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# Nucleosides, Nucleotides and Nucleic Acids

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# Solid-Phase Synthesis of Oligodeoxyribonucleotides without Base Protection Utilizing O-Selective Reaction of Oxazaphospholidine Derivatives

Natsuhisa Oka $^{\rm a}$ , Yukihiro Maizuru $^{\rm a}$ , Mamoru Shimizu $^{\rm a}$  & Takeshi Wada $^{\rm a}$ 

<sup>a</sup> Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba, Japan Published online: 24 Feb 2010.

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### SOLID-PHASE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES WITHOUT BASE PROTECTION UTILIZING *O*-SELECTIVE REACTION OF OXAZAPHOSPHOLIDINE DERIVATIVES

#### Natsuhisa Oka, Yukihiro Maizuru, Mamoru Shimizu, and Takeshi Wada

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba, Japan

 $\Box$  A study on the development of a novel method to synthesize oligodeoxyribonucleotides without base protection is described. We found that nucleoside 3'-O-oxazaphospholidine derivatives exclusively react with the hydroxy group of nucleosides in the presence of unprotected nucleobase amino groups. Since the O-chemoselectivity of the oxazaphospholidine derivatives is likely due to their ring structure, which allows the regeneration of the oxazaphospholidine derivatives from the corresponding base phosphitylation adducts via an intramolecular recyclization, the method is expected to be compatible with any kinds of acidic activators.

**Keywords** Oligonucleotide; oxazaphospholidine; base protection; phosphoramidite; *O*-selective reaction

#### INTRODUCTION

Chemically synthesized oligonucleotides and their analogues have been used for a wide range of applications (e.g., diagnostics, therapeutics, nanotechnology),<sup>[1]</sup> and the growing demands for them in recent years have created interest in the development of a more efficient method to synthesize oligonucleotides and their analogues than the conventional phosphoramidite method.<sup>[2]</sup> The use of monomers without base protection is one of the most promising approaches to date owing to their improved synthetic efficiency by eliminating at least several steps for protection and deprotection.<sup>[3,4]</sup> The method is also advantageous for the synthesis of base-labile oligonucleotide analogues because the deprotection of nucleobases, which is usually carried out under strongly basic conditions, is not required.<sup>[3,4]</sup>

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Address correspondence to Takeshi Wada, Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa 277-8562, Japan. E-mail: wada@k.u-tokyo.ac.jp

For the synthesis of oligonucleotides without base protection, a highly O-chemoselective condensation between the phosphorus atom of monomer units and the terminal hydroxy group of oligonucleotides in the presence of highly reactive unprotected nucleobase amino groups<sup>[5]</sup> is requisite. The following two approaches have achieved the O-selective condensation and the resultant synthesis of oligonucleotides without base protection. The first one is the H-phosphonate method, in which base unprotected nucleoside 3'-H-phosphonate monoesters are used as monomer units. The H-phosphonate monomers exclusively react with hydroxy groups of nucleosides in the presence of nucleobase amino groups. Relatively short oligonucleotides have been synthesized utilizing this chemoselectivity. However, it is difficult to synthesize long oligomers due to the inherent instability of the H-phosphonate diester internucleotide linkages.<sup>[4]</sup> The second approach uses base unprotected nucleoside 3'-phosphoramidites as monomer units. Since the condensation of nucleoside phosphoramidite derivatives promoted by commonly used acidic activators, such as 1H-tetrazole, does not have a high O/N-chemoselectivity, various acidic activators have been developed to achieve a highly chemoselective condensation between the phosphoramidites and nucleoside hydroxy groups by base protonation or generating reactive intermediates that have a high O-chemoselectivity. However, complete O-chemoselectivity has not been achieved in many cases, which still suffer from the generation of N-phosphitylation byproducts.<sup>[3]</sup>

On the other hand, during the course of our study on the synthesis of stereoregulated backbone-modified oligonucleotide analogues by using nucleoside 3'-O-(1,3,2-oxazaphospholidine) monomer units,<sup>[6]</sup> we have found that the oxazaphospholidine monomers do not give any N-phosphitylation byproducts when mixed with N-unprotected nucleosides and an acidic activator.<sup>[6c]</sup> This finding led us to develop a new method to synthesize oligonucleotides without base protection. The results of this study are described below.

