Chemical Synthesis of Oligodeoxyribonucleotides Using *N*-Unprotected *H*-Phosphonate Monomers and Carbonium and Phosphonium Condensing Reagents: *O*-Selective Phosphonylation and Condensation

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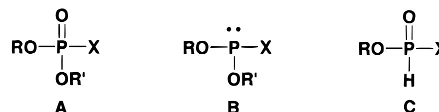
Abstract: Oligodeoxyribonucleotides were synthesized using *H*-phosphonate monomers without amino protection. The *H*-phosphonate monomers of deoxyadenosine, deoxycytidine, and deoxyguanosine bearing the free amino groups were synthesized in good yields by *O*-selective phosphonylation of the parent 5′-*O*-(dimethoxytrityl)deoxyribonucleosides. It was found that the amino groups of the nucleosides were not modified during internucleotidic bond formation where (benzotriazol-1-yloxy)carbonium and -phosphonium compounds were employed as condensing reagents. The most effective condensing reagent for rapid internucleotidic bond formation was found to be 2-(benzotriazol-1-yloxy)-1,1-dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (BOMP). In the present *H*-phosphonate method, 2-(phenylsulfonyl)-3-(3-nitrophenyl)oxaziridine (PNO) was employed as a new oxidizing reagent for the oxidation of internucleotidic *H*-phosphonate linkages under anhydrous conditions in the presence of *N,O*-bis(trimethylsilyl)acetamide. The reaction mechanism for the *O*-selective condensation was investigated in detail by means of ^{31}P NMR spectroscopy. Unprecedented oxidation of the *H*-phosphonate monomers was observed during activation of the monomers with (benzotriazol-1-yloxy)phosphonium and -carbonium condensing reagents in the absence of the 5′-hydroxyl components. A mechanism for the *O*-selective condensation was proposed on the basis of *ab initio* molecular orbital calculations for the model compounds at the HF/6-31G* level.

Introduction

From the chemical point of view, the amino groups of nucleosides have sufficient reactivities toward electrophiles. It is well known that a pentavalent phosphorus(V)-type phosphorylating reagent (type A)[1−10] and a trivalent phosphorus(III)-type phosphitylating reagent (type B)[10−13] will react with the exocyclic amino groups of nucleosides (Scheme 1). In some cases, reagents of both the types react with the 6-*O*-position of the guanine and the 4-*O*-position of the uracil and thymine bases.[14−22] Therefore, in the current synthesis of DNA and RNA as well as their analogs, the nucleobases are generally blocked by base-labile protecting groups during chain elongation.[23] However, highly base-sensitive DNA analogs, which readily decompose under the standard basic conditions prescribed for removal of the protecting groups, could not be synthesized with such protection modes. Several examples have been reported of using amino protecting groups which can be deprotected under neutral or mild basic conditions for the synthesis of methylphosphonate,[24−27] phenylphosphonate,[28] alkyl phosphate,[28−33] and phosphoramidate[34,35] analogs of oligodeoxyribonucleotides. A more straightforward approach for

Scheme 1



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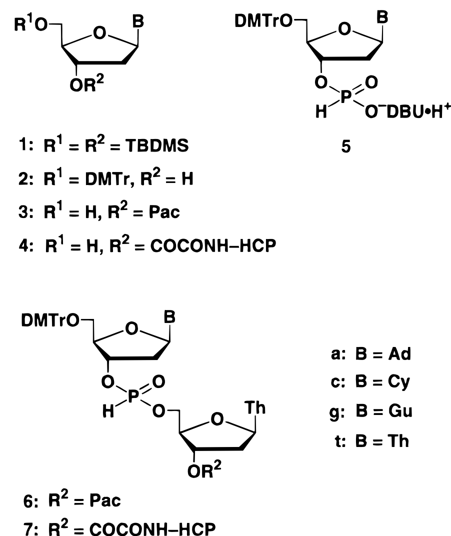
synthesizing such labile DNA analogs is a method utilizing nucleotide building blocks having unprotected nucleobases. Indeed, Letsinger *et al.* have described a pioneering study of the phosphoramidite method which utilizes monomers with unprotected bases.[11,12] They have reported that the free amino groups of the nucleobases are phosphitylated with activated phosphoramidite reagents, making an additional step for P−N bond cleavage ultimately necessary for oligonucleotide synthesis.

On the other hand, *N*-protected *H*-phosphonate oligonucleotides have been used as versatile intermediates for the synthesis of oligodeoxyribonucleotides[36] and their analogs bearing phosphorothioate,[37,38] phosphoramidate,[38,39] methyl phosphate,[38] methylphosphonate,[40] alkylphosphonate,[41] *S*-aryl phosphorothioate,[41] acylphosphonate,[41] phosphorofluoridate,[42] phosphorodithioate,[43] selenophosphate,[44] (hydroxymethyl)phosphonate,[45] and boranophosphate[46] internucleotidic linkages. Jones *et al.* have also described one variation of the *H*-phosphonate method which uses monomers having unprotected bases.[47] However, the free amino groups of the nucleobases are acylated to some extent with the condensing reagents. These modifications are removed by treatment with aqueous ammonia, but such a treatment is only possible for the sufficiently stable oligonucleotide derivatives. In addition, it has been observed that in the *H*-phosphonate approach, undesirable side reactions such as modifications of the 5′-hydroxyl group by condensing reagents,[48−50] overactivation of *H*-phosphonate monomers,[40,51,52] acylation of the internucleotidic linkages,[49,53,54] and hydrolysis of the *H*-phosphonate backbone during the oxidation step[36a,55] sometimes occur. In order to exclude these troublesome reactions, a completely different approach in *H*-phosphonate chemistry should be explored.

Scheme 2



In this paper, we report in detail our studies of a novel *H*-phosphonate method which utilizes *N*-unprotected monomers, phosphonium and carbonium condensing reagents, and a new type of oxidizing reagent.

Results and Discussion

Synthesis of *H*-Phosphonate Monomers with Unprotected Bases. Few examples of the *O*-selective phosphorylation of *N*-unprotected nucleosides have been reported in the literature. Yoshikawa *et al.* have described the phosphorylation of *N*-unprotected ribonucleosides with phosphorus oxychloride in the presence of water.[56] Hayakawa *et al.* have recently described a new method for the *O*-selective phosphorylation of *N*-unprotected nucleosides.[57] In this case, the *O*-selective activation by the metalloorganic bases resulted in *O*-selective phosphorylation. On the contrary, we have recently reported the synthesis of *N*-phosphorylated ribonucleoside derivatives.[10] In connection with this study, we have screened a wide range of reactions of *O*-protected deoxyadenosine, deoxycytidine, and deoxyguanosine derivatives (**1a**, **1c**, **1g**; Scheme 2) having free amino groups with a number of types of active phosphorus compounds. It was found that the commonly used trivalent phosphorus reagents (Scheme 1, type B) prescribed for the synthesis of *H*-phosphonate monoesters such as tris(1,2,4-triazol-1-yl)phosphine,[55] tris(imidazol-1-yl)phosphine,[58] and 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one[59] react with the *N*-unprotected nucleosides under the standard conditions. In contrast,

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phosphonylating reagents containing the P−H function (Scheme 1, type C) such as pyrophosphonate,[60] bis(1,1,1,3,3,3-hexafluoro-2-propyl) phosphonate,[61] and diphenyl phosphonate[62−64] were completely inert to the amino groups of any nucleosides. By using these reagents, *O*-selective phosphonylation of *N*-unprotected nucleosides was examined. For instance, 5′-*O*-dimethoxytritylated deoxyadenosine (**2a**), deoxycytidine (**2c**), or deoxyguanosine (**2g**) was allowed to react with diphenyl phosphonate in pyridine at room temperature (rt) for 20 min.[62] After the subsequent hydrolysis in the presence of triethylamine, the triethylammonium salts of the *H*-phosphonate monomers having free amino groups were obtained in high yields. It was found that when the deoxycytidine unit was rendered anhydrous by repeated coevaporation with pyridine, triethylamine was gradually lost from the monomer unit and marginally soluble precipitates were formed. Therefore, at the final step of its preparation, the triethylammonium salt was converted to the more stable DBU salt.[36a,65] In this manner, the DBU salts of the *H*-phosphonate monomers **5a**, **5c**, and **5g** were isolated in 88, 91, and 94% yields, respectively.[66] When pyrophosphonate[60,67] was used in place of diphenyl phosphonate, the reaction proceeded slowly, and several hours were required to complete the reaction. In these cases, the yields of the monomers were 74−87%.

***O*-Selective Formation of *H*-Phosphonate Internucleotidic Linkages.** In order to synthesize the *H*-phosphonate oligonucleotide intermediates bearing unmodified bases, neither the activated monomers nor the condensing reagents must react with the exocyclic amino groups. It has been reported that condensing reagents such as pivaloyl chloride,[47] adamantanecarbonyl chloride,[47] and diphenyl phosphorochloridate,[6,7] which are commonly used in the *H*-phosphonate method, react with the unprotected nucleobases. In the *H*-phosphonate approach using *N*-unprotected monomers and acyl chloride as a condensing reagent, there is no evidence of the *N*-phosphonylation during condensation.[47] Careful TLC monitoring of the reactions between *O*-protected adenosine (**1a**), cytidine (**1c**), and guanosine (**1g**), which have free amino groups, with 10 equiv of thymidine 3′-*H*-phosphonate **5t** in the presence of 50 equiv of pivaloyl chloride in pyridine indicated that, in the case of **1c** and **1g**, only the *N*-acylated products were observed. In contrast, the deoxyadenosine derivative **1a** gave both *N*-acylated and *N*-phosphitylated products.[68] A detailed analysis of these reactions by means of ^{31}P NMR indicated that thymidine 3′-bis(pivaloyl) phosphite[51] (125.05 ppm) is the major product in all cases. In the case of the deoxyadenosine derivative **1a**, additional signals were observed at 132.61 and 132.87 ppm.[69] These results strongly suggest that there is a base modification of the adenine base by the monomer **5t**, which is activated by the condensing reagent. It was found that *N,N*-bis(2-oxo-3-oxazolidin-1-yl)phosphinic chloride (BOP-Cl)[70] did not react with the free amino groups of the nucleosides, but a large excess of the *H*-phosphonate monomer **5t** activated by the condensing reagent[52] reacted to some extent with the amino groups of **1a** and **1c**.

