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A Novel Method for the Synthesis of Dinucleoside Boranophosphates by a Boranophosphotriester Method

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2′-Deoxyribonucleoside-3′-boranophosphates (nucleotide monomers), including four kinds of nucleobases, were synthesized in good yields by the use of new boranophosphorylating reagents. We have explored various kinds of condensing reagents as well as nucleophilic catalysts for the boranophosphorylation reaction with nucleosides. In the synthesis of dinucleoside boranophosphates, undesirable side reactions occurred at the *O*-4 of thymine and the *O*-6 of N^2-phenylacetylguanine bases. To avoid these side reactions, additional protecting groups, benzoyl (Bz) and diphenylcarbamoyl (Dpc) groups, were introduced to thymine and guanine bases, respectively. As a result, the condensation reactions proceeded smoothly without any side reactions, and the dimers including four kinds of nucleobases were obtained in excellent yields. In the deprotection of the 5′-DMTr group, Et_3SiH was found to be effective as a scavenger for the DMTr cation which caused a P−B bond cleavage. After removal of the other protecting groups by the conventional procedure, four kinds of dinucleoside boranophosphates were obtained in good yields.

Introduction

A new class of nucleic acid analogues, oligodeoxyribonucleotides bearing internucleotidic boranophosphate linkages (boranophosphate DNAs), are regarded as potentially useful antisense molecules.[1] The DNA analogues are isosteric with methylphosphonate DNAs and phosphorothioate DNAs, but the BH_3 group is slightly larger than the oxygen in native phosphate DNAs.[2] Boranophosphate DNAs are also isoelectronic with phosphate diesters, but imparts a considerable increase in hydrophobicity compared to native DNAs.[2] In addition, a duplex consisting of a boranophosphate DNA and its complementary RNA is a good substrate for ribonuclease H (RNase H) compared with the corresponding native DNA/RNA and phosphorothioate DNA/RNA duplexes.[3] Boranophosphate DNAs are, furthermore, stable and are more resistant to nucleases than phosphorothioate DNAs.[4] From another point of view, boranophosphate DNAs are applicable to boron neutron capture therapy (BNCT)[5] due to the ^{10}B atom property. Thus, boranophosphate DNAs have many advantages as nucleic acid analogues. It has been reported that the hybridization ability of boranophosphate DNAs are much poorer than that of native DNAs; the T_m value of the duplex of dodecathymidylate boranophosphate with native dodecadeoxyadenylate is lower than those of the corresponding native DNA/DNA and phosphorothioate DNA/DNA duplexes.[4] Nevertheless, the duplexes of boranophosphate DNAs including cytosine and guanine bases are expected to have higher T_m values.

The methods previously reported for the synthesis of boranophosphate DNAs are either enzymatic or chemical. In an enzymatic approach for the synthesis of boranophosphate DNAs, 2′-deoxynucleoside 5′-(α-*P*-borano)-triphosphates are employed as substrates and polymerized by the use of T7 DNA polymerase.[6] However, the enzyme can recognize only one of the diasteromers (the R_P isomer), and the S_P isomer cannot be incorporated into a DNA strand. Hence, the enzymatic method yields only boranophosphate oligonucleotides with the S_P configuration. Furthermore, the enzymatic method is obviously not suitable for large-scale synthesis. On the other hand, the chemical synthesis of boranophosphate oligonucleotides may be accomplished via the phosphoramidite or *H*-phosphonate approach, followed by the boronation of the P(III) intermediates.[4,7−9] However, this chemical approach requires the use of a borane reagent to give rise to undesirable side reactions at the base moieties in the boronation step; these base modifications are mainly caused by the reduction of *N*-acyl protecting groups to

(1) Summers, J. S.; Shaw, B. R. *Curr. Med. Chem.* **2001**, *8*, 1147−1155.
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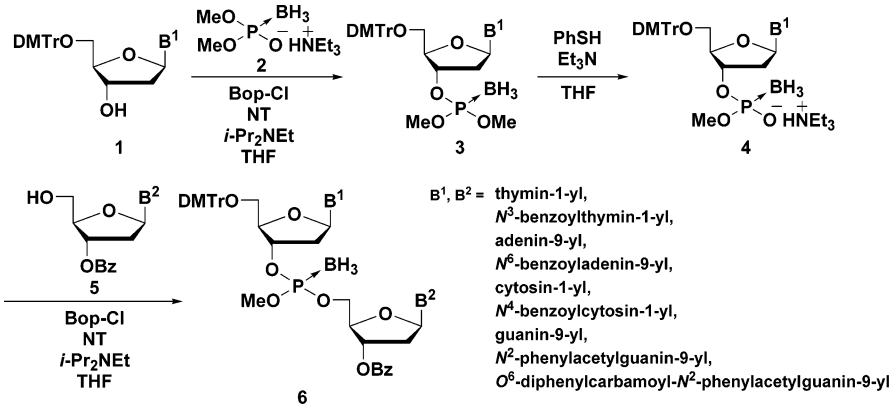
SCHEME 1