#### **RESULTS AND DISCUSSION**

To examine the reactivity of nucleoside 3'-O-oxazaphospholidine derivatives toward nucleobase amino groups in the presence of 1*H*-tetrazole, one of the conventional activators for the phosphoramidite method,<sup>[2]</sup> we analyzed the reaction between a 3',5'-protected deoxycytidine derivative (Scheme 1, 1c) and an oxazaphospholidine derivative (*trans-3*)<sup>[6a-c]</sup> in the presence of 1*H*-tetrazole by <sup>31</sup>P NMR spectroscopy. Since *trans-3* was synthesized from racemic 2-methylamino-1-phenylethanol as a mixture of two *P*-diastereomers, the corresponding two <sup>31</sup>P NMR signals are observed (Figure 1B, 136.5, 135.6 ppm). A reaction, in which the oxazaphospholidine *trans-3* was replaced by a regular phosphoramidite monomer (2), was also



**SCHEME 1** Phosphitylation of cytosine exocyclic amino group (1c) with conventional nucleoside 3'-phosphoramidite (2) and nucleoside 3'-O-oxazaphospholidine (3).

monitored by <sup>31</sup>P NMR. The <sup>31</sup>P NMR analysis showed that the phosphoramidite **2** gave *N*-phosphitylation adducts, such as **4**,<sup>[3d]</sup> by the appearance of several new <sup>31</sup>P NMR signals around 125 ppm (Figure 1A). In sharp contrast, the reaction between the oxazaphospholidine trans-3 and 1c did not give any N-phosphitylation adducts, although the epimerization of trans-3 which generated a mixture of cis-3 (139.5, 137.6 ppm) and trans-3 was observed as reported in the previous reports (Figure 1B).<sup>[6a-c]</sup> We confirmed that trans-3 also epimerized in the absence of 1c. Therefore, the epimerization of *trans*-**3** is probably due to the repetitive attacks of 1H-tetrazole to the chiral phosphorus atom of 3, although the involvement of the intermediate 5 cannot be excluded. The experiments using the 3',5'-protected deoxyadenosine counterpart (1a) gave virtually the same results (data not shown). Thus, the experiments demonstrated that the nucleoside 3'-O-oxazaphospholidine derivative did not give any N-phosphitylation adducts in the presence of unprotected nucleobases and 1*H*-tetrazole in contrast to the conventional phosphoramidite. It can be attributed to the structure of the oxazaphospholidine ring, which allows the regeneration of the oxazaphospholidine derivative 3 from the corresponding base phosphitylation adducts via an intramolecular recyclization (Figure 1, 5), although further investigations are necessary for full clarification of the mechanism.



FIGURE 1 <sup>31</sup>P NMR analysis. A: 1c + 2 + 1H-tetrazole; B: 1c + trans-3 + 1H-tetrazole.

The results shown above prompted us to develop a new method to synthesize oligodeoxyribonucleotides by using base unprotected nucleoside 3'-O-oxazaphospholidine monomers. First, the synthesis of nucleoside 3'-O-oxazaphospholidine monomers without protection of the nucleobases was investigated. As the first design of the oxazaphospholidine monomers, we adopted the simplest ring structure derived from 2-(methylamino)ethanol.<sup>[7]</sup> In general, the nucleoside 3'-O-oxazaphospholidine derivatives are synthesized by the reaction between the 3'-OH of appropriately protected nucleosides and 2-chlorooxazaphospholidine derivatives, which are prepared from the corresponding 1,2-amino alcohols and PCl<sub>3</sub> in the presence of a tertiary amine.<sup>[7]</sup> However, the 2-chlorooxazaphospholidine derivatives prepared by this method suffer from contamination by the ammonium salt, which is difficult to remove completely even by filtration and distillation. To circumvent this problem, we chose a new synthetic route, in which N,O-bis(trimethylsilyl)-2-(methylamino)ethanol  $\mathbf{6}^{[8]}$ was allowed to react with  $PCl_3$ . Salt-free 7 was obtained after a simple vacuum distillation in good yield and used for the following synthesis of the nucleoside 3'-O-oxazaphospholidine monomers (Table 1).