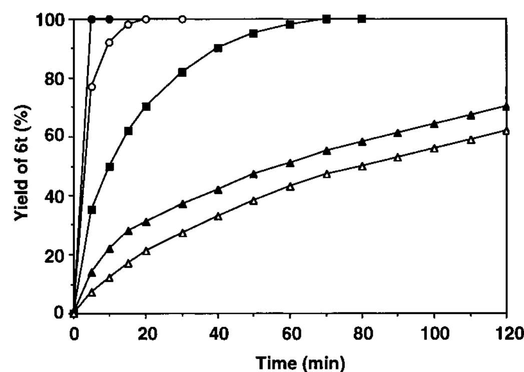

Figure 1. Condensation of **5t** with **3t** (2 equiv) in the presence of a carbonium type of condensing reagent (5 equiv) in CD_3CN−pyridine (1:1, v/v) at 25 °C. Data in parentheses refer to the half-lifetimes of **5t**: ●, **10b** (<1 min); ○, **8d** (2 min) ■, **8b** + 10 equiv of MeIm (10 min); ▲, **9b** (60 min); △, **8b** (80 min).

A few examples have been reported of carbonium (**8**−**10**) and phosphonium (**11** and **12**) condensing reagents, which were originally developed for peptide synthesis, as activators for phosphorus compounds such as phosphates,[71] dithiophosphates,[72] alkylphosphonates,[73a] and phenylphosphonates.[73b] Takaku *et al.* have reported the use of 2-chloro-1,3-dimethylimidazolium chloride (**10i**) as a condensing reagent in the *H*-phosphonate method.[74,75] However, we found that the activated *H*-phosphonate monomer **5t** by this reagent reacted significantly with the amino groups of **1a**, **1c**, and **1g**. In a similar manner, halophosphonium types of reagents (**12i**[76] and **12j**[77]) resulted in modifications of the base moieties of nucleosides. Consequently, these reagents are only effective for the method utilizing *N*-protected monomers.

After extensive screening, we ultimately found that a (benzotriazol-1-yloxy)carbonium or a -phoshonium type of condensing reagent and the activated monomers produced by these reagents did not affect the amino groups of the nucleosides. The *H*-phosphonate monomer **5t** was condensed with 2 equiv of 3′-*O*-(phenoxyacetyl)thymidine **3t** in the presence of a condensing reagent (5 equiv) in CD_3CN−pyridine (1:1, v/v) at 25 °C, and the reaction was monitored by ^{31}P NMR (Figures 1 and 2). HBTU (**8b**)[78] and BOP (**11b**),[79] widely used for peptide synthesis, gave relatively slow rates of internucleotidic bond

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(69) After addition of water to the reaction mixture, these signals were converted to the signals at 7.64 ppm (J_{PH} = 683.6 Hz) and 8.53 ppm (J_{PH} = 679.9 Hz). Characterization of these products is now in progress.
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Scheme 3

$(Me_2N)_2\text{-}\overset{+}{C}\text{-}X\ PF_6^-$ **8**; $(\text{pyrrolidin-1-yl})_2\text{-}\overset{+}{C}\text{-}X\ PF_6^-$ **9**; cyclic carbonium $X\ PF_6^-$ **10**; $(Me_2N)_3\text{-}\overset{+}{P}\text{-}X\ PF_6^-$ **11**; $(\text{pyrrolidin-1-yl})_3\text{-}\overset{+}{P}\text{-}X\ PF_6^-$ **12**; PF_6^- **BOMP**

X = **b**, **d**, CF_3 **e**, **f**, F **h**, Cl **i**, Br **j**

Figure 2. Condensation of **5t** with **3t** (2 equiv) in the presence of a phosphonium type of condensing reagent (5 equiv) in CD_3CN−pyridine (1:1, v/v) at 25 °C. Data in parentheses refer to the half-lifetimes of **5t**: ●, **12e** and **BOMP** (<1 min); ○, **12b** + 10 equiv of MeIm (2 min); ■, **12d** (4 min); □, **12b** (12 min); ▲, **11b** (125 min); △, **12f** (18 h).

formation. When these reagents were used, the reactions were not complete even after 2 h. Therefore, we tested several condensing reagents having various core structures and leaving groups to accelerate the condensation (Scheme 3). In general, the introduction of an electron-withdrawing group to the benzotriazolyl moiety resulted in an accelerated rate of condensation. By use of HATU (**8d**)[80] and PyAOP (**12d**)[81] having a 7-azabenzotriazol-1-yloxy group (OAt), the reactions were completed in 18 and 28 min, respectively. The 6-(trifluoromethyl)benzotriazol-1-yloxy group (OTfbt) was found to be a highly effective leaving group for the phosphonium reagents. The time required for completion of the condensation using PyFOP (**12e**)[76] was less than 5 min. In the case using the phosphonium reagent PyPOP (**12h**)[76] having the pentafluorophenoxy group, no reaction took place after 30 min. The (4-oxo-1,2,3-benzotriazin-3-yl)oxy group (Dhbt) was found to be ineffective for rapid condensation in the case of PyDOP (**12f**).[76] In comparison with BOP (**11b**) and PyBOP (**12b**),[82] the pyrrolidin-1-yl group is superior to the dimethylamino group as a substituent on the phosphonium center for rapid condensation.[76] When **11b** and **12b** were used, the half-lifetimes of **5t** were 125 and 12 min, respectively. In the cases of the carbonium reagents HBTU (**8b**) and HBPyU (**9b**),[83] no remarkable differences were observed between the rates of condensation. When **8b** and **9b** were used, the half-lifetimes of **5t** were 82 and 60 min, respectively. Consequently, a new phosphonium condensing reagent, 2-(benzotriazol-1-yloxy)-1,3-dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (BOMP), was found to be highly effective in the present method. In spite of having an unsubstituted benzotriazolyloxy group in this reagent, condensation was completed within 5 min. A cyclic carbonium compound, BOI (**10b**),[84] was found to have a reactivity similar to that of BOMP. However, BOI (**10b**) is poorly soluble in pyridine, and the reagent is not suitable for a solid-phase synthesis. In the condensation with carbonium- and phosphonium-type condensing reagents, pyridine plays an essential role in the activation of the condensing reagents. When the reaction was carried out in CD_3CN in the absence of pyridine, the condensation proceeded very slowly. It was also found that the addition of *N*-methylimidazole (MeIm)[85] as a nucleophilic catalyst accelerated the reaction.

Next, the *H*-phosphonate monomers **5a**, **5c**, and **5g** were condensed with 2 equiv of 3′-*O*-(phenoxyacetyl)thymidine **3t** in the presence of BOMP (5 equiv) in pyridine−CD_3CN (1:1, v/v). One of the best ways to confirm the side reactions caused by the activated monomers and the phosphonium condensing reagents is through a direct observation of the reaction mixture by ^{31}P NMR spectroscopy because highly unstable intermediates containing a phosphorus atom, which readily decompose during TLC or HPLC analysis, can be detected.[20−22,54] ^{31}P NMR monitoring of the condensation mixtures indicated that the reactions were completed within 5 min in all cases to give the *H*-phosphonate dimers **6a**, **6c**, and **6g** bearing the unprotected bases without any side products (Table 4).[68] It was noteworthy that no modification of the 5′-hydroxyl group of **3t** by the excess amount of condensing reagent was detected by the ^{31}P NMR and TLC analysis.

Ab Initio **Calculations of Condensing Reagents.** The reactivity of the (benzotriazolyoxy)carbonium and -phosphonium condensing reagents depends on their core structures as well as the nature of their leaving groups. It is obvious that a bulky group neighboring a carbonium or phosphonium center may decrease the reactivity of the reagent, and an electron-withdrawing substituent on a leaving group may increase the reactivity. It was found that cyclic carbonium and phosphonium reagents such as BOI (**10b**) and BOMP had unexpected high condensing abilities. In order to elucidate the structure−reactivity relationship of the condensing reagents, *ab initio* calculations of both acyclic and cyclic reagents were carried out.

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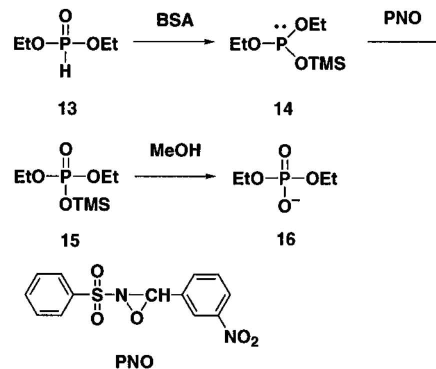
Recently, the crystal structure of HBTU (**8b**) was determined to have a guanidinium form by X-ray analysis.[86] First, molecular orbital calculations of the isomeric structures of **8b**, a uronium form having a O−C$^+$ bond and a guanidinium form having a N−C$^+$ bond, were examined. Geometry optimization was carried out at the HF/6-31G* level, and the single-point energy of each structure was calculated at the MP2/6-31G* level. As seen in the total energies of the isomeric structures, the guanidinium form was more stable by 4.92 kcal/mol than the uronium form.[68] In the case of **10b**, the energy difference between the isomeric structures was calculated to be 5.10 kcal/mol.[68] These results are consistent with the crystal structure of **8b**. In the uronium form of **8b** and **10b**, there is no large LUMO on the carbonium center, and the lowest unoccupied molecular orbital (UMO) having a large coefficient on the carbonium center is the LUMO + 1.[68] In the case of the guanidinium form of **8b** and **10b**, the lowest UMO having a large coefficient on the carbonium center is the LUMO.[68] However, superiority of **10b** over **8b** cannot be explained by comparing the energy levels and coefficients of the UMOs of these compounds. In contrast, the atomic charge on the carbonium center of the guanidinium form of **10b** (0.901 e) is appreciably greater than that of **8b** (0.758 e).[68] Therefore, **10b** is advantageous to **8b** in the charge-controlled reactions with *H*-phosphonate anions.

