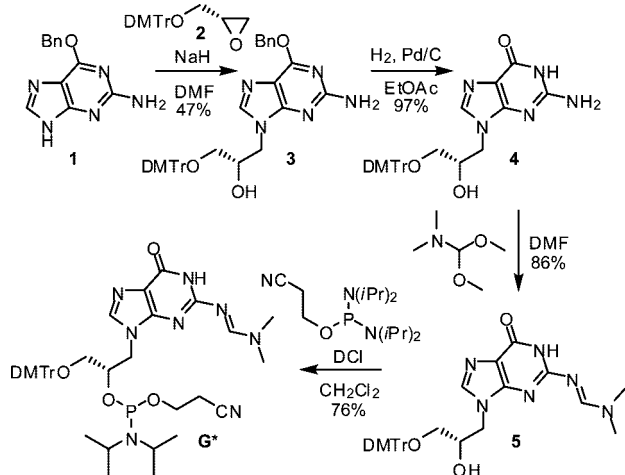


FIGURE 2. Previous (A, G, C, T) and optimized new (A*, G*, C*) GNA phosphoramidite building blocks.

SCHEME 1. Synthesis of Guanine Phosphoramidite G*

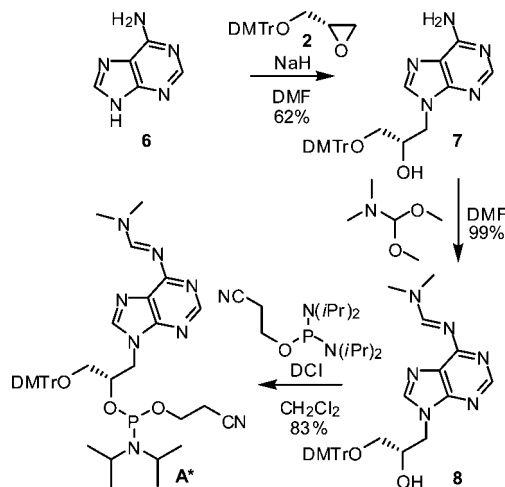


its *N*²-isobutyryl counterpart **G**. In addition, this new synthesis of **G*** has a significantly improved overall yield of 30% over five steps compared to 8% over the same number of steps for the previous phosphoramidite **G**.

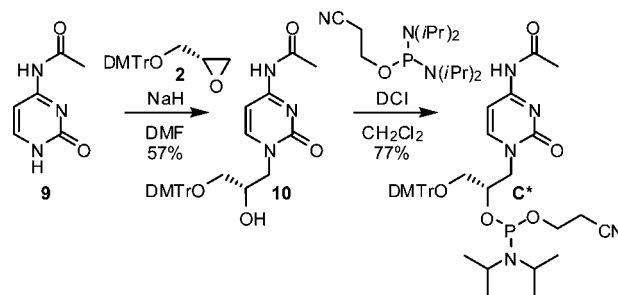
Amidine protection has been previously investigated in the context of adenosine nucleosides in an attempt to minimize acid catalyzed depurination during oligonucleotide synthesis.¹⁰ We thus next explored *N*⁶-dimethylformamidine protection of (*S*)-9-(3-(4,4'-dimethoxytrityloxy)-2-hydroxypropyl)adenine (**7**),⁸ obtained from adenine (**6**) in one step by the reaction with the tritylated glycidol **2** in the presence of catalytic amounts of NaH.⁸ The reaction of compound **7** with dimethylformamide dimethyl acetal in DMF afforded compound **8** in 99% yield (Scheme 2). The following conversion to phosphoramidite **A*** using 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite and DCI in CH₂Cl₂ could be accomplished in 83% yield. This new synthesis has a vastly improved overall yield of 51% over four steps for **A*** compared to only 26% over the same number of steps for the previous phosphoramidite **A**.

Next, in order to render the cytosine GNA building block more amenable toward milder deprotection we replaced the

SCHEME 2. Synthesis of Adenine Phosphoramidite A*



SCHEME 3. Synthesis of Cytosine Phosphoramidite C*



benzoyl protection group of **C** against an acetyl group in **C*** (Figure 2). *N*⁴-Acetylcytosine DNA phosphoramidites are widely used in mild and ultramild oligonucleotide synthesis. Accordingly, epoxide ring-opening of compound **2** using commercially available *N*⁴-acetylcytosine (**9**) in DMF with catalytic amounts of NaH afforded compound **10** in 57% yield (Scheme 3). Subsequent conversion to phosphoramidite **C*** was achieved using 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite and DCI in CH₂Cl₂ proceeded in 77%. It should be noted that attempts to purify compound **C*** using flash chromatography over silica gel were unsuccessful. However, we found that pure phosphoramidite **C*** can be isolated using basic alumina (Brockmann type II). This new synthesis has an overall yield of 44% over three steps for **C*** which is slightly better than the 39% over the same number of steps for the previous phosphoramidite **C**.