give the corresponding *N*-alkyl derivatives.[10] Therefore, the method is applicable only to thymine derivatives, in which no acyl protecting group is required. This means that oligonucleotide chains must be constructed without base protection or with base protection by nonacyl-type *N*-protecting groups in order to synthesize boranophosphate DNAs including four kinds of nucleobases, A, C, and G as well as T, by this method. However, the base-unprotected method has some limitations, due to the difficulty of the synthesis of long *H*-phosphonate DNAs in acceptable yields,[11] and due to the reduction of unprotected thymine base moiety by a borane reagent in the boronation step.[9]

These results strongly suggest that the development of a new boranophosphorylating agent is very fascinating for the effective synthesis of boranophosphate DNAs. Imamoto et al. have reported a boranophosphorylation reaction of nucleosides.[12] In this method, tetramethyl pyroboranophosphate is used as a boranophosphorylating reagent. However, this reagent is less reactive for the nucleophilic attack of an alcohol; the reaction has to be carried out by the use of *t*-BuLi for the activation of the hydroxy function at −78 °C in THF. Therefore, this reaction is not applicable to solid-phase synthesis.

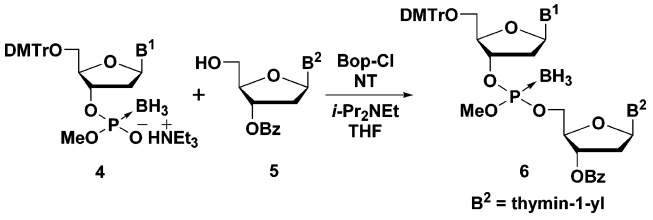
Recently, we have reported a new strategy for the synthesis of boranophosphate DNAs (the boranophosphotriester method).[13] In this method, a subsequently boronated phosphorylating reagent was used in order to introduce a BH_3 group into an internucleotidic phosphate linkage; consequently, side reactions, caused by borane reagents in the conventional methods for the synthesis of boranophosphate DNAs, have been completely avoided. In this paper, we report detailed study on the boranophosphotriester method involving the boranophosphorylation, internucleotidic bond formation, and deprotection reactions.

Results and Discussion

Synthesis of Dimers. We have recently reported a novel strategy for the boranophosphorylation of nucleosides (Scheme 1).[13] Applying this boranophosphotriester method, dithymidine boranophosphate was synthesized in >90% yield by using *N*,*N*′-bis(2-oxo-3-oxazolidinyl)-phosphonic chloride (Bop-Cl) as a condensing reagent in the presence of 3-nitro-1,2,4-triazole (NT) and *i*-Pr_2NEt in THF.

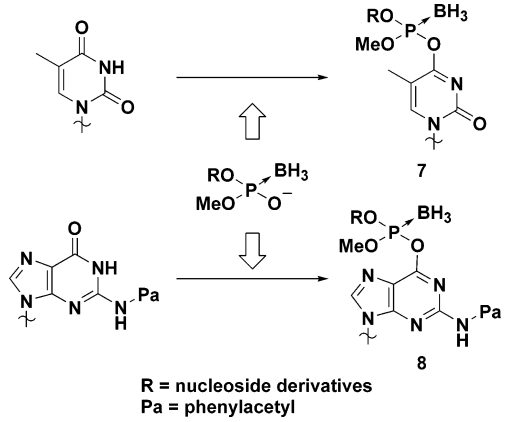
In a similar manner, we attempted to synthesize dimers including other nucleobases, A, C, and G. However, in contrast to pyrimidine−pyrimidine dimers (dTp^{b}dT and dCp^{b}dT), purine−pyrimidine dimers (dAp^{b}dT and dGp^{b}dT) were obtained only in 40−60% yields (Table 1). A precise monitoring of the reaction mixture by TLC indicated that the monomer **4** reacted with the O^4 of the thymin-1-yl moiety and the O^6 of the N^2-phenylacetylguanin-9-yl moiety in the nucleoside derivatives

TABLE 1. Synthesis of Dimers



entry	B^1	yield of **6** (%)
1	N^6-benzoyladenin-9-yl	59
2	N^4-benzoylcytosin-1-yl	94
3	N^2-phenylacetylguanin-9-yl	47
4	thymin-1-yl	92