Next, the molecular orbital calculations for the phosphonium compounds BOP (**11b**) and BOMP were carried out. These compounds have similar atomic charges on the phosphonium centers.[68] In these compounds, the lowest UMO having a large coefficient on the phosphonium center is the LUMO + 2.[68] Compared with the UMOs of **11b** and BOMP, the high reactivity of BOMP can be attributed to the existence of the significantly large LUMO + 2 on the phosphorus atom.[68]

Oxidation of *H*-Phosphonate Diesters under Anhydrous Conditions. In the current *H*-phosphonate approach, the most troublesome step in the solid-phase synthesis is the oxidation of the *H*-phosphonate backbone.[36a] During the oxidation using aqueous I_2, the internucleotidic *H*-phosphonate linkages are partially hydrolyzed to decrease the yield of oligomers.[55] Improved oxidation procedures have been reported by some groups.[36a,55,87] However, using the oxidation conditions with aqueous I_2, a complete suppression of the competitive hydrolysis of the *H*-phosphonate backbone cannot be achieved. Therefore, a new method for the oxidation of the *H*-phosphonate diesters under anhydrous conditions should be explored. Van Boom has reported the oxidation of dinucleoside *H*-phosphonate *via* the corresponding trimethylsilyl phosphite using *t*-BuOOH as an oxidizing reagent.[40] In fact, the ^{31}P NMR monitoring indicated that oxidation of diethyl trimethylsilyl phosphite (**14**) with *t*-BuOOH in CD_3CN proceeded very quickly and was completed within 5 min. However, the silyl phosphite is highly moisture-sensitive and is readily converted to the *H*-phosphonate diester which cannot be oxidized by *t*-BuOOH. When the reaction was carried out in the presence of excess *N,O*-bis(trimethylsilyl)acetamide (BSA) to eliminate all traces of water, the oxidation was incomplete after several hours. In this reaction, *t*-BuOOH was converted to a less reactive species of *t*-BuOOTMS. When the silyl phosphite **14** was treated with 5 equiv of *t*-BuOOTMS, the time required for complete oxidation was 24 h. The addition of TMSOTf, which was employed as

Scheme 4



a catalyst for activation of trimethylsilyl peroxide by Hayakawa,[88] was not effective in accelerating the reaction rate in this case.

Consequently, 2-(phenylsulfonyl)-3-(3-nitrophenyl)oxaziridine (PNO)[89] was found to be effective for the oxidation of silyl phosphites under neutral and anhydrous conditions. For instance, diethyl phosphonate (**13**) was treated with 2 equiv of PNO in the presence of 5 equiv of BSA in $CDCl_3$ (Scheme 4). Monitoring of the reaction mixture by ^{31}P NMR indicated that the oxidation was completed within 5 min quantitatively to give diethyl trimethylsilyl phosphate **15** (−8.18 ppm, $^3J_{PH}$ = 8.5 Hz).[68] The trimethylsilyl group of **15** was readily removed by treatment with MeOH in the presence of triethylamine to give diethyl phosphate **16** (−0.02 ppm, $^3J_{PH}$ = 7.3 Hz).[68] When the *O*-protected nucleosides **1a**, **1c**, **1g**, and **1t** were treated with a large excess amount of PNO in the presence or absence of BSA, oxidation of the base moieties was not detected after 6 h by TLC analysis. In addition, PNO will be useful for the oxidation of phosphite intermediates in the phosphoramidite approach under anhydrous conditions.[12,88]

Solid-Phase Synthesis of Oligodeoxyribonucleotides. In order to confirm the *O*-selective condensation and the new oxidation method at the oligomer level, a solid-phase synthesis was examined. The condensation using the *N*-unprotected monomers **5** and BOMP was successfully applied to the solid-phase method. The yields of dimers **7a**, **7c**, **7g**, and **7t** anchored to a highly cross-linked polystyrene (HCP)[90] *via* an oxalyl linker[91] were estimated to be 97−99% by DMTr assay with use of the monomer units **5** (0.05 M, 20 equiv to **4t**) and BOMP (0.2 M, 4 equiv to **5**) in pyridine[92] for 2 min. By use of these coupling conditions, a tetranucleoside *H*-phosphonate, d(Cp-(H)Ap(H)pGp(H)T), was synthesized on the polymer support with an average coupling yield of 99%. The *H*-phosphonate intermediate was successively treated with a 0.2 M solution of PNO in dry CH_2Cl_2 in the presence of BSA (0.5 M) for 10 min. After being washed with CH_2Cl_2, the tetramer was released from the support by treatment with $PrNH_2$−MeOH (1:4, v/v) for 30 min. The resulting crude d(CAGT) was analyzed by anion-exchange HPLC (Figure 3A). The purity of the tetramer was estimated to be 87%. The tetramer was isolated by anion-exchange HPLC in 58% yield from the anchored

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(92) The reactions of **5c** and **4t** in the presence of BOMP in CH_3CN−pyridine (1:1, v/v) resulted in the formation of hardly soluble precipitates. The use of pyridine in place of CH_3CN−pyridine (1:1, v/v) gave a clear solution.

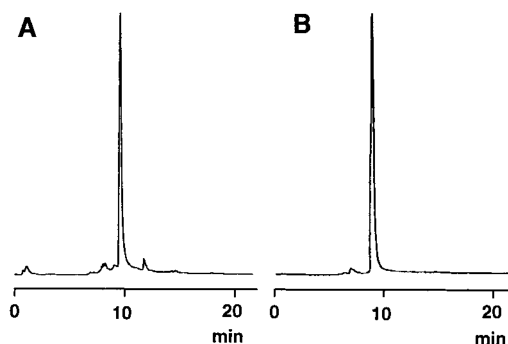

Figure 3. Anion-exchange HPLC profiles: (A) crude d(CAGT); (B) d(CAGT) purified by anion-exchange HPLC.

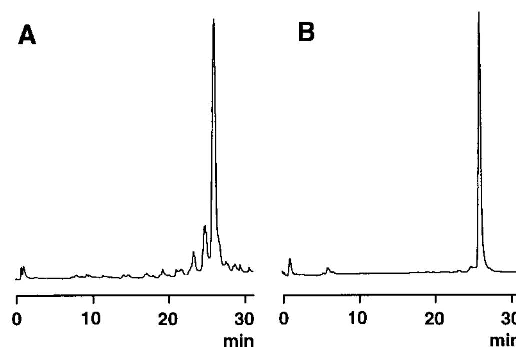

Figure 4. Anion-exchange HPLC profiles: (A) crude $d(CAGT)_3$; (B) $d(CAGT)_3$ purified by anion-exchange HPLC.

nucleoside. The purified tetramer (Figure 3B) was treated with snake venom phosphodiesterase followed by calf intestinal alkaline phosphatase to give dC, dG, T, and dA in the ratio of 0.99:1.09:0.91:1.00. No base modifications were detected by the enzymatic characterization. In a similar manner, a 12-mer $d(CAGT)_3$ was synthesized with an average coupling yield of 99%, and the crude product was analyzed by anion-exchange HPLC (Figure 4A). In this case, the purity of the 12-mer was estimated to be 74%.[93] The 12-mer was further purified by anion-exchange HPLC (Figure 4B) and digested with the enzymes as described for the tetramer to give dC, dG, T, and dA in the ratio of 0.97:1.08:0.89:1.06. These results indicate that the present new condensation and oxidation methods are effective for the synthesis of oligodeoxyribonucleotides without *N*-protection.

^{31}P NMR Studies of the Reaction Mechanism. In the general *H*-phosphonate method in solution, *H*-phosphonate monoesters react with excess amounts of condensing reagents in the absence of the 5′-hydroxyl component to form trivalent species such as diacyl phosphites[51,53] and trimetaphosphites.[52] This overactivation was not observed in the presence of an equimolar or an excess amount of the 5′-hydroxyl component. In a similar manner, the *H*-phosphonate monomer **5t** was allowed to react with 2 equiv of the 5′-hydroxyl component **3t** in the presence of 5 equiv of the phosphonium condensing reagent **12b**, resulting in the quantitative formation of the dinucleoside *H*-phosphonate **6t** (Figure 5). In contrast, when the reaction of the *H*-phosphonate monomer **5t** with **12b** was carried out in the absence of the 5′-hydroxyl component **3t**, an unprecedented oxidation of the monomer unit was observed (Figure 6). In this reaction, no signals corresponding to trivalent phosphorus compounds over 100 ppm were detected. A signal corresponding to the initial product was observed at −9.53 ppm

(93) During the chain elongation, partial decomposition of the *H*-phosphonate backbone occurred to a degree of 1−2% per cycle. A detailed analysis of this reaction will be reported elsewhere.

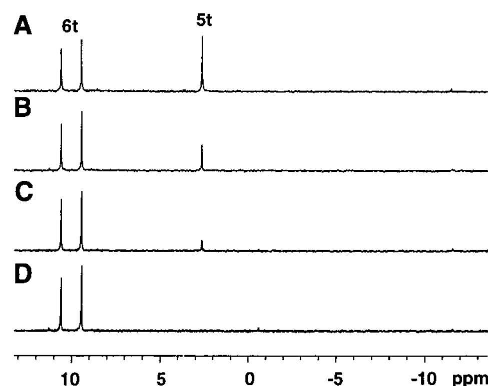

Figure 5. ^{31}P NMR spectra of the reaction mixture obtained by the reaction of **5t** and **3t** (2 equiv) in the presence of **12b** (5 equiv) in CD_3CN−pyridine (1:1, v/v) at 25 °C: (A) after 15 min; (B) after 30 min; (C) after 60 min; (D) after 80 min.