Having the three new phosphoramidites **A***, **G***, and **C*** along with the previously reported **T** phosphoramidite⁸ in hand, GNA oligonucleotides were prepared using general procedures for DNA oligonucleotides, except that the coupling time was extended to 3 min. Subsequent cleavage from the solid support and deprotection of the exocyclic amino protection groups was accomplished in only 15–20 min at 55 °C using a 1:1 mixture of 40% aqueous methylamine and 25% aqueous ammonium hydroxide (AMA).¹⁵ After cooling to room temperature, the entire solution of crude tritylated oligonucleotide was applied directly to a Sep-Pak Classic reversed-phase column and subsequently washed, detritylated with 1.5% aqueous TFA, and then eluted from the column using 20% aqueous acetonitrile.

(15) Reddy, M. P.; Hanna, N. B.; Farooqui, F. *Tetrahedron Lett.* **1994**, 35, 4311–4314.

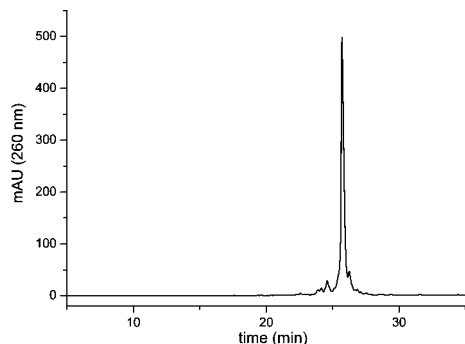


FIGURE 3. Crude HPLC trace of the 13-mer GNA strand 3'-CATGTCGTGCGTA-2' after synthesis and deprotection. The solid support for the 2'-end A nucleotide was synthesized from compound **8** as previously described.⁸ The strand was eluted (1 mL/min) over a Waters XTerra column (MS C18, 4.6 × 50 mm, 2.5 μm) at 60 °C with aqueous triethylammonium acetate buffer (50 mM, pH = 7.0) and a linear gradient of 2–8% acetonitrile over 30 min. See the Supporting Information for a MALDI-TOF spectrum of this crude GNA strand.

This improved deprotection and workup procedure represents a saving of at least 12 h for the removal of the protection groups and one HPLC purification compared to the conventional procedure previously reported for GNA oligonucleotides.⁸ The crude HPLC trace of a representative oligonucleotide solution obtained according to this protocol is shown in Figure 3 and demonstrates the high quality of the crude product.

In conclusion, we have presented an improved protection group scheme which provides a more economical route for the synthesis of GNA phosphoramidite building blocks and also quicker access to GNA oligonucleotides. These synthetic routes should be applicable to the large scale synthesis of GNA phosphoramidites and work along these lines is in progress.

Experimental Section

(S)-9-(3-(4,4'-Dimethoxytrityloxy)-2-hydroxypropyl)-O⁶-benzylguanine (3). Compound **1** (3.10 g, 12.8 mmol) was partially dissolved in anhydrous DMF (25.0 mL) under a nitrogen atmosphere. NaH was added (105 mg, 2.63 mmol, 60% in mineral oil), and the solution was allowed to stir under nitrogen for 1 h. In a separate flask, compound **2** (4.60 g, 12.2 mmol) was dissolved in 26.0 mL of DMF, added to the first solution, and then heated to 90 °C overnight. The next morning, the solution was cooled, all solvent removed, and the resulting oil coevaporated with toluene, redissolved in ethyl acetate, and concentrated again. The product was purified via column chromatography starting with 2:1:0.01 hexanes/acetone/Et₃N, then eluting with 3:2:0.01 hexanes/acetone/Et₃N to afford compound **3** as a light yellow foam (3.73 g, 47%): ¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.53 (s, 1H), 7.51 (d, *J* = 7.1 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.35–7.25 (m, 9H), 7.21 (t, *J* = 7.3 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 4H), 5.52 (s, 2H), 5.20 (br, 1H), 4.85 (s, 2H), 4.28 (m, 1H), 4.16 (m, 2H), 3.78 (s, 6H), 3.21 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.03 (dd, *J* = 9.4, 5.6 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) 161.0, 158.8, 158.7, 154.0, 144.8, 140.8, 136.5, 136.0, 135.9, 130.1, 128.5, 128.4, 128.2, 128.1, 128.0, 127.0, 115.4, 113.3, 86.4, 69.5, 68.3, 64.7, 55.3, 48.5; IR (film) ν (cm⁻¹) = 3515, 1401, 3341, 3212, 3065, 3034, 2934, 2834, 1616, 1589, 1510, 1456, 1410, 1385, 1356, 1333, 1300, 1252, 1175, 1154, 1101, 1061, 1028, 907, 828, 789, 756, 727, 698, 633, 581; HRMS calcd for C₃₆H₃₅N₅O₅Na (M + Na)⁺ 640.2530, found (M + Na)⁺ 640.2529.