SCHEME 2



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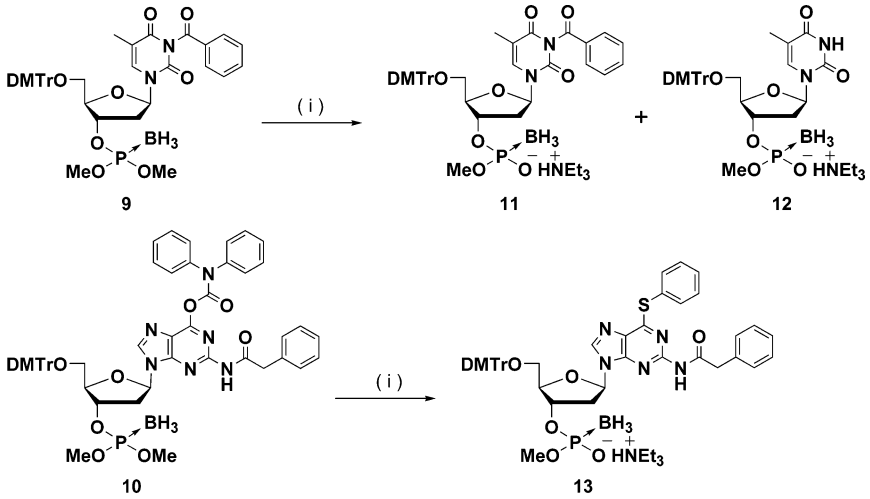
SCHEME 3

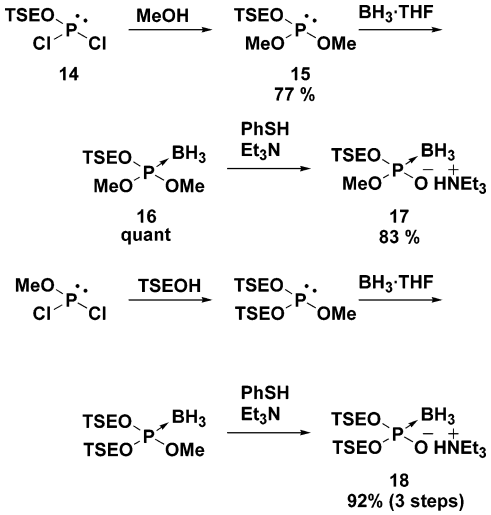


to give the corresponding boranophosphotriesters **7** and **8** (Scheme 2). In the formation of the dimers **6**, the boranophosphotriesters **7** and **8** with pyrimidine nucleoside derivatives would be reactive with the 5′-OH of the nucleosides **5** to give the desired dimers, while those with purine nucleoside derivatives may be less reactive due to the bulky purine bases, resulting in low yields of the dimers. Although similar side-reactions should take place in the formation of the monomers, the side reactions did not become a serious problem; since the boranophosphorylating reagent **2** is not bulky, even if a P−O bond was formed at the base moieties, it would be readily hydrolyzed during the aqueous workup of the reaction mixture.

Synthesis of New Boranophosphorylating Reagents. To prevent the side reactions described above, additional protecting groups should be introduced at the N^3 of the thymin-1-yl moiety and the O^6 of the N^2-phenylacetylguanin-9-yl moiety. For newly introduced protecting groups, the following requirements should be satisfied: (1) stable under acidic and basic conditions and (2) deprotected under the same conditions prescribed for the removal of the *N*-acyl protecting groups of A, C, and G. To meet these criteria, we designed the heretofore undescribed fully protected monomers, **9** and **10** (Scheme 3). In **9**, a benzoyl (Bz) group was used to block the N^3 of thymine, whereas in **10** a diphenylcarbamoyl (Dpc) group was used to additionally block the O^6 of N^2-phenylacetylguanine. However, the Bz group in **9** was found to be partially lost during demethylation with PhSH/Et_3N according to the literature method,[16] yielding a mixture of **11** and **12**. Moreover, in agreement with a previous report of displacement of the Dpc group during demethylation with PhSH/Et_3N,[17] treatment of **10** with this reagent led to the sulfide **13**.

Therefore, we decided to change the phosphorus protecting group of the boranophosphorylating reagent from a methyl group to one that could be removed without loss of the nucleobase protecting groups. An attractive candidate for this purpose was the 2-(trimethylsilyl)ethyl (TSE) group,[18] which can be cleaved by a fluoride ion under neutral conditions. Thus, we synthesized the new TSE-protected boranophosphorylating reagent **17**, according to Scheme 4. In a similar manner, triethylammonium bis-2-(trimethylsilyl)ethyl boranophosphate (**18**) was also synthesized.