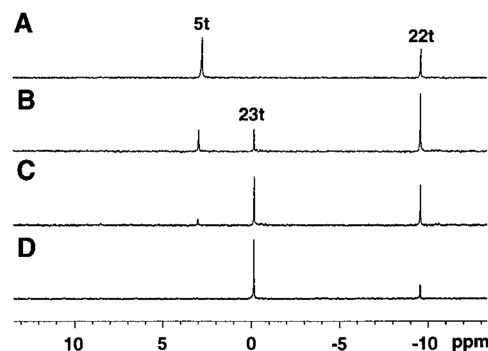

Figure 6. ^{31}P NMR spectra of the reaction mixture obtained by the reaction of **5t** with **12b** (5 equiv) in CD_3CN−pyridine (1:1, v/v) at 25 °C: (A) after 10 min; (B) after 15 min; (C) after 30 min; (D) after 60 min.

with a J_{PH} value of 8.5 Hz. This signal was gradually converted to a new signal at −0.15 ppm with a J_{PH} value of 6.1 Hz. These signals were found to be essentially unchanged by the addition of water after a few minutes. These results indicate that these products have no residues derived from the condensing reagent. With the use of the carbonium reagent **9b** in place of **12b**, formation of the same products was observed. In addition, the reaction of *H*-phosphonate monomer **5t** was allowed to react with 5 equiv of a carbonium- or phosphonium-type condensing reagent in the presence of *N*-unprotected nucleosides **1a**, **1c**, or **1g**, resulting in the formation of the same oxidation products, and the amino groups of the nucleosides proved to be unchanged.[68] The chemical shift of the initial product is quite similar to those of pyrophosphate or phosphorazolide derivatives.[94] Identification of the oxidation products was successfully performed as follows. The *H*-phosphonate monomer **5t** was treated with 1-(trimethylsilyl)-1,2,3-benzotriazolide[95] in the presence of CCl_4 and triethylamine in CD_3CN for 30 min to give the corresponding trimethylsilyl ester of phosphorobenzotriazolide **26t** (−10.22 ppm, $^3J_{PH}$ = 8.5 Hz) (Scheme 6).[68,96] This product might be generated *via* the corresponding bis(trimethylsilyl) phosphite **24t** and phosphorochloridate **25t**. After the addition of methanol, the signal of **24t** was readily converted to the signal of **22t** at −9.62 ppm with a J_{PH} value of 8.5 Hz,

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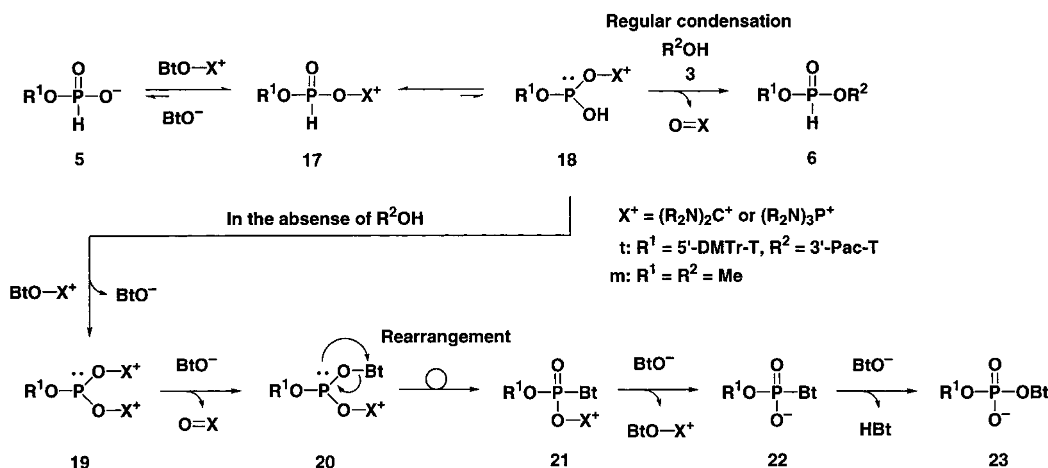
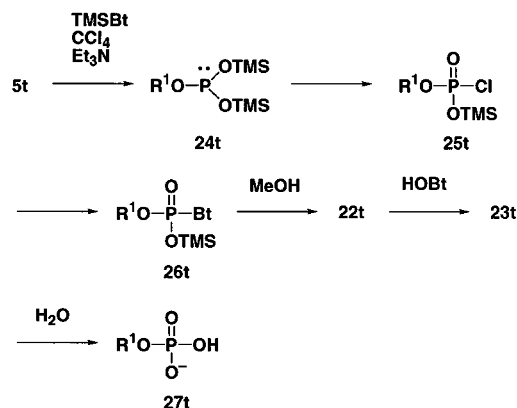
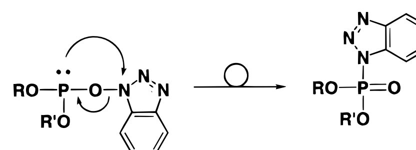
Scheme 5



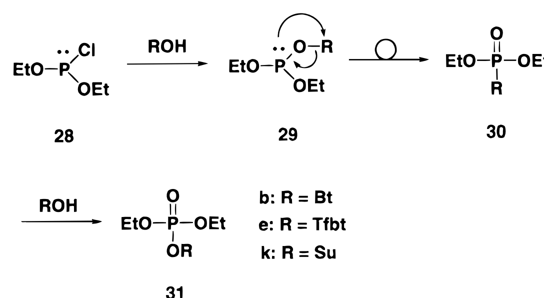
Scheme 6



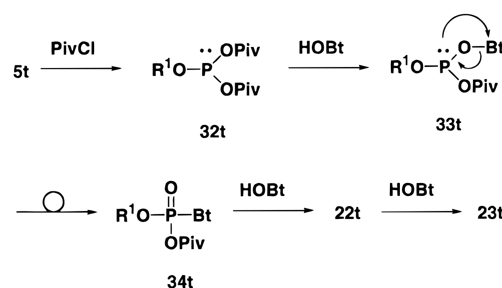
Scheme 7



Scheme 8



Scheme 9



which was identical to that of the initial oxidation product.[68] The addition of excess HOBt to the reaction mixture resulted in the formation of the corresponding benzotriazolyloxy ester **23t**.[68] Further, **23t** was hydrolyzed by addition of water to give the phosphate monoester **27t** (1.50 ppm, $J_{PH} = 7.3$ Hz). The time required for the complete hydrolysis of **23t** was more than 20 h. In the solid-phase synthesis using large excess amounts of monomer units and condensing regents, the excess monomers may have been converted to **22** and **23**. However, these compounds have no phosphorylation activity with the 5′-hydroxyl group.

Next, the reaction mechanism for the unique oxidation of the *H*-phosphonate monomers was investigated with model reactions. We found that this type of oxidation generally occurred in the reaction of the active phosphite derivatives with *N*-hydroxyl compounds. For example, diethyl phosphorochloridite **28** was allowed to react with 4 equiv of HOBt in C_5D_5N−pyridine (1:9, v/v).[68] After 2 min, the signal of **28** (167.31 ppm) disappeared and the new signal of **29b** was observed at 145.38 ppm, along with the formation of the oxidized products of **30b** (−8.27 ppm) and **31b** (−1.03 ppm) (Scheme 8). After 30 min, the signal of **29b** disappeared and the signal of **30b** was almost converted to that of **31b**. When the more acidic 6-(trifluoromethyl)-1-hydroxybenzotriazole (HOTfbt) was used in place of HOBt, the reaction proceeded very quickly and the signal of **31e** (−1.22 ppm) was observed directly after 2 min.[68] In the case using the less acidic *N*-hydroxysuccinimide (HOSu), the trivalent intermediate **29k** (144.37 ppm) remained even after several hours, and the formation of the oxidized products **30k** and **31k** proceeded slowly.[68] A similar oxidation was also observed for phosphite derivatives of the nucleosides. The nucleoside 3′-bis(pivaloyl) phosphite **32t**,[51,53] obtained from the reaction of **5t** with 10 equiv of pivaloyl chloride, was allowed to react with 20 equiv of HOBt in C_5D_5N−pyridine (1:9, v/v).[68] After 5 min, the formation of the nucleoside 3′-phosphorobenzotriazolide **22t** was observed. The signal was completely converted to that of **23t** after 15 min. The oxidation of the phosphite **32t** may have proceeded *via* the intermediate **33t** (Scheme 9). The ^{31}P NMR data of these compounds are listed in Table 4.