(S)-9-(3-(4,4'-Dimethoxytrityloxy)-2-hydroxypropyl)guanine (4). Compound **3** (3.30 g, 5.3 mmol) and Pd/C (1.70 g, 10% on carbon) were suspended in EtOAc (125 mL), and the solution was purged with nitrogen, then hydrogen, and allowed to stir under

a hydrogen atmosphere. After 3 h, TLC showed completion of the reaction, and the mixture was filtered through Celite and washed with 5:1 CH₂Cl₂/MeOH to afford compound **4** as a tan solid (2.73 g, 97%): ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm) 10.69 (br, 1H), 7.59 (s, 1H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.35–7.17 (m, 7H), 6.88 (dd, *J* = 8.7, 1.6 Hz, 4H), 6.48 (br, 2H), 5.40 (d, *J* = 4.0 Hz, 1H), 4.11–3.90 (m, 3H), 3.74 (s, 6H), 2.98 (dd, *J* = 8.6, 3.7 Hz, 1H), 2.90 (dd, *J* = 8.6, 3.4 Hz, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ (ppm) 158.0, 156.9, 153.4, 151.3, 144.9, 138.0, 135.6, 129.7, 127.7, 126.6, 116.4, 113.1, 85.3, 68.0, 65.7, 55.0, 46.3; IR (film) ν (cm⁻¹) = 3412 (br), 3127, 2949, 2832, 1699, 1607, 1578, 1541, 1508, 1481, 1462, 1443, 1412, 1379, 1302, 1250, 1173, 1152, 1113, 1074, 1024, 986, 899, 828, 789, 777, 752, 725, 692, 629; HRMS calcd for C₂₉H₂₉N₅O₅Na (M + Na)⁺ 550.2061, found (M + Na)⁺ 550.2061.

(S)-9-(3-(4,4'-Dimethoxytrityloxy)-2-hydroxypropyl)-N²-[(dimethylamino)methylene]guanine (5). To a solution of **4** (2.63 g, 4.99 mmol) in anhydrous DMF (16.0 mL) was added dimethylformamide dimethyl acetal (2.35 mL, 17.4 mmol) and the mixture heated to 60 °C for 1 h. After cooling and removal of the DMF, the residue was redissolved in methylene chloride, washed once with saturated aqueous NaHCO₃, dried over Na₂SO₄, and finally concentrated. The product was purified via column chromatography starting with 100:1 EtOAc/Et₃N then eluting with 40:3:0.01 EtOAc/MeOH/Et₃N to afford compound **5** as a white foam (2.50 g, 86%): ¹H NMR (300 MHz, CDCl₃) δ (ppm) 9.21 (br, 1H), 8.48 (s, 1H), 7.51–7.45 (m, 3H), 7.39–7.15 (m, 7H), 6.83 (dd, *J* = 9.0, 2.3 Hz, 4H), 4.44 (m, 2H), 4.01 (dd, *J* = 14.4, 8.2 Hz, 1H), 3.78 (s, 6H), 3.36 (dd, *J* = 9.5, 4.8 Hz, 1H), 3.08 (dd, *J* = 9.4, 7.3 Hz, 1H), 3.01 (s, 3H), 2.89 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 158.7, 158.2, 157.5, 156.5, 150.3, 145.1, 139.5, 136.2, 136.1, 130.1, 128.2, 128.0, 126.9, 120.0, 113.33, 113.30, 86.4, 69.3, 65.0, 55.4, 48.6, 41.3, 35.2; IR (solid) ν (cm⁻¹) = 2929, 2836, 1630, 1558, 1506, 1444, 1416, 1399, 1345, 1326, 1300, 1245, 1174, 1110, 1066, 1024, 981, 827, 755, 726, 701, 644, 581; HRMS calcd for C₃₂H₃₄N₆O₅Na (M + Na)⁺ 605.2483, found (M + Na)⁺ 605.2477.