The thymidine monomer **8a** was synthesized by the reaction between 5'-*O*-DMTr-thymidine and **7** in the presence of  $Et_3N$  at room temperature according to the procedure reported in the literature (Table 1, entry 1).<sup>[7]</sup> In

TMS-ON 6	TMS 0 then	PCl <sub>3</sub> °C then rt, 1 h vacuum distillation	CI PN 7 72%	5'- <i>O</i> -DMTr-nucleoside <u>Et<sub>3</sub>N</u> THF, -78 °C then rt, 30 min (8a) or -78 °C, 12 h (8b-d)	DMTrO O B O N O N Ba-d
Entry	8	В		Isolated yield (%)	Purity (%) <sup>a</sup>
1	а	thymin-1-yl		72	98
2	b	cytosine-1-y	/l	65	92
3	с	adenine-9-yl		85	94
4	d	guanine-9-y	yl	84	90

TABLE 1 Synthesis of N-unprotected nucleoside 3'-O-oxazaphospholidine monomers 8a-d

<sup>a</sup>Determined by <sup>31</sup>P NMR.

contrast, the other monomers **8b–d** have a nucleobase amino group, which can react with **7** under the same conditions. In fact, when base unprotected 5'-O-DMTr-2'-deoxycytidine, deoxyadenosine, and deoxyguanosine were reacted with **7** under the same conditions, serious base-phosphitylations were observed by <sup>31</sup>P NMR (signals at 130–120 ppm). We found that the basephosphitylations were minimized when the reactions were carried out at  $-78^{\circ}$ C. Thus, the 2-chlorooxazaphospholidine **7** reacted with the 3'-OH of 5'-O-DMTr-2'-deoxycytidine, deoxyadenosine, and deoxyguanosine almost exclusively in THF  $-78^{\circ}$ C to afford the desired monomer units **8b–d** in modest to good yields. The monomer units of sufficient purity were obtained by simple precipitation. The monomer units were stable at least for several months under proper storage conditions (at  $-30^{\circ}$ C in a glass vial flushed with an inert gas).

With the base-unprotected nucleoside 3'-O-oxazaphospholidine monomer units 8 in hand, a d[CAGT] 4mer was manually synthesized on a controlled-pore glass (CPG). The synthesis cycle is given in Table 2. The

Step	Operation	Reagents and solvents	Time
1	detritylation	3% DCA in CH <sub>2</sub> Cl <sub>2</sub>	30 seconds
2	washing	(i) CH <sub>2</sub> Cl <sub>2</sub> (ii) dry CH <sub>3</sub> CN (iii) drying in vacuo.	—
3	coupling	8 (0.09 M, 40 equiv) and 1 <i>H</i> -tetrazole (0.45 M, 200 equiv) in dry CH <sub>3</sub> CN under argon	3 minutes
4	washing	<ul><li>(i) dry CH<sub>3</sub>CN (ii) dry NMP (for dG)</li><li>(iii) drying in vacuo.</li></ul>	—
5	oxidation	<i>t</i> -BuOOH (1 M) in dry toluene	3 minutes
6	washing	(i) dry CH <sub>3</sub> CN (ii) CH <sub>2</sub> Cl <sub>2</sub>	—

TABLE 2 Procedure for manual solid-phase synthesis (0.5  $\mu$ mol)



**FIGURE 2** A) Anion-exchange HPLC profile: a linear gradient of 0–30% 1 M NaCl in 10 mM phosphate buffer (pH 6.9) at 50°C for 30 minutes at a rate of 0.5 mL/min using Gen-Pak FAX column (4.6 × 100 mm) (Waters). B) RP-HPLC profile: a linear gradient of 0–20% acetonitrile in 0.1 M ammonium acetate buffer (pH 7.0) at 50°C for 60 minutes at a rate of 0.5 mL/min using a  $\mu$ Bondasphere 5  $\mu$ m C<sub>18</sub> column (3.9 × 150 mm) (Waters, Milford, MA, USA).