Considering of the above results, the oxidation of trivalent phosphorus compounds with HOBt can be explained in terms of the intramolecular rearrangement of the benzotriazolyl group in the phosphite intermediates along with the oxidation of the phosphorus atom and the reduction of the nitrogen atom, as shown in Scheme 7. The intramolecular rearrangements are exothermic and irreversible reactions. The *ab initio* single-point energy calculations of **29b** and **30b** at the MP2/6-31G* level

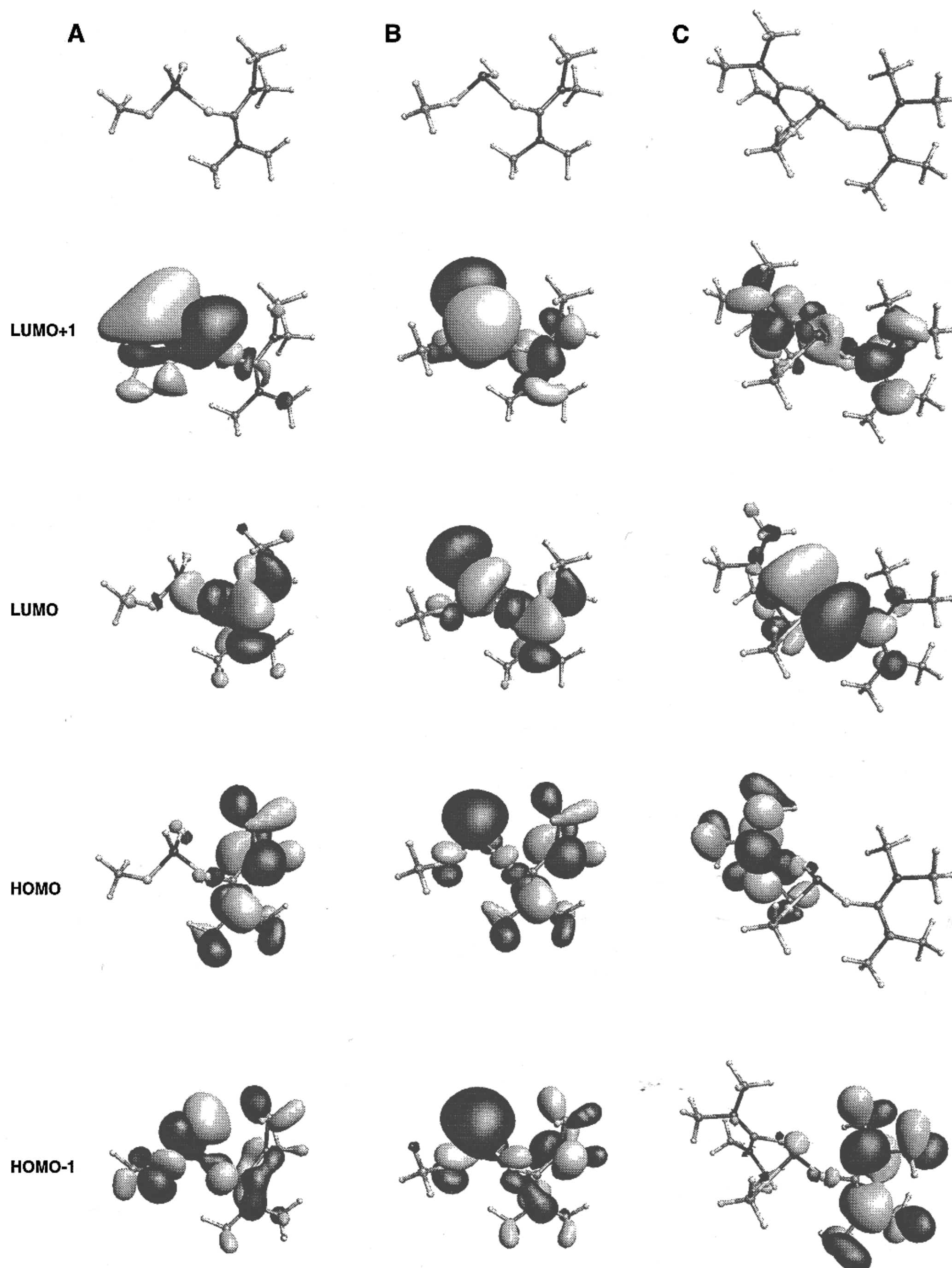


Figure 7. Optimized geometries and MOs of the model compounds calculated at the HF/6-31G* level: (A) **17m**; (B) **18m**; (C) **19m**. The MOs are visualized on the threshold of 0.030.

indicate that **30b** is 68.86 kcal/mol more stable than **29b**.[97] The oxidation of the *H*-phosphonate monomers with a carbonium or a phosphonium reagent might occur *via* trivalent phosphorus intermediates **20** in a similar mechanism. In addition, the rate of condensation is much faster than that of the oxidation of the *H*-phosphonate monomer under regular condensation conditions. Interestingly, it was found that both condensation and oxidation of the monomer unit did not proceed in the presence of 10 equiv of HOBt. These results suggest that the formation of the intermediate **17** is reversible and that the benzotriazolyloxy anion attacks at the onium center of **17** (Scheme 5). In the absence of the 5′-hydroxyl component, the intermediate **17** was further activated with a condensing reagent *via* the tautomeric phosphite **18** to give the doubly-activated species **19**. In the next step, the benzotriazolyloxy anion attacks the phosphorus atom of the phosphite **19** to give the key intermediate **20**. Once the phosphite intermediate **20** having a benzotriazolyloxy group is formed, the intramolecular rearrangement proceeds instantaneously to give the phosphorobenzotriazolide **21**. The benzotriazolyloxy anion existing in the reaction mixture attacks at the onium center of **21**, giving the initially observable oxidation product **22** as well as regenerating one equimolar condensing reagent.

***Ab Initio* Calculations for the Reaction Intermediates.** In order to elucidate the mechanism for the *O*-selective condensation using the *N*-unprotected *H*-phosphonate monomers and the carbonium and phosphonium condensing reagents, *ab initio* calculations for the reaction intermediates were examined. A

(97) A transition state search for the reactions of **29b** to **30b** and **18m** with nucleophiles is now in progress.

Table 1. Total Energies, Orbital Energies (au), and Mulliken Charges (e) of the Model Compounds Calculated at the MP2/6-31G* Level

	17m	**18m**	**19m**
total energy	−912.30963	−912.30267	−1217.61243
LUMO + 1	0.06568	0.02366	−0.09445
LUMO	−0.00394	0.00738	−0.10868
HOMO	−0.53393	−0.52013	−0.61985
HOMO − 1	−0.60582	−0.52879	−0.62162
charge on P	1.160	1.012	1.072
charge on C^+	0.827	0.841	0.816, 0.802

general question in the reaction mechanism elucidated by ^{31}P NMR is why the benzotriazolyloxy anion attacks at the onium center of **17t** and the trivalent phosphorus center of **19t**. Therefore, calculations for the model compound **17m**, its tautomeric structure **18m**, and **19m** were carried out at the HF/6-31G* level. In these calculations, the nucleoside residue was substituted for the methyl group and the *N,N,N′N′*-tetramethyluronium group was adopted as a leaving group. The optimized geometries and molecular orbitals of these compounds are shown in Figure 7. The single-point energy calculations of the total energies for **17m** and its tautomeric structure **18m** at the MP2/6-31G* level suggest that the *H*-phsophonate **17m** is 4.36 kcal/mol more stable than the phosphite **18m**. In the structure of the predominant *H*-phosphonate form **17m**, a large LUMO is located on the carbonium center, but no large orbitals exist on the back side of the leaving group of the phosphorus atom (Figure 7A). This is why the benzotriazolyloxy anion attacks the carbonium center of **17**. In contrast, the phosphite intermediate **18m** has a large LUMO on the back side of the leaving group of the phosphorus atom (Figure 7B). An alcohol may attack at this orbital to give the *H*-phosphonate diester **6**. The *H*-phosphonate intermediate **17m** has a large LUMO + 1 on the back side of the leaving group of the phosphorus atom (Figure 7A). However, the energy difference between the LUMO and the LUMO + 1 is relatively large compared with those of **18m** and **19m** (Table 1). Therefore, the interaction of the HOMO of the nucleophile and the LUMO + 1 of **17m** seems to be unfavorable. In the overactivated species **19m**, there is a significantly large LUMO on the phosphorus atom (Figure 7C). In addition, the energy level of the LUMO of **19m** is much lower than that of **17m** and **18m** (Table 1). On the other hand, the energy level of the HOMO of benzotriazolyoxy anion is calculated to be −0.130 58 au (Table 3), which is very close to the energy level of the LUMO of **19m** (−0.108 68 au). Therefore, **19** readily reacts with the benzotriazoyloxy anion existing in the reaction mixture, which results in the formation of **20**. When compared with the MOs of the benzotriazoyloxy anion and protonated HOBt, the benzotriazoyloxy anion is a plausible nucleophile in the reaction because of the large coefficient and the high-energy level of its HOMO (Table 3).[68] In the solid-phase synthesis using excess amounts of the monomer units and a condensing reagent, the phosphite intermediates **19** and **20**, which are potentially reactive with the amino groups of the nucleosides, might be formed in the solution above a solid support. However, these intermediates are immediately oxidized to an inert species by an intramolecular reaction which does not affect the unprotected amino groups during condensation.

The second question is why condensation proceeds without oxidation of the monomer units in the presence of the excess 5′-hydroxyl component. The HOMO on the oxygen atom of P−OH in the phosphite **18m** is relatively small and is therefore at a disadvantage when reacting with a condensing reagent. In comparison, the interaction between the large HOMO on the

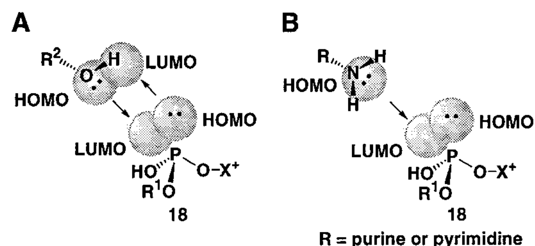


Figure 8. Interaction of frontier molecular orbitals between the phosphite intermediate **18** and nucleophiles: (A) the hydroxyl group of nucleoside as a nucleophile; (B) the amino group of nucleoside as a nucleophile.

Table 2. MO Energies (au) and Mulliken Charges (e) on the Amino Group of the Nucleobases Calculated at the MP2/6-31G* Level

	9-MeAd	1-MeCy	9-MeGu
LUMO + 1	0.16945	0.19595	0.17280
LUMO	0.13926	0.12543	0.16118
HOMO	−0.30391	−0.32853	−0.29001
HOMO − 1	−0.35871	−0.36928	−0.38082
charge on N	−0.840	−0.833	−0.868

oxygen atom of an alcohol[68] and the large LUMO on the phosphorus atom of **18m** results in the fast formation of the *H*-phosphonate diester **6** without further activation of **18m**.