SCHEME 4



Preparation of Fully Protected Thymidine and 2′-Deoxyguanosine 3′-Boranophosphate Monomers. We at first attempted to synthesize thymidine derivatives. When 5′-*O*-dimethoxytrityl-N^3-benzoylthymidine (**1**, B^1 = N^3-benzoylthymin-1-yl) was condensed with **18** in the presence of Bop-Cl, NT, and *i*-Pr_2NEt in THF, the reaction was very slow, and the desired compound was obtained only in 52% yield after 1 h. In this case, due to the bulkiness of the TSE group, the nucleophilic catalyst, NT, would not be able to attack at the phosphorus center of tetrakis(2-(trimethylsilyl)ethyl) pyroboranophosphate, which was considered to be the probable intermediate in the boranophosphorylation reaction. To avoid the formation of such a sterically crowded intermediate, **17** was used as the boranophosphorylating reagent instead of **18**. The reaction proceeded quickly, and **19** (B^1 = N^3-benzoylthymin-1-yl) was obtained in 91% yield after 30 min. In a similar manner, **1** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) was condensed with **17** to give **19** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) in 85% yield (Scheme 5).

SCHEME 5

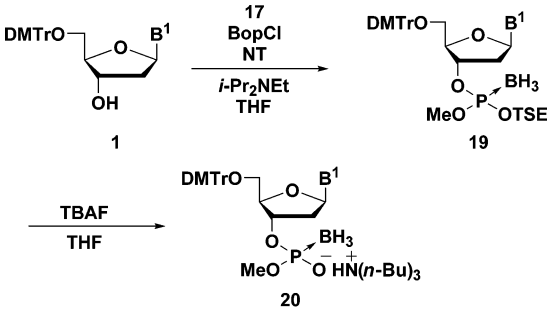
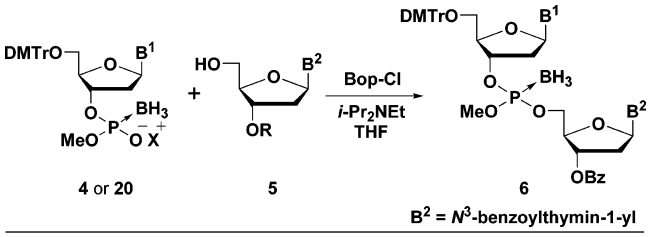


TABLE 2. Synthesis of Fully Base-Protected Dimers



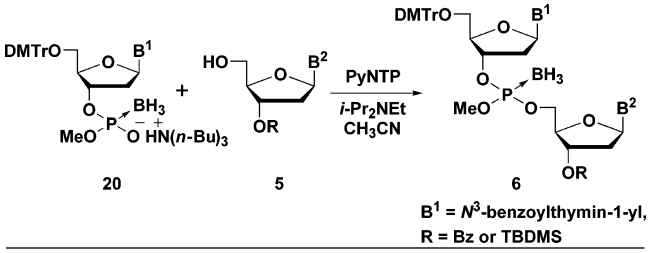
entry	B^1	X	yield of **6** (%)
1	N^6-benzoyladenin-9-yl	$HNEt_3$	85
2	N^4-benzoylcytosin-1-yl	$HNEt_3$	quant
3	O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl	$HN(n\text{-}Bu)_3$	83
4	N^3-benzoylthymin-1-yl	$HN(n\text{-}Bu)_3$	quant

Although a TSE group is known to be relatively stable toward fluoride ion,[18] it was found that cleavage of **19** to **20** was complete within 1 min upon treatment with tetrabutylammonium fluoride (TBAF). After purification by silica gel column chromatography, the corresponding boranophosphate diesters **20** ($B^1 = N^3$-benzoylthymin-1-yl) and **20** ($B^1 = O^6$-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) were obtained in good yields; unexpectedly, the diesters were not tetrabutylammonium salts, but tributylammonium salts, of which the identity was confirmed by ^{1}H NMR and elemental analysis.[19]

Synthesis of Dimers by Using Fully Base-Protected Derivatives. We next tried to synthesize dinucleoside boranophosphates by the use of four kinds of fully base-protected monomers (**4** ($B^1 = N^6$-benzoyladenin-9-yl), **4** ($B^1 = N^4$-benzoylcytosin-1-yl), **20** ($B^1 = N^3$-benzoylthymin-1-yl), and **20** ($B^1 = O^6$-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl)). When the fully base-protected monomers were allowed to react with the protected nucleoside **5** ($B^1 = N^3$-benzoylthymin-1-yl), the yields of the dimers **6** were highly improved (Table 2; entries 1−4).