average coupling yield estimated by DMTr<sup>+</sup> assay was 96%. It should be noted that the solid-support was washed with *N*-methylpyrrolidone (NMP) after the cycle to incorporate a dG to wash away the dG-monomer and/or its residue, which had rather low solubility to the organic solvents used for the synthesis. After the chain elongation, the DMTr-on CPG was treated with conc. NH<sub>3</sub> at 55°C overnight for deprotection and cleavage from the support. The 5'-O-DMTr group was then removed by treatment with 80% AcOH, and the crude d[CAGT] was analyzed by anion-exchange and reserve-phase HPLC. The analysis showed that the desired 4mer was obtained as a major product (Figure 2).

Since the deprotection of the internucleotidic phosphates required somewhat harsh conditions (The RP-HPLC profile indicates that small amounts of oligos having one or two protected phosphate groups remained even after the treatment with conc.  $NH_3$  at 55°C for about 12 hours), it would be necessary to redesign the oxazaphospholidine ring structure for the synthesis of base-labile oligonucleotide analogues. Fortunately, preliminary experiments with a dithymidine phosphate derivative showed that the deprotection of the phosphate was completed under milder conditions (conc  $NH_3$ , room temperature, 30 minutes or 0.2 M DBU in MeCN, room temperature, 30 minutes) when the oxazaphospholidine monomer *trans-3* was used in the place of **8**, indicating that the method developed in this study is potentially applicable to the synthesis of base-labile oligonucleotides, such as oligoribonucleotides.

#### CONCLUSION

In conclusion, we demonstrated that the nucleoside 3'-O-oxazaphospholidine derivatives had a complete O/N-chemoselectivity and were useful for the synthesis of DNA oligomers without base protection. Since the chemoselectivity of the oxazaphospholidine derivatives is likely due to their ring structure, which allows the regeneration of the oxazaphospholidine derivatives from the corresponding base phosphitylation adducts via an intramolecular recyclization, it is expected to be compatible with any kinds of acidic activators. Further investigation on the synthesis of oligonucleotides and their analogues based on this new concept is now in progress.

#### **EXPERIMENTAL**

#### General

Infrared (IR) spectra were recorded on a JASCO (Tokyo, Japan) FT/IR-480 Plus spectrophotometer. All NMR spectra were recorded on a Varian (Palo Alto, CA, USA) Mercury 300. <sup>1</sup>H NMR spectra were obtained at 300 MHz with tetramethylsilane (TMS;  $\delta$  0.0) as an internal standard in CDCl<sub>3</sub>. <sup>13</sup>C NMR spectra were obtained at 75.5 MHz with CDCl<sub>3</sub> as an internal standard ( $\delta$  77.0) in CDCl<sub>3</sub>. <sup>31</sup>P NMR spectra were obtained at 121.5 MHz with 85% H<sub>3</sub>PO<sub>4</sub> ( $\delta$  0.0) as an external standard in CDCl<sub>3</sub>. Dry THF was prepared by distillation from sodium benzophenone ketyl under argon prior to use. Other dry organic solvents were prepared by appropriate procedures. The other organic solvents were reagent grade and used as received. Manual solid-phase synthesis was performed by using a glass filter (10 mm × 50 mm) with a stopper at the top and a stopcock at the bottom as a reaction vessel. Controlled-pore glass (CPG) was purchased from 3-Prime (Aston, PA, USA).