The third question is why this condensation proceeds *O*-selectively without base modifications. In the phosphite intermediate **18m**, there is a large HOMO on the phosphorus atom which corresponds to the lone-pair electrons (Figure 7B). On the other hand, a primary alcohol such as methanol has a large LUMO located on the hydrogen atom of the hydroxyl group.[68] In the initial phase of the reaction of **18m** with an alcohol, remarkable interactions between the frontier molecular orbitals (FMOs) of these molecules can be expected, as shown in Figure 8A.[97] This FMO interaction raises the HOMO energy of the alcohol and decreases the LUMO energy of **18m**. As a result, the formation of the *H*-phosphonate diester **6** *via* the phosphite intermediate **18m** proceeds smoothly. On the other hand, several *ab initio* calculations for the monomeric nucleobases have been reported at various theoretical levels.[98] In order to elucidate the reactivity of nucleobases toward phosphorylating reagents, calculations for the model compounds 9-methyladenine, 1-methylcytosine, and 9-methylguanine were carried out at the HF/6-31G* level (Table 2).[68,98p] It was found that 9-methyladenine and 9-methylguanine had relatively large HOMOs on the nitrogen atom of the exocyclic amino groups.[68] In the case of 1-methylcytosine, the highest OMO existing on the exocyclic amino group is the HOMO − 1.[68] In contrast to alcohols, there are no LUMOs located on the hydrogen atom of the amino groups of nucleobases.[68] In the case of 9-methyladenine, 1-methylcytosine, and 9-methylguanine, the relatively

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Table 3. MO Energies (au) and Mulliken Charges (e) of the Nucleophiles Calculated at the MP2/6-31G* Level

	MeOH	$^{-}$OBt	HOBt
LUMO + 1	0.28033	0.31894	0.16017
LUMO	0.22681	0.16871	0.09716
HOMO	−0.44324	−0.13058	−0.32171
HOMO − 1	−0.49730	−0.16828	−0.34008
charge on O	−0.660	−0.671	−0.524

Table 4. ^{31}P NMR Data

compound	δ (ppm)	$^{1}J_{PH}$ (Hz)	$^{3}J_{PH}$ (Hz)	solvent
5a	3.74	612.8	8.5	*a*
5c	3.83	612.8	8.6	*a*
5g	3.07	618.9	8.5	*a*
5t	3.78	611.6	8.5	*a*
6a	9.34, 10.44	717.8, 714.1	8.1, 8.8	*b*
6c	9.45, 10.56	719.0, 716.5	8.2, 8.3	*b*
6g	9.50, 10.54	717.8, 715.3	8.2, 8.6	*b*
6t	9.44, 10.58	719.0, 716.6	7.7, 8.6	*b*
15	−8.18		8.5	*a*
16	−0.02		7.3	*a*
22t	−9.53		8.5	*a*
23t	−0.15		6.1	*b*
26t	−10.22		8.5	*b*
27t	1.50		7.3	*b*
29b	145.38			*c*
29k	144.37			*c*
30b	−8.27		8.5	*c*
30k	−7.84		8.5	*c*
31b	−1.03		7.3	*c*
31e	−1.12		7.3	*c*
31k	−0.11		7.3	*c*

[a] $CDCl_3$. [b] CD_3CN−pyridine (1:1, v/v). [c] C_5D_5N−pyridine (1:9, v/v).

large UMOs which can interact with the HOMO of **18m** are LUMO + 3, LUMO + 2, and LUMO + 2, respectively. Because of the absence of an appreciable interaction between the LUMO of the nucleobases and the HOMO of **18m**, the amino groups of nucleosides are unreactive to the phosphite intermediate **18m** (Figure 8B).[97] In the doubly-activated phosphite **19m**, there is no large HOMO on the trivalent phosphorus because of the electron-withdrawing effect of the two leaving groups (Figure 7C). *O*-Selective condensation is no longer expected *via* the overactivated intermediate **19m** because of the large coefficient and the low energy level of its LUMO.

Consequently, a mechanism for the *O*-selective condensation of the *H*-phosphonate monomer and the 5′-hydroxyl component with the (benzotriazolyloxy)carbonium and -phosphonium condensing reagents can be explained in terms of the interactions of the FMOs of the phosphite intermediate **18** and the nucleophiles.

Conclusion

The present new *H*-phosphonate approach using *N*-unprotected monomers and carbonium- and phosphonium-type condensing reagent has enabled us to synthesize *N*-unmodified oligonucleotides bearing *H*-phosphonate internucleotidic linkages. These intermediates will be useful for the synthesis of a wide variety of backbone-modified DNA analogs which cannot be achieved by the conventional method utilizing base-labile *N*-protecting groups. It is well known that the *N*-unprotected deoxyadenosine derivatives are more stable under the acidic conditions than the corresponding *N*-acylated ones currently used in oligonucleotide synthesis. Therefore, the present method is suitable to suppress the depurination of deoxyadenosine. We should emphasize that the newly synthesized condensing reagent BOMP is useful for internucleotidic bond formation without modification of the base moieties, the 5′-hydroxyl group, or the internucleotidic bonds. In addition, BOMP will also be available as a highly efficient condensing reagent in other fields such as peptide chemistry. The new oxidation procedure using PNO under anhydrous conditions excludes the hydrolysis of the *H*-phosphonate backbone. When the present approach is used, *N*-protecting groups are unnecessary for the synthesis of oligodeoxyribonucleotides as well as their analogs. The present *O*-selective phosphonylation and condensation will be useful for the direct *O*-phosphorylation of natural products containing the *N*-unprotected nucleoside residues.

Experimental Section

General Information. ^{1}H NMR spectra were obtained at 270 MHz on a JEOL-EX-270 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$. ^{13}C NMR spectra were obtained at 67.8 MHz on a JEOL-EX-270 spectrometer with tetramethylsilane as an internal standard. ^{31}P NMR spectra were obtained at 109.25 MHz on a JEOL-EX-270 spectrometer using 85% H_3PO_4 as an external standard. UV spectra were recorded on a Hitachi 220A spectrophotometer. Thin-layer chromatography (TLC) was performed on precoated glass plates of Kieselgel 60 F_{254} (Merck, No. 5715) in CH_2Cl_2−MeOH (6:1, v/v). Silica gel column chromatography was carried out using Wakogel C-200. Reversed phase HPLC was carried out on a Waters LC module 1 with a column of μBondasphere 5-μm C18,100 Å, 3.9 mm × 150 mm (Waters), with a linear gradient of 0−30% acetonitrile in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C for 30 min at a rate of 1.0 mL/min. Anion-exchange HPLC was carried out on a Waters LC module 1 with a column of Gen-Pak Fax, 4.6 mm × 100 mm (Waters), with a linear gradient of 10−63% 1.0 M NaCl containing 25 mM NaH_2PO_4 (pH 6.0) in 25 mM NaH_2PO_4 (pH 6.0) at 50 °C for 40 min at a rate of 1.0 mL/min. 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)adenosine (**1a**), 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)cytidine (**1c**), and 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)guanosine (**1g**) were prepared according to the procedure described by Ogilvie.[99] 5′-*O*-(Dimethoxytrityl)adenosine (**2a**), 5′-*O*-(dimethoxytrityl)cytidine (**2c**), and 5′-*O*-(dimethoxytrityl)-guanosine (**2g**) were prepared according to the procedure described by Michelson.[100] Condensing reagents **8b**,[78] **9b**,[82] **10i**,[101] **11b**,[79] and **12b**[77] were purchased from Tokyo Kasei Co., **12i**[76] and **12j**[77] were purchased from Fluka Chemie AG, and **8d**[80] was purchased from Peptide Institute Inc. Condensing reagents **12d**, **12e**, **12f**, and **12h** were prepared according to the procedure described by Høeg-Jensen,[76] and **10b** was prepared according to the procedure described by Kiso.[101] 2-(Phenylsulfonyl)-3-(3-nitrophenyl)oxaziridine (PNO) was prepared according to the procedure described by Davis.[89b] Other reagents were purchased from Tokyo Kasei Co. or Aldrich Chemical Co. unless noted. Organic solvents were purified and dried by the appropriate procedure. Aminomethylated highly closs-linked polystyrene (Perkin-Elmer Applied Biosystems)[90] was functionalized with 5′-*O*-(dimethoxytrityl)thymidine 3′-*O*-oxalate according to the procedure described by Letsinger.[91] Solid-phase synthesis was performed on an Applied Biosystems 380A synthesizer or manually 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. Snake venom phosphodiesterase and calf intestinal alkaline phosphatase were purchased from Boeringer Mannheim GmbH.

***Ab Initio* Calculations.** All *ab initio* molecular orbital calculations were carried out using the Gaussian 94 program[102] on a Cray C-916/12256 supercomputer. The results were analyzed using UniChem (version 4.0, Oxford Molecular Ltd.) software on a Silicon Graphics

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Inc. Indigo2 IMPACT workstation. Geometry optimizations were carried out at the HF/6-31G* level, and single-point energy calculations were carried out at the MP2/6-31G* level involving electronic correlation to obtain accurate energies and atomic charges.

Monitoring of the Reactions by ^{31}P NMR Spectroscopy. A reaction was carried out in an NMR sample tube (5 mm × 180 mm) at 25 °C. In each measurement, data accumulation was carried out 10 times with a pulse width of 6.9 μs, an acquisition time of 0.189 s, and a pulse delay of 5.0 s. A spectrum was acquired with 65 536 data points for a spectral width of 40 000 Hz.