Despite the improvement of the coupling yields, the condensing reagent Bop-Cl gave rise to an HCl salt which was hardly soluble in the reaction solvent. To solve this problem, we applied 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP), recently developed in our laboratory,[20] because it conveniently releases the soluble nucleophilic catalyst NT in situ and does not form an insoluble HCl salt. The yields of dimers obtained with PyNTP and with Bop-Cl as the coupling reagents were similar (Table 3; entries 1−4).

TABLE 3. Synthesis of Fully Bese-Protected Dimers



entry	B^2	yield of **6** (%)
1	N^6-benzoyladenin-9-yl	92
2[a]	N^4-benzoylcytosin-1-yl	99
3	O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl	94
4	N^3-benzoylthymin-1-yl	quant

[a] The 3′-OH was protected with a TBDMS group, since **5** ($B^2 = N^4$-benzoylcytosin-1-yl) protected with a Bz group was insoluble in CH_3CN.

Removal of the DMTr Group. It is well-known that 4,4′-dimethoxytrityl cation ($DMTr^+$) causes the decomposition of boranophosphate DNAs.[9,21] Thus a $DMTr^+$ scavenger is necessary to suppress this major side reaction. We used **3** (B^1 = thymin-1-yl) as a prototypical boranophosphate triester and tested two types of scavengers (Table 4). When the DMTr group was removed with dichloroacetic acid (DCA), byproducts **22**−**24** were observed (Table 4, entry 1). On the other hand, when Et_3SiH was included as a scavenger the formation of byproducts was decreased or entirely eliminated (Table 4, entries 2−4). Although byproducts were likewise not observed when MeOH or H_2O was used as the scavenger (Table 4, entries 5−8), complete removal of the DMTr group was much slower. Thus, Et_3SiH appeared to be a more effective scavenger than MeOH or H_2O in this deprotection step.

Removal of the Other Protecting Groups. Once the DMTr had been removed under the best conditions described above (Table 4; entry 4), the other protecting groups were cleaved by the conventional procedure using aqueous ammonia.[7] However, considerable degradation of the dinucleoside boranophosphates **27** was observed by an HPLC analysis. From this result, we concluded that aqueous ammonia was unsuitable for the demethylation of boranophosphotriesters. Accordingly, we remove the methyl group with $PhSH/Et_3N$ prior to cleaving the other base-labile protecting groups with $MeOH/NH_3$. In this manner, we were able to obtain excellent yields of the dinucleoside boranophosphate **27** (B^1 = A, C, G, or T) via **25** and **26** as shown in Table 5.

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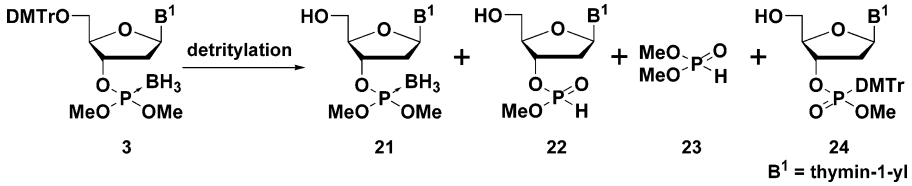
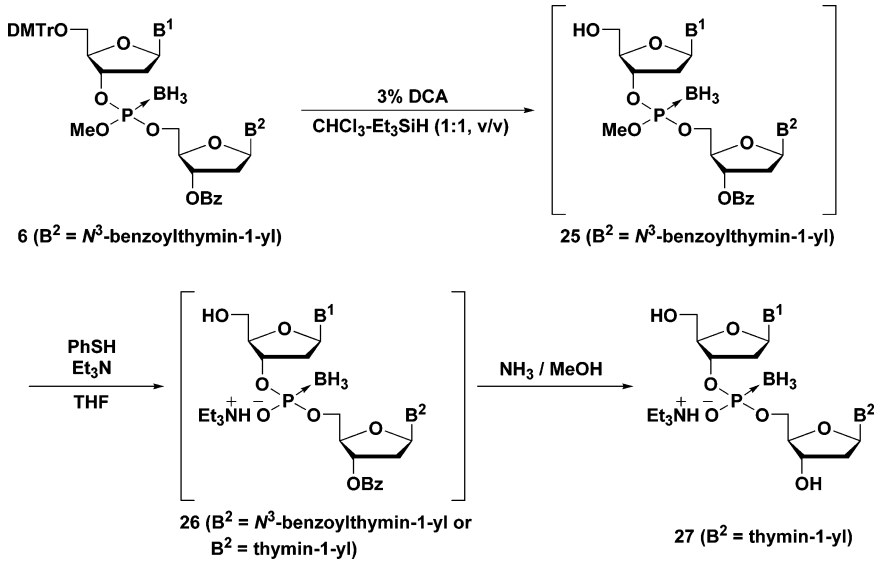
TABLE 4. Detritylation of Boranophosphate Triester