*N,O-Bis(trimethylsilyl)-2-(methylamino)ethanol* (6). Compound 6 was synthesized according to the procedure described in the literature.<sup>[8]</sup> 2-(Methylamino)ethanol (8.03 mL, 100 mmol) was dissolved in dry Et<sub>2</sub>O (100 mL) under argon and the mixture was cooled with a dry ice–hexane bath. A 2.67 M solution of BuLi in hexane (78.7 mL, 210 mmol) was added dropwise to the mixture over 30 minutes with stirring. The cooling bath was removed and the mixture was stirred for 30 minutes. The mixture was then cooled again with a dry ice–hexane bath and TMSCl (26.5 mL, 210 mmol) was added dropwise over 10 minutes. The mixture was then stirred for 2 hours at room temperature. Any volatile reagents were removed by distillation at atmospheric pressure under argon (bath temperature = 80°C) and the residue was purified by vacuum distillation to afford **6** (15.9 g, 72 mmol, 72%) as a colorless liquid. b.p. 78–79 °C/12 mmHg (lit.<sup>[8]</sup> 86°C/17 mmHg).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  3.54 (t, J = 6.9 Hz, 2H), 2.83 (t, J = 6.9 Hz, 2H), 2.48 (s, 3H), 0.13 (s, 9H), 0.06 (s, 9H).

2-Chloro-3-methyl-1,3,2-oxazaphospholidine (7).<sup>[7,9]</sup> N,O-Bis (trimethylsilyl)-2- (methylamino) ethanol (6) (15.9 g, 72 mmol) was added dropwise to PCl<sub>3</sub> (6.28 mL, 72 mmol) at 0°C under argon, and the mixture was stirred for 1 hour at room temperature. The resultant TMSCl was removed by distillation at atmospheric pressure under argon (bath temperature = 100°C) and the residue was purified by vacuum distillation to afford 7 (9.3 g, 67 mmol, 93%) as a colorless liquid. b.p. 77–78°C/15 mmHg (lit.<sup>[9]</sup> 57–58°C/2 mmHg). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  4.61 (brs, 1H), 4.33 (brs, 1H), 3.18 (m, 2H), 2.77 (d, J = 13.5 Hz, 3H). <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>)  $\delta$  169.5.

5'-O-DMTr-thymidine 3'-O-oxazaphospholidine (8a).<sup>[7]</sup> 5'-O-DMTr-thymidine (0.545 g, 1.0 mmol) was dried by repeated coevaporations with dry pyridine and dry toluene under argon and dissolved in freshly distilled THF (12.0 mL). Et<sub>3</sub>N (0.67 mL, 5.0 mmol) was added, and the mixture was cooled to  $-78^{\circ}$ C. The 2-chlorooxazaphospholidine 7 (0.167 g, 1.2 mmol) was then added dropwise, and the mixture was stirred for 30 minutes at room temperature. The mixture was poured into a saturated NaHCO<sub>3</sub> aqueous solution (100 mL) and extracted with CHCl<sub>3</sub> (100 mL). The organic layer was washed with saturated NaHCO<sub>3</sub> aqueous solutions  $(2 \times 100 \text{ mL})$ and combined aqueous layers were back-extracted with  $CHCl_3$  (100 mL). The organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to dryness under reduced pressure. The residue was precipitated from dry hexane (100 mL) and dried in vacuo to afford 8a (0.470 g, 0.72 mmol, 72%) as a white powder. 98% purity (<sup>31</sup>P NMR). 50:50 mixture of P-diastereomers (<sup>1</sup>H NMR). IR (KBr) 3422, 3194, 3060, 2931, 2836, 1691, 1608, 1509, 1466, 1274, 1252, 1177, 1065, 1033, 927, 830, 792, 757, 728, 702,  $585 \text{ cm}^{-1}$ . <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.93 (brs, 1H), 7.60 (d, I = 0.9 Hz, 1H), 7.40–7.22 (m, 9H), 6.82 (d, J = 8.7 Hz, 4H), 6.35 (m, 1H), 4.77 (m, 1H), 4.27 (m, 1H), 4.14 (m, 1H), 4.02 (m, 1H), 3.78 (s, 6H), 3.54-3.44 (m, 1H), 3.32 (ddd, J = 10.4, 6.2, 2.7 Hz, 1H), 3.07 (m, 1H), 2.93 (m, 1H), 2.68(d, I = 12.3 Hz, 1.5H), 2.62 (d, I = 12.3 Hz, 1.5H), 2.48-2.25 (m, 2H), 1.42(d, I = 0.9 Hz, 1.5 H), 1.41 (d, I = 0.9 Hz, 1.5 H).<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 163.5, 158.4, 150.1, 144.0, 143.9, 135.3, 135.1, 135.0, 135.0, 129.9, 127.9, 127.8, 126.9, 113.0, 111.1, 86.8, 86.7, 85.5, 85.4, 84.4, 84.3, 72.6 (d,  ${}^{2}J_{PC} =$ 14.7 Hz), 72.3 (d,  ${}^{2}J_{PC} = 15.3$  Hz), 68.8 (d,  ${}^{2}J_{PC} = 3.5$  Hz), 68.7 (d,  ${}^{2}J_{PC} =$ 3.5 Hz), 62.9, 62.8, 55.2, 49.0 (d,  ${}^{2}I_{PC} = 4.9$  Hz), 49.0 (d,  ${}^{2}I_{PC} = 4.9$  Hz), 40.4 (d,  ${}^{2}I_{PC} = 4.3$  Hz), 40.2, 31.5 (d,  ${}^{2}I_{PC} = 21.1$  Hz), 31.5 (d,  ${}^{2}I_{PC} = 21.2$ Hz), 11.7, 11.7. <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>) δ 139.9, 139.7.