2-(Benzotriazol-1-yloxy)-1,3-dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium Hexafluorophosphate (BOMP). 1,3-Dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidine[103] (18.7 g, 0.1 mol) was added dropwise over 10 min to a vigorously stirred suspension of 1-hydroxybenzotriazole (13.5 g, 0.1 mol) and CCl_4 (19.2 mL, 50 mol) in dry CH_2Cl_2 (200 mL) at −78 °C under argon. After being stirred for 2 h at the same temperature, the mixture was gradually warmed to rt and washed with water (300 mL) containing KPF_6 (18.4 g, 0.1 mol). The organic layer was dried over Na_2SO_4, filtered, and concentrated to half-volume. Ethyl acetate (100 mL) was added to the above mixture, and it was cooled to 0 °C. The white crystals were collected by filtration, washed with cold ethyl acetate, and dried over P_2O_5 under reduced pressure to give BOMP (21.1 g) in 45% yield: mp 128−129 °C dec; ^{31}P NMR ($CDCl_3$) δ 41.52 (s, P^+), −142.90 (septet, J = 711.6 Hz, PF_6^-); ^{1}H NMR ($CDCl_3$) δ 2.14 (4H, ddd, J = 7.7, 5.3, 1.3 Hz, 3,4-CH_2 of pyrroiydinyl), 2.87 (6H, d, J = 11.2 Hz, N-CH_3), 2.91 (2H, ddd, J = 8.9, 7.3, 5.6 Hz, 2- or 5-CH_2 of pyrrolidinyl), 3.50 (2H, ddd, J = 12.9, 9.1, 5.8 Hz, 2- or 5-CH_2 of pyrrolidinyl), 3.64 (4H, ddd, J = 8.9, 4.3, 2.3 Hz, 4,5-CH_2 of diazaphospholidine), 7.52 (1H, ddd, J = 8.3, 6.8, 1.3 Hz, 6-H of benzotriazolyl), 7.72 (1H, dd, J = 8.6, 6.8 Hz, 5-H of benzotriazolyl), 7.78 (1H, d, J = 8.6 Hz, 4-H of benzotriazolyl), 8.07 (1H, d, J = 8.3 Hz, 7-H of benzotriazolyl); ^{13}C NMR ($CDCl_3$) δ 26.45 (d, J = 9.8 Hz, N-CH_3), 30.95 (d, J = 6.1 Hz, 3,4-C of pyrrolidinyl), 45.78 (d, J = 15.8 Hz, 4,5-C of diazaphospholidine), 48.68 (d, J = 4.9 Hz, 2,5-C of pyrrolidinyl), 108.19, 120.63, 126.15, 127.58, 130.60, 142.89 (benzotriazolyl); FAB$^+$ m/z calcd for $C_{14}H_{22}N_6OP$ [M − PF_6]$^+$ 321.159, found 321.158. Anal. Calcd for $C_{14}H_{22}F_6N_6OP_2$: C, 36.06; H, 4.76; N, 18.02. Found: C, 35.88; H, 4.59; N, 18.15.

1,8-Diazabicyclo[5.4.0]undec-7-enium 5′-*O*-(Dimethoxytrityl)-deoxyadenosin-3′-yl Phosphonate (5a). Method A. 5′-*O*-(Dimethoxytrityl)deoxyadenosine **2a** (0.554 g, 1 mmol) was dried by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (5 mL). To the solution was added diphenyl phosphonate (1.64 g, 7 mmol). After being stirred for 20 min, the mixture was treated with H_2O−Et_3N (1:1, v/v, 2 mL) and stirred at rt for an additional 20 min. The mixture was diluted with MeOH (5 mL), and 1 M triethylammonium hydrogen carbonate (10 mL) was added. The mixture was washed three times with Et_2O (10 mL × 3), and the organic layer was back-extracted with 1 M triethylammonium hydrogen carbonate. The aqueous layer and washings were combined and extracted three times with $CHCl_3$−MeOH (2:1, v/v, 10 mL × 3), and the aqueous layer was back-extracted several times with $CHCl_3$−MeOH (2:1, v/v). The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a column of silica gel (30 g of silica gel). Chromatography was performed with $CHCl_3$ containing 0.5% triethylamine, applying a gradient of methanol (0−15%). The fractions containing **5a** were combined and concentrated to dryness. The product was dissolved in $CHCl_3$−MeOH (2:1, v/v, 10 mL) and washed with 0.2 M 1,8-diazabicyclo[5.4.0]undec-7-enium hydrogen carbonate (10 mL). The aqueous layer was back-extracted several times with $CHCl_3$−MeOH (2:1), and the organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to give **5a** (0.631 g, 82%) as a colorless foam.

Method B. To a solution of phosphonic acid (0.082 g, 10 mmol, dried by repeated coevaporation with dry pyridine) in dry pyridine (10 mL) was added bis(2-oxo-3-oxazolidinyl)phosphinic chloride (1.40 g, 5.5 mmol). The reaction mixture was stirred at rt for 20 min. To the mixture was added 5′-*O*-(dimethoxytrityl)deoxyadenosine **2a** (0.554 g, 1 mmol). After the mixture was stirred for 6 h, H_2O (1 mL) was added to the mixture. It was diluted with $CHCl_3$−MeOH (2:1, v/v, 10 mL) and washed three times with 1 M triethylammonium hydrogen carbonate (10 mL × 3), and the aqueous layer was back-extracted several times with $CHCl_3$−MeOH (2:1, v/v). The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product (triethylammonium salt) was purified by silica gel column chromatography and converted to the DBU salt as described for method A to give **5a** (0.600 g, 78%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 3.74 (dd, J = 612.8, 8.5 Hz); ^{1}H NMR ($CDCl_3$) δ 1.66 (2H, m, CH_2 of DBU), 1.73 (4H, m, CH_2 of DBU), 1.97 (2H, quintet, J = 5.6 Hz, CH_2 of DBU), 2.42−2.80 (2H, m, 2′,2″-H), 2.83 (2H, m, CH_2 of DBU), 3.36−3.44 (8H, m, 5′,5″-H, CH_2 of DBU), 3.77 (6H, s, OCH_3 of DMTr), 4.22 (1H, m, 4′-H), 4.91 (1H, m, 3′-H), 5.96 (2H, br s, 6-NH_2), 6.52 (1H, t, J = 6.8 Hz, 1′-H), 6.78 (4H, d, J = 8.9 Hz, 3,3′,5,5′-H of DMTr), 6.96 (1H, d, J = 612.3 Hz, P-H), 7.15−7.42 (9H, m, ArH of DMTr except 3,3′,5,5′-H), 7.99 (1H, s, 2-H), 8.26 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 19.50, 23.99, 26.85, 28.99, 32.11, 37.90, 39.86 (2′-C), 48.56, 54.22, 55.15, 63.65 (5′-C), 73.67 (d, J = 3.7 Hz, 3′-C), 84.17 (1′-C), 85.61 (d, J = 7.3 Hz, 4′-C), 86.38, 113.08, 119.84 (5-C), 126.76, 127.75, 128.18, 130.06, 135.67, 135.74, 138.78 (8-C), 144.55, 149.72 (2-C), 152.81 (4-C), 155.40 (6-C), 158.40, 166.11; FAB$^+$ m/z calcd for $C_{31}H_{33}N_5O_7P$ [M − DBU + H]$^+$ 618.212, found 618.212. Anal. Calcd for $C_{40}H_{48}N_7O_7P{\cdot}5H_2O$: C, 55.87; H, 6.80; N, 11.40. Found: C, 55.83; H, 6.72; N, 11.28.

1,8-Diazabicyclo[5.4.0]undec-7-enium 5′-*O*-(Dimethoxytrityl)-deoxycytidin-3′-yl Phosphonate (5c). With use of methods A and B as described in the case of **5a**, compound **5c** was obtained from 5′-*O*-(dimethoxytrityl)deoxycytidine **2c** (0.530 g, 1 mmol) as a colorless foam in 91% (0.679 g) and 74% (0.552 g) yields, respectively: ^{31}P NMR ($CDCl_3$) δ 3.83 (dd, J = 612.8, 8.6 Hz); ^{1}H NMR ($CDCl_3$) δ 1.66 (2H, m, CH_2 of DBU), 1.71 (4H, m, CH_2 of DBU), 1.97 (2H, quintet, J = 5.6 Hz, CH_2 of DBU), 2.22 (1H, m, 2′-H), 2.66 (1H, m, 2″-H), 2.81 (2H, m, CH_2 of DBU), 3.35−3.51 (8H, m, 5′,5″-H, CH_2 of DBU), 3.78 (6H, s, OCH_3 of DMTr), 4.40 (1H, m, 4′-H), 5.05 (1H, m, 3′-H), 5.51 (2H, d, J = 7.3 Hz, 5-H), 6.30 (1H, t, J = 6.1 Hz, 1′-H), 6.32 (2H, br s, 4-NH_2), 6.82 (4H, d, J = 8.9 Hz, 3,3′,5,5′-H of DMTr), 6.92 (1H, d, J = 613.3 Hz, P-H), 7.17−7.41 (9H, m, ArH of DMTr except 3,3′,5,5′-H), 7.80 (1H, d, J = 7.3 Hz, 6-H); ^{13}C NMR ($CDCl_3$) δ 19.32, 23.87, 26.62, 30.44, 32.04, 37.83, 40.42 (2′-C), 48.41, 54.11, 55.13, 62.97 (5′-C), 72.60 (3′-C), 84.82 (1′-C), 85.59 (4′-C), 86.47, 94.74 (5-C), 113.08, 126.77, 127.76, 128.01, 129.97, 135.38, 135.47, 140.56 (6-C), 144.46, 155.89 (2-C), 158.38, 165.48 (4-C), 165.89; FAB$^+$ m/z calcd for $C_{30}H_{33}N_3O_8P$ [M − DBU + H]$^+$ 594.201, found 594.203. Anal. Calcd for $C_{39}H_{48}N_5O_8P{\cdot}4H_2O$: C, 57.27; H, 6.90; N, 8.56. Found: C, 57.38; H, 7.06; N, 8.82.