entry	acid	solvent (0.04 M)[a]	time required for detritylation	ratio of compds[b] **21**/**22**/**23**/**24**
1	3% DCA	$CDCl_3$	<1 min	78:8:6:8
2	3% DCA	$Et_3SiH - CDCl_3$ (1:9, v/v)	<1 min	89:6:3:2
3	3% DCA	$Et_3SiH - CDCl_3$ (1:3, v/v)	<1 min	91:7:2:0
4	3% DCA	$Et_3SiH - CDCl_3$ (1:1, v/v)	<1 min	100:0:0:0
5	3% DCA	CD_3OD (d_4)	3 h	100:0:0:0
6	1% TFA	$MeOH - CDCl_3$ (1:1, v/v)	2 h	100:0:0:0
7	1% TFA	$MeOH - CDCl_3$ (1:3, v/v)	2 h	100:0:0:0
8	80% AcOH	D_2O [c]	>14 h	100:0:0:0

[a] Concentration of **3**. [b] Estimated by ^{31}P NMR (after 30 min). [c] Heterogeneous.

TABLE 5. Deprotection of Dinucleoside Boranophosphate



entry	B^1 of **27**	yield of **27** (%)
1	adenin-9-yl	75
2	cytosin-1-yl	89
3[a]	guanin-9-yl	92
4	thymin-1-yl	86

[a] Methanolic ammonia treatment was carried out for the compounds **25** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) in order to remove the Dpc group at the *O*-6 position of the guanin-9-yl moiety, since the Dpc group was displaced by PhS^-.

Conclusion

We have developed a new efficient method for the synthesis of boranophosphates. The present new boranophosphotriester method enabled us to synthesize dinucleoside borabophosphates with all possible nucleobases, A, C, G, and T. The present strategy essentially eliminates the troublesome side reactions and thus should find widespread applications in solid-phase synthesis of unnatural oligonucleotide analogues.

Experimental Section[22]

Triethylammonium Dimethyl Boranophosphate 2. To a solution of dimethyl trimethylsilyl phosphite (12.9 g, 70.8 mmol) in dry THF (70 mL) under argon atmosphere was added dropwise a 1.1 M solution of BH_3·THF in THF (78 mL, 85.8 mmol) at 0 °C. The mixture was stirred for 2 h at rt and then cooled to 0 °C. To the mixture was slowly added dry MeOH (28.4 mL, 700 mmol) at 0 °C. After being stirred for 30 min at rt, the solution was treated with redistilled Et_3N (19.6 mL, 140 mmol) at rt, and stirring at rt was continued for 12 h. The solution was concentrated to dryness under reduced pressure and then dried by repeated coevaporation with dry toluene and $CHCl_3$ to obtain **2** (14.5 g, 91%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 12.58 (1H, bs), 3.56 (6H, d, J_{POCH} = 10.5 Hz), 3.13−3.02 (6H, m), 1.33 (9H, t, J = 7.5 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 50.1, 50.0, 45.2, 8.2; ^{31}P NMR ($CDCl_3$) δ 96.52 (q, J_{PB} = 136.5 Hz); IR (KBr) 3401, 2986, 2946, 2840, 2362, 1643, 1476, 1399, 1142, 1029, 840, 782, 712, 634 cm^{-1}. Anal. Calcd for $C_8H_{25}BNO_3P\cdot{}^1/_5CHCl_3$: C, 39.56; H, 10.20; N, 5.63. Found: C, 39.40; H, 10.40; N, 5.58.

(22) A general statement describing materials and methods is provided in the Support Information.