5'-O-DMTr-2'-deoxycytidine 3'-O-oxazaphospholidine (8b). 5'-O-DMTr-2'deoxycytidine (0.530 g, 1.0 mmol) was dried by repeated coevaporations with dry pyridine and dry toluene under argon and dissolved in freshly distilled THF (12.0 mL). Et<sub>3</sub>N (0.67 mL, 5.0 mmol) was added, and the mixture was cooled to  $-78^{\circ}$ C. The 2-chlorooxazaphospholidine 7 (0.210 g,

1.5 mmol) was then added dropwise, and the mixture was stirred for 12 hours at  $-78^{\circ}$ C. The mixture was poured into a saturated NaHCO<sub>3</sub> aqueous solution (100 mL) and extracted with  $CHCl_3$  (100 mL). The organic layer was washed with saturated NaHCO<sub>3</sub> aqueous solutions ( $2 \times 100$  mL) and combined aqueous layers were back-extracted with CHCl<sub>3</sub> (100 mL). The organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to dryness under reduced pressure. The residue was precipitated from dry Et<sub>2</sub>O (100 mL) and dried in vacuo to afford **8b** (0.413 g, 0.65 mmol, 65%) as a white powder. 92% purity (<sup>31</sup>P NMR). 50:50 mixture of P-diastereomers (<sup>31</sup>P NMR). IR (KBr) 3351, 3194, 2934, 2837, 1652, 1509, 1410, 1362, 1286, 1252, 1178, 1115, 1071, 1033, 927, 829, 791, 757, 727, 702, 585 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.89 (d, J = 6.6 Hz, 0.5H), 7.87 (d, I = 6.6 Hz, 0.5 H), 7.43-7.22 (m, 9H), 6.84 (d, I = 8.4 Hz, 4 H), 6.25 (t, I = 8.4 Hz, 4 Hz), 6.25 (t, I = 8.4 Hz),I = 5.7 Hz, 1H), 5.49 (d, I = 7.8 Hz, 0.5H), 5.46 (d, I = 7.5 Hz, 0.5H), 4.72 (m, 1H), 4.26 (m, 1H), 4.12 (m, 1H), 4.00 (m, 1H), 3.78 (s, 6H), 3.41 (m, 1H), 3.05 (m, 1H), 2.91 (m, 1H), 2.65 (d, J = 12.3 Hz, 1.5H), 2.59 (d, J = 12.3 Hz, 1.5H), 2.5H), 2.5H, 1.5H), 2.5H, 1.5H, 2.5H, 1.5H), 2.5H, 1.5H, 1.5H), 2.5H, 1.5H, 2.5H, 1.5H, 1.5H), 2.5H, 1.5H, 1.5H, 2.5H, 1.5H), 2.5H, 1 12.3 Hz, 1.5H), 2.54–2.43 (m, 1H), 2.26–2.16 (m, 1H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) & 165.6, 158.5, 155.8, 144.4, 144.3, 140.9, 135.4, 135.4, 135.3, 130.1, 128.1, 127.9, 126.9, 113.1, 86.6, 85.6, 85.5, 84.9, 71.2 (d,  ${}^{2}J_{PC} = 14.6$  Hz), 70.8 (d,  ${}^{2}I_{PC} = 14.7 \text{ Hz}$ ), 68.8, 68.7, 62.1, 61.9, 55.2, 55.2, 49.0 (d,  ${}^{2}I_{PC} = 4.9$ Hz), 49.0 (d,  ${}^{2}I_{PC} = 5.2$  Hz), 41.1, 40.9, 31.5 (d,  ${}^{2}I_{PC} = 21.4$  Hz), 31.4 (d,  ${}^{2}I_{PC} = 21.0 \text{ Hz}$ ).  ${}^{31}P$  NMR (121 MHz, CDCl<sub>3</sub>)  $\delta$  140.8, 140.4.