Phosphoramidite G*. To a solution of **5** (1.80 g, 3.09 mmol) in 15.5 mL of anhydrous methylene chloride under nitrogen was added a 1 M solution of dicyanoimidazole (2.20 mL in acetonitrile). 2-Cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (1.03 mL, 3.24 mmol) was then added dropwise and the solution stirred at room temperature. After 2 h, the reaction mixture was diluted with methylene chloride, washed twice with saturated aqueous NaHCO₃, dried over Na₂SO₄, and then concentrated. The product was purified via column chromatography starting with 1:1:0.01 hexanes/acetone/Et₃N, then eluting with 1:2:0.01 hexanes/acetone/Et₃N to afford compound **G*** as a white foam (1.85 g, 76%): ³¹P NMR (162 MHz, CDCl₃) δ (ppm) 150.3, 150.0; HRMS calcd for C₄₁H₅₂N₈O₆P (M + H)⁺ 783.3742, found (M + H)⁺ 783.3736.

(S)-9-(3-(4,4'-Dimethoxytrityloxy)-2-hydroxypropyl)-N⁶-[(dimethylamino)methylene]adenine (8). To a solution of **7** (1.32 g, 2.58 mmol) in 7.5 mL of anhydrous DMF was added dimethylformamide dimethyl acetal (1.21 mL, 9.04 mmol) and the mixture heated to 60 °C for 1 h. After cooling and removal of the DMF, the residue was redissolved in methylene chloride, washed once with saturated aqueous NaHCO₃, dried over Na₂SO₄, and finally concentrated. The product was purified via column chromatography starting with 100:1 EtOAc/Et₃N then eluting with 40:3:0.01 EtOAc/MeOH/Et₃N to afford compound **8** as a white foam (1.45 g, 99%): ¹H NMR (300 MHz, CDCl₃) δ (ppm) 8.89 (s, 1H), 8.44 (s, 1H), 7.84 (s, 1H), 7.43 (m, 2H), 7.35–7.17 (m, 7H), 6.82 (m, 4H), 4.98 (br, 1H), 4.45 (m, 1H), 4.32–4.17 (m, 2H), 3.79 (s, 6H), 3.29 (dd, *J* = 9.4, 4.7 Hz, 1H), 3.23 (s, 3H), 3.21 (s, 3H), 3.10 (dd, *J* = 9.4, 5.7 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 159.5, 158.7, 158.4, 152.1, 151.8, 144.8, 143.0, 135.91, 135.83, 130.1, 128.11, 128.00, 127.0, 125.8, 113.3, 86.5, 69.6, 64.8, 55.3, 48.7, 41.4, 35.2; IR (solid) ν (cm⁻¹) = 2929, 2836, 1630, 1558, 1506, 1444, 1416, 1399, 1345, 1326, 1300, 1245, 1174, 1110, 1066, 1024, 981, 827,

755, 726, 701, 644, 581; HRMS calcd for $C_{32}H_{35}N_6O_4$ ($M + H$)⁺ 567.2714, found ($M + H$)⁺ 567.2709.

Phosphoramidite A*. To a solution of **8** (1.82 g, 3.21 mmol) in 16.0 mL of anhydrous methylene chloride under nitrogen was added a 1 M solution of dicyanoimidazole (2.20 mL in acetonitrile). 2-Cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (1.07 mL, 3.37 mmol) was then added dropwise and the solution stirred at room temperature. After 2 h, the reaction mixture was diluted with methylene chloride, washed twice with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 , and then concentrated. The product was purified via column chromatography starting with 3:2:0.01 hexanes/acetone/ Et_3N then eluting with 1:1:0.01 hexanes/acetone/ Et_3N to afford compound **A*** as a white foam (2.05 g, 83%). ³¹P NMR (162 MHz, $CDCl_3$) δ (ppm) 150.4, 149.9; HRMS calcd for $C_{41}H_{52}N_8O_5P$ ($M + H$)⁺ 767.3793, found ($M + H$)⁺ 767.3781.