1,8-Diazabicyclo[5.4.0]undec-7-enium 5′-*O*-(Dimethoxytrityl)-deoxyguanosin-3′-yl Phosphonate (5g). With use of methods A and B as described in the case of **5a**, compound **5g** was obtained from 5′-*O*-(dimethoxytrityl)deoxyguanosine **2g** (0.570 g, 1 mmol) as a colorless foam in 94% (0.739 g) and 87% (0.684 g) yields, respectively: ^{31}P NMR ($CDCl_3$) δ 3.07 (dd, J = 618.9, 8.5 Hz); ^{1}H NMR ($CDCl_3$) δ 1.58−1.78 (6H, m, CH_2 of DBU), 1.95 (2H, quintet, J = 5.6 Hz, CH_2 of DBU), 2.31−2.54 (2H, m, 2′,2″-H), 2.62−2.78 (2H, m, CH_2 of DBU), 3.28−3.48 (8H, m, 5′,5″-H, CH_2 of DBU), 3.78 (6H, s, OCH_3 of DMTr), 4.37 (1H, m, 4′-H), 4.99 (1H, m, 3′-H), 6.17 (1H, dd, J = 9.6 Hz, 4.3 Hz, 1′-H), 6.48 (2H, br s, 2-NH_2), 6.82 (4H, d, J = 8.6 Hz, 3,3′,5,5′-H of DMTr), 7.02 (1H, d, J = 619.3 Hz, P-H), 7.16−7.44 (9H, m, ArH of DMTr except for 3,3′,5,5′-H), 7.65 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 19.89, 24.17, 26.97, 29.09, 33.26, 39.01, 41.33 (2′-C), 48.55, 54.13, 55.20, 63.90 (5′-C), 74.90 (3′-C), 83.70 (1′-C), 85.16 (4′-C), 86.43, 113.19, 117.29 (5-C), 126.82, 127.81, 128.12, 130.03, 130.06, 133.69, 135.67 (8-C), 135.70, 144.56, 150.71 (4-C), 155.58 (2-C), 158.49 (6-C), 165.57; FAB$^+$ m/z calcd for $C_{31}H_{33}N_5O_8P$ [M − DBU + H]$^+$ 634.207, found 634.208. Anal. Calcd for $C_{40}H_{48}N_7O_8P{\cdot}6H_2O$: C, 53.74; H, 6.76; N, 10.97. Found: C, 53.58; H, 6.54; N, 10.87.

Analysis of Base Modifications by Condensing Reagents. An appropriate 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)nucleoside (**1a**, **1c**, or **1g**; 10 μmol) was dried by repeated coevaporation with dry pyridine and dissolved in C_5D_5N−pyridine (1:9, v/v, 600 μL). The mixture was

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transferred into an NMR sample tube, and a condensing reagent (100 μmol) was added. The reaction was monitored by ^{31}P NMR and TLC.

Analysis of Base Modifications by Condensing Reagents and *H*-Phosphonate Monomers. An appropriate 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)nucleoside (**1a**, **1c**, or **1g**; 10 μmol) and an *H*-phosphonate monomer, **5t** (15.2 mg, 20 μmol), were dried by repeated coevaporation with dry pyridine and dissolved in C_5D_5N−pyridine (1:9, v/v, 600 μL). The mixture was transferred into an NMR sample tube, and a condensing reagent (100 μmol) was added. The reaction was monitored by ^{31}P NMR and TLC.

^{31}P NMR Study of Internucleotidic Bond Formation. An *H*-phosphonate monomer (**5a**, **5c**, **5g**, or **5t**; 20 μmol) and 3′-*O*-(phenoxyacetyl)thymidine (15.1 mg, 40 μmol) were dried by repeated coevaporation with dry pyridine and dissolved in CD_3CN−pyridine (1:1, v/v, 400 μL). The mixture was transferred into an NMR sample tube, and a condensing reagent (100 μmol) was added. The reaction was monitored by ^{31}P NMR, and the yield of the dimer **6** was estimated on the basis of the integration of the signals of **6** and **5t**.

Oxidation of Diethyl Phosphonate (13) with PNO. A mixture of diethyl phosphonate (**13**; 13 μL, 0.1 mmol) and *N,O*-bis(trimethylsilyl)acetamide (BSA, 135 μL, 0.5 mmol) in $CDCl_3$ (250 μL) was transferred into an NMR sample tube. 2-(Phenylsulfonyl)-3-(3-nitrophenyl)oxaziridine (PNO; 0.067 g, 0.2 mmol) was added to the above mixture. After 5 min, quantitative formation of **15** was observed. To the above mixture was added MeOH−Et_3N (200 μL). After 5 min, quantitative formation of **16** was observed.

Reactions of *H*-Phosphonate Monomers with Condensing Reagents. An *H*-phosphonate monomer, **5t** (15.2 mg, 20 μmol), was dried by repeated coevaporation with dry pyridine and dissolved in CD_3CN−pyridine (1:1, v/v, 400 μL). The mixture was transferred into an NMR sample tube, and a condensing reagent (100 μmol) was added. The reaction was monitored by ^{31}P NMR.

Identification of the Oxidation Products by ^{31}P NMR Spectroscopy. An *H*-phosphonate monomer, **5t** (15.2 mg, 20 μmol), in an NMR sample tube (5 mm × 180 mm) was dissolved in a mixture of 1-(trimethylsilyl)-1,2,3-benzotriazole (38 mg, 0.2 mmol) and Et_3N (28 μl, 0.2 mmol) in CCl_4−CD_3CN (1:1, v/v, 400 μL). After 30 min, a complete conversion of **5t** to **26t** was observed. The mixture was transferred into a round-bottomed flask and coevaporated with MeOH under reduced pressure. The residue was dissolved in CD_3CN−pyridine (1:1, v/v, 400 μL) and transferred into an NMR sample tube. To the mixture was added HOBt (27 mg, 0.2 mmol). After 1 h, complete conversion of **22t** to **23t** was observed.

Typical Procedure for Solid-Phase Synthesis. Each cycle of chain elongation consisted of detritylation (1% TFA in CH_2Cl_2; 15 s), washing (CH_2Cl_2 followed by 1% quinoline in CH_2Cl_2), coupling (0.05 M monomer **5** and 0.2 M BOMP in pyridine; 2 min), washing (pyridine), capping (0.05 M triethylammonium isopropyl phosphonate, 0.2 M BOMP in pyridine; 2 min), and washing (pyridine followed by CH_2Cl_2). Generally, the average yield per cycle was estimated to be 97−99% by DMTr assay. After chain elongation, the DMTr group was removed by treatment with 1% TFA in CH_2Cl_2 for 15 s and washed with CH_2Cl_2. The *H*-phosphonate oligomer on the HCP resin was oxidized by treatment with a mixture of PNO (0.2 M) and BSA (0.5 M) in CH_2Cl_2 (400 μL) for 10 min. After being washed with CH_2Cl_2, the oligomer was released from the HCP resin by treatment with $PrNH_2$−MeOH (1:4, v/v, 500 μL) for 30 min. The HCP resin was removed by filtration and washed with MeOH. The filtrate was concentrated to dryness. The resulting crude product was dissolved in distilled water (1 mL) and purified by anion-exchange HPLC followed by reversed-phase HPLC.

Enzymatic Studies. The isolated oligomer (1.0 A_{260} unit) was dissolved in 50 mM Tris−HCl buffer (pH 8.0, 80 μL). Snake venom phosphodiesterase (10 units) was added, and the reaction mixture was incubated at 37 °C for 3 h. After the enzyme was inactivated by heat treatment (100 °C, 1 min), calf intestinal alkaline phosphatase (20 units) was added. After the reaction mixture was incubated at 37 °C for 3 h, the enzyme was inactivated by heat treatment (100 °C, 1 min). The reaction mixture was analyzed by reversed-phase HPLC at 260 nm.

Tetranucleotide d(CAGT). With use of the typical procedure described above, 5′-*O*-(dimethoxytrityl)thymidine oxalate bound to HCP (66 mg, 0.5 μmol) gave d(CAGT) (9.9 A_{260} units, 53% based on the assumption of 15% hypochromicity): UV (H_2O) λ_{max} 257.4 nm, λ_{min} 228.0 nm. Enzymatic digestion of the product gave a mixture of dC, dG, T, and dA in the ratio of 0.99:1.09:0.91:1.00.

Dodecanucleotide d(CAGT)$_3$. 5′-*O*-(Dimethoxytrityl)thymidine oxalate bound to HCP (66 mg, 0.5 μmol) gave d(CAGT)$_3$ (14.6 A_{260} units, 30% based on the assumption of 25% hypochromicity): UV (H_2O) λ_{max} 256.8 nm, λ_{min} 229.0 nm. Enzymatic digestion of the product gave a mixture of dC, dG, T, and dA in the ratio of 0.97:1.08:0.89:1.06.

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Supporting Information Available: The ^{1}H, ^{13}C, and ^{31}P NMR spectra of BOMP, **5a**, **5c**, and **5g**, the ^{31}P NMR analysis of the reaction of **1a**, **1c**, or **1g** with **5t** in the presence of PivCl or BOMP, the ^{31}P NMR analysis of the reaction of **5a**, **5c**, **5g**, or **5t** with **3t** in the presence of BOMP, the ^{31}P NMR analysis of the reaction of **13** with PNO in the presence of BSA (Scheme 4), the ^{31}P NMR analysis of the reaction of **5t** with TMSBt in the presence of CCl_4 (Scheme 6), the ^{31}P NMR analysis of the reaction of **28** with HOBt, HOTfbt, or HOSu (Scheme 8), the ^{31}P NMR analysis of the reaction of **5t** with PivCl in the presence of HOBt (Scheme 9), and the optimized geometries and MOs of **8b**, **10b**, **11b**, BOMP, 9-methyladenine, 1-methylcytosine, 9-methylguanine, MeOH, $^-$OBt, and HOBt calculated at the HF/6-31G* level (29 pages). See any current masthead page for ordering and Internet access instructions.

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