5'-O-DMTr-2'-deoxyadenosine 3'-O-oxazaphospholidine (8c). 5'-O-DMTr-2'deoxyadenosine (0.554 g, 1.0 mmol) was dried by repeated coevaporations with dry pyridine and dry toluene under argon and dissolved in freshly distilled THF (12.0 mL). Et<sub>3</sub>N (0.67 mL, 5.0 mmol) was added, and the mixture was cooled to  $-78^{\circ}$ C. The 2-chlorooxazaphospholidine 7 (0.210 g, 1.5 mmol) was then added dropwise, and the mixture was stirred for 12 hours at  $-78^{\circ}$ C. The mixture was poured into a saturated NaHCO<sub>3</sub> aqueous solution (100) mL) and extracted with CHCl<sub>3</sub> (100 mL). The organic layer was washed with saturated NaHCO<sub>3</sub> aqueous solutions ( $2 \times 100$  mL) and combined aqueous layers were back-extracted with  $CHCl_3$  (100 mL). The organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to dryness under reduced pressure. The residue was precipitated from dry hexane (100 mL) and dried in vacuo to afford 8c (0.556 g, 0.85 mmol, 85%) as a white powder. 94% purity (<sup>31</sup>P NMR). 55:45 mixture of *P*-diastereomers (<sup>31</sup>P NMR). IR (KBr) 3328, 3174, 2931, 2836, 1642, 1605, 1509, 1470, 1445, 1418, 1364, 1331, 1301, 1251, 1177, 1073, 1033, 927, 829, 798, 756, 727, 702, 651, 585 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.30 (s, 0.5H), 8.30 (s, 0.5H), 8.00 (s, (0.5H), 7.99 (s, 0.5H), 7.42–7.18 (m, 9H), 6.80 (d, I = 7.8 Hz, 4H), 6.42 (m, 1H), 5.86 (brs, 2H), 4.88 (m, 1H), 4.35 (m, 1H), 4.17 (m, 2H), 3.78 (s, 6H), 3.43-3.31 (m, 2H), 3.12 (m, 1H), 2.96 (m, 1H), 2.84 (m, 1H), 2.69 (d, J =12.0 Hz, 1.5H), 2.66 (d, I = 12.0 Hz, 1.5H), 2.57–2.47 (m, 1H). <sup>13</sup>C NMR (75) MHz, CDCl<sub>3</sub>)  $\delta$  158.5, 155.4, 152.9, 149.6, 144.5, 144.4, 138.9, 138.8, 135.7, 135.6, 135.6, 130.0, 128.1, 127.8, 126.9, 120.0, 113.1, 86.5, 85.7, 84.3, 84.2, 72.6 (d,  ${}^{2}J_{PC} = 10.0$  Hz), 69.0, 68.8, 63.2, 63.1, 55.2, 55.2, 49.3 (d,  ${}^{2}J_{PC} = 5.4$  Hz), 49.1 (d,  ${}^{2}J_{PC} = 5.5$  Hz), 40.0, 39.8, 31.6 (d,  ${}^{2}J_{PC} = 21.9$  Hz).  ${}^{31}$ P NMR (121 MHz, CDCl<sub>3</sub>)  $\delta$  139.1, 138.0.