(S)-1-(3-(4,4'-Dimethoxytrityloxy)-2-hydroxypropyl)-*N*⁴-acetylcytosine (10). To a suspension of **9** (1.16 g, 7.57 mmol) in 15.0 mL of anhydrous DMF was added NaH (60 mg, 1.5 mmol, 60% in mineral oil) and the mixture allowed to stir under nitrogen for 1 h. A solution of **2** (2.71 g, 7.20 mmol) in 15.0 mL of anhydrous DMF was added to the above solution, and the reaction was heated to 110 °C overnight. The next morning, the solution was cooled, all solvent removed, and the resulting oil coevaporated with toluene, redissolved in ethyl acetate, and concentrated again. The product was purified via column chromatography starting with 3:2:0.01 hexanes/acetone/ Et_3N then eluting with 1:1:0.01 hexanes/acetone/ Et_3N to afford compound **10** as a light yellow foam (2.30 g, 57%): ¹H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.81 (br, 1H), 7.57 (d, J = 7.3 Hz, 1H), 7.41 (m, 2H), 7.34–7.17 (m, 8H), 6.82 (m, 4H), 4.33 (dd, J = 13.5, 2.6 Hz, 1H), 4.21 (br, 1H), 4.07 (br, 1H), 3.83–3.73 (m, 7H), 3.24 (dd, J = 9.6, 5.1 Hz, 1H), 3.08 (dd, J = 9.6, 6.0 Hz, 1H), 2.22 (s, 3H). ¹³C NMR (75 MHz, $CDCl_3$) δ (ppm) 171.1, 162.9, 158.7, 157.4, 150.5, 144.7, 135.75, 135.71, 130.1, 128.09, 128.03, 127.0, 113.3, 96.7, 86.4, 68.9, 64.5, 55.3, 54.6, 24.9. IR (solid) ν (cm^{-1}) = 3256, 2963, 2929, 2836, 1630, 1558, 1507, 1445, 1417, 1347, 1299, 1245, 1174, 1112, 1069, 1030, 827, 789, 755, 727, 701, 645, 582; HRMS calcd for $C_{30}H_{31}N_3O_6Na$ ($M + Na$)⁺ 552.2105, found ($M + Na$)⁺ 552.2104.

Phosphoramidite C*. To a solution of **10** (1.06 g, 2.00 mmol) in 10.0 mL of anhydrous methylene chloride under nitrogen was added a 1 M solution of dicyanoimidazole (1.40 mL in acetonitrile). 2-Cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (0.67 mL, 2.1 mmol) was then added dropwise and the solution stirred at room temperature. After 2 h, the reaction mixture was diluted with methylene chloride, washed once with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 , and then concentrated. The product was purified via column chromatography (basic alumina, Brockmann type II) starting with 100:1 EtOAc/ Et_3N then eluting with 50:1:0.01 EtOAc/MeOH/ Et_3N to afford compound **C*** as a white foam (1.12 g, 77%): ³¹P NMR (162 MHz, $CDCl_3$) δ (ppm) 150.3, 150.1; HRMS calcd for $C_{39}H_{49}N_5O_7P$ ($M + H$)⁺ 730.3364, found ($M + H$)⁺ 730.3358.

GNA Oligonucleotide Synthesis and Purification. GNA oligonucleotides were prepared on an ABI 394 DNA/RNA synthesizer on a 1 μ mol scale. GNA phosphoramidites (**A***, **G***, **C***, and **T**) were used at a concentration of 100 mM with a standard protocol for 2-cyanoethyl phosphoramidites, except that the coupling time was extended to 3 min. After the trityl-on synthesis, the resin was incubated with AMA solution (1.5 mL) for 15–20 min at 55 °C. After cooling, the entire solution was applied directly to a Sep-Pak Classic reversed-phase column (Waters, 360 mg) and washed sequentially with 3% NH_4OH (15 mL), water (10 mL), 1.5% aqueous TFA (10 mL), and finally water (10 mL). The oligo was then eluted with 20% aqueous acetonitrile and further purified using a Waters XTerra column (MS C18, 4.6 $\times$ 50 mm, 2.5 μ m) at 60 °C with aqueous TEAA (50 mM) and acetonitrile as the eluent. All identities were confirmed by MALDI-TOF MS.

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Supporting Information Available: ¹H and ¹³C NMR spectra of compounds **3–5**, **8**, and **10**; ³¹P NMR spectra for compounds **G***, **A***, and **C***. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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