Triethylammonium Methyl 2-(Trimethylsilyl)ethyl Boranophosphate 17. To a solution of **14** (34.9 g, 159 mmol) in dry THF (160 mL) under argon atmosphere was slowly added a mixture of MeOH (14.2 mL, 352 mmol) and distilled *i*-Pr_2NEt (59.7 mL, 352 mmol) at 0 °C. The mixture was allowed to warm to rt and stirred at rt for 1 h. Then, the mixture was diluted with $CHCl_3$ (300 mL) and washed with satd $NaHCO_3$ aq (3 × 200 mL), and the aqueous layer was back-extracted with $CHCl_3$ (4 × 100 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was purified by distillation (3 mmHg, 65−67 °C) to obtain **15** (25.8 g, 77%) as a colorless oil. To a solution of **15** (25.8 g, 123 mmol) in dry THF (123 mL) under argon atmosphere was added dropwise a 1.0 M solution of BH_3·THF in THF (147 mL, 147 mmol) at 0 °C, and the solution was stirred for 1 h at rt. The mixture was concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (100 g) using CH_2Cl_2 as an eluent. The fractions containg dimethyl 2-(trimethylsilyl)ethyl boranophophate (**16**) were combined and concentrated to dryness under reduced pressure to obtain **16** (27.5 g, quantitative) as a colorless oil. To a solution of **16** (26.9 g, 120 mmol) in dry THF (120 mL) was added a mixture of Et_3N (66.5 mL, 480 mmol) and PhSH (48.9 mL, 480 mmol) at rt. After being stirred at rt for 24 h, the mixture was concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (100 g) using a gradient of MeOH (0−4%) in CH_2Cl_2 with 0.5% Et_3N as an eluent. The fractions containg triethylammonium methyl 2-(trimethylsilyl)ethyl boranophosphate (**17**) were combined and concentrated to dryness under reduced pressure. Excess Et_3N was removed by repeated coevaporation with toluene, and the residue was dissolved in $CHCl_3$ (100 mL). The solution was washed with satd NaCl aq (3 × 100 mL), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 50 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to obtain **17** (31 g, 83%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.00−3.88 (2H, m), 3.55 (3H, d, J_{POCH} = 9.0 Hz), 3.13−2.98 (6H, m), 1.31 (9H, t, J = 7.5 Hz), 1.03 (2H, m), 1−0 (3H, bq), 0.01 (9H, s); ^{13}C NMR ($CDCl_3$) δ 61.2, 50.5, 50.4, 45.5, 20.2, 20.1, 8.7, −1.2; ^{31}P NMR ($CDCl_3$) δ 94.70 (q, J_{PB} = 139.4 Hz); IR (KBr) 2951, 2895, 2838, 2359, 1475, 1398, 1249, 1177, 1139, 1045, 997, 936, 839, 779, 696 cm^{-1}. Anal. Calcd for $C_{12}H_{35}BNO_3PSi$·0.5H_2O·0.25$CHCl_3$: C, 42.02; H, 10.44; N, 4.00. Found: C, 42.04; H, 10.62; N, 3.91.

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl Dimethyl Boranophosphate (3, B^1 = *N*^{6}-Benzoyladenin-9-yl). **1** (B^1 = N^6-benzoyladenin-9-yl) (197.3 mg, 0.300 mmol) and triethylammonium dimethyl boranophosphate **2** (203 mg, 0.900 mmol) were dried by repeated coevaporation with dry toluene followed by dry pyridine and finally dissolved in dry THF (3.00 mL). To the solution were successively added *i*-Pr_2NEt (0.510 mL, 3.00 mmol), NT (171 mg, 1.50 mmol), and Bop-Cl (382 mg, 1.50 mmol). After being stirred at rt for 30 min, the mixture was diluted with $CHCl_3$ (10 mL). The solution was washed with satd $NaHCO_3$ aq (3 × 10 mL), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 10 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (30 g) using a gradient of MeOH (0−4%) in CH_2Cl_2 as an eluent. The fractions containing 5′-*O*-dimethoxytrityl-N^6-benzoyl-2′-deoxyadenosin-3′-yl dimethyl boranophosphate (**3**, B^1 = N^6-benzoyladenin-9-yl) were combined and concentrated to dryness under reduced pressure to obtain **3** (B^1 = N^6-benzoyladenin-9-yl) (202 mg, 88%) as a colorless foam: ^{1}H NMR ($CDCl_3$)-δ 9.03 (1H, bs), 8.72 (1H, s), 8.17 (1H, s), 8.07−7.18 (14H, m), 6.81 (4H, d, J = 8.1 Hz), 6.52 (1H, t, J = 7.1 Hz), 5.25 (1H, m), 4.38 (1H, m), 3.78 (6H, s), 3.73, 3.72 (6H, 2d, J_{POCH} = 4.5, 6.6 Hz), 3.44 (2H, m), 3.12−2.99 (1H, m), 2.81−2.70 (1H, m), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 164.5, 158.6, 152.7, 152.6, 151.5, 149.5, 144.3, 141.4, 141.3, 135.4, 133.6, 132.8, 130.0, 129.9, 128.9, 128.0, 127.8, 127.1, 123.4, 113.3, 113.1, 86.8, 85.3, 84.6, 84.5, 77.6, 63.0, 55.3, 55.2, 55.1, 53.4, 39.0; ^{31}P NMR ($CDCl_3$) δ 120.0−116.8 (m). IR (KBr) 3420, 2953, 2837, 2400, 1702, 1610, 1582, 1509, 1457, 1300, 1252, 1178, 1072, 1033, 952, 830, 799, 756, 708, 645 cm^{-1}. Anal. Calcd for $C_{40}H_{43}BN_5O_8P$·$^1/_3CHCl_3$: C, 60.30; H, 5.44; N, 8.72. Found: C, 60.06; H, 5.50; N, 8.73.