5'-O-DMTr-2'-deoxyguanosine 3'-O-oxazaphospholidine (3d). 5'-O-DMTr-2'deoxyguanosine (0.285 g, 0.50 mmol) was dried by repeated coevaporations with dry pyridine and dry toluene under argon and dissolved in freshly distilled THF (6.0 mL). Et<sub>3</sub>N (0.35 mL, 2.5 mmol) was added, and the mixture was cooled to  $-78^{\circ}$ C. The 2-chlorooxazaphospholidine 7 (0.105 g, 0.75 mmol) was then added dropwise, and the mixture was stirred for 12 hours at  $-78^{\circ}$ C. The mixture was poured into a saturated NaHCO<sub>3</sub> aqueous solution (50 mL) and extracted with CHCl<sub>3</sub> (50 mL). The organic layer was washed with saturated NaHCO<sub>3</sub> aqueous solutions  $(2 \times 50 \text{ mL})$  and combined aqueous layers were back-extracted with CHCl<sub>3</sub> (50 mL). The organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to dryness under reduced pressure. The residue was precipitated from dry Et<sub>2</sub>O (50 mL) and dried in vacuo to afford 8d (0.285 g, 0.42 mmol, 84%) as a white powder. 90% purity (<sup>31</sup>P NMR). 51:49 mixture of *P*-diastereomers (<sup>31</sup>P NMR). IR (KBr) 3343, 3206, 2933, 2837, 1688, 1607, 1532, 1509, 1465, 1445, 1413,  $1360, 1302, 1251, 1177, 1073, 1033, 927, 830, 784, 756, 728, 702, 584 \text{ cm}^{-1}$ . <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  10.4, 7.83 (d, I = 3.6 Hz, 1H), 7.36–7.21 (m, 9H), 6.85 (d, I = 8.7 Hz, 2H), 6.85 (d, I = 9.0 Hz, 2H), 6.50 (brs, 2H), 6.11(m, 1H), 4.68 (m, 1H), 4.25 (m, 1H), 4.07 (m, 1H), 3.88 (m, 1H), 3.73 (s, 6H), 3.24-3.09 (m, 2H), 3.03-2.90 (m, 2H), 2.79-2.70 (m, 1H), 2.56 (d, J =11.7 Hz, 1.5H), 2.54 (d, J = 12.6 Hz, 1.5H), 2.40–2.28 (m, 1H). <sup>13</sup>C NMR (75) MHz, CDCl<sub>3</sub>) δ 159.0, 157.7, 154.7, 152.0, 145.7, 136.4, 135.8, 130.6, 128.8, 128.6, 127.7, 117.7, 114.1, 86.6, 85.5, 83.1, 72.9 (d,  ${}^{2}J_{PC} = 9.5$  Hz), 69.8, 69.7, 64.3, 64.1, 56.0, 55.9, 49.7 (d,  ${}^{2}J_{PC} = 5.5$  Hz), 49.4 (d,  ${}^{2}J_{PC} = 5.4$  Hz), 39.3, 39.0, 31.9 (d,  ${}^{2}J_{PC} = 22.5$  Hz).  ${}^{31}P$  NMR (121 MHz, CDCl<sub>3</sub>)  $\delta$  136.5, 135.3.

A general procedure for manual solid-phase synthesis of d[CAGT]. 5'-O-DMTrthymidine-loaded CPG (0.5  $\mu$ mol) via a succinyl linker was used for the synthesis. Chain elongation was performed by repeating the steps in Table 2. After the chain elongation, the CPG was treated with conc NH<sub>3</sub> (5 mL) in a tightly-sealed flask at 55°C overnight. The CPG was then filtered off, and the filtrate was concentrated under reduced pressure. The residue was treated with an 80% AcOH aqueous solution at room temperature for 1 hour and concentrated under reduced pressure. The residue was dissolved in H<sub>2</sub>O (5 mL), washed with Et<sub>2</sub>O (3 × 5 mL), and concentrated to dryness under reduced pressure. The resultant crude d[CAGT] was analyzed by anion-exchange and reverse-phase HPLC. The main product was identical to an authentic sample prepared by the conventional phosphoramidite method.<sup>[2]</sup>

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