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl Dimethyl Boranophosphate (3, B^1 = *N*^{4}-Benzoylcytosin-1-yl).[23] This compound was obtained from **1** (B^1 = N^4-benzoylcytosin-1-yl) as a colorless foam (96% yield) in a similar manner as **3** (B^1 = N^6-benzoyladenin-9-yl).

5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl Methyl 2-(Trimethylsilyl)ethyl Boranophosphate (19, B^1 = *N*^{3}-Benzoylthymin-1-yl). **1** (B^1 = N^3-benzoylthymin-1-yl) (130 mg, 0.200 mmol) and **17** (187 mg, 0.600 mmol) were dried by repeated coevaporation with dry toluene followed by dry pyridine and finally dissolved in dry THF (2.00 mL). To the solution were successively added *i*-Pr_2NEt (0.510 mL, 3.00 mmol), NT (114 mg, 1.00 mmol), and Bop-Cl (255 mg, 1.00 mmol). After being stirred at rt for 30 min, the mixture was diluted with $CHCl_3$ (20 mL). The reaction mixture was washed with satd $NaHCO_3$ aq (3 × 20 mL), and the aqueous layer was back-extracted with $CHCl_3$ (2 × 20 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (30 g) using EtOAc/hexane (1:1 v/v) as an eluent. The fractions containing 5′-*O*-dimethoxytrityl-N^3-benzoylthymidin-3′-yl methyl 2-(trimethylsilyl)-ethyl boranophosphate (**19**, B^1 = N^3-benzoylthymin-1-yl) were combined and concentrated to dryness under reduced pressure to obtain **19** (B^1 = N^3-benzoylthymin-1-yl) (153 mg, 91%) as a light yellow foam: ^{1}H NMR ($CDCl_3$) δ 7.95, 7.93 (2H, m), 7.72 (1H, s), 7.68−7.21 (12H, m), 6.87 (4H, d, J = 9.0 Hz), 6.45, 6.42 (1H, dd, $J_{1′,2′}/J_{1′,2″}$ = 5.7, 8.7 Hz), 5.21 (1H, t, J = 7.5 Hz), 4.25 (1H, m), 4.10 (2H, m), 3.80 (6H, s), 3.68, 3.63 (3H, 2d, J_{POCH} = 9.0, 12.0 Hz), 3.48−3.44 (2H, m), 2.66−2.57 (1H, m), 2.53−2.41 (1H, m), 1.46 (3H, s), 1.09−0.99 (2H, m), 1−0 (3H, bq), 0.04, 0.02 (9H, 2s); ^{13}C NMR ($CDCl_3$) δ 168.9, 162.7, 158.8, 158.7, 149.3, 144.1, 135.2, 135.0, 134.9, 131.6, 130.5, 130.2, 130.0, 129.3, 129.1, 128.2, 128.1, 128.0, 127.3, 126.1, 113.6, 113.4, 111.6, 87.3, 85.2, 85.1, 85.0, 84.9, 84.7, 84.6, 66.0, 65.9, 63.3, 63.2, 55.2, 55.1 55.0, 53.1 53.0 39.7, 39.6, 19.7, 19.6, 19.5 19.4, 11.7, −1.6; ^{31}P NMR ($CDCl_3$) δ 118.79−115.48 (m). IR (KBr) 3448, 2954, 2835, 2397, 1751, 1704, 1664, 1608, 1509, 1445, 1395, 1280, 1253, 1178, 1110, 1034, 987, 834, 762, 701 cm^{-1}. Anal. Calcd for $C_{44}H_{54}BN_2O_{10}PSi$: C, 62.86; H, 6.47; N, 3.33. Found: C, 63.07; H, 6.44; N, 3.27.

5′-*O*-Dimethoxytrityl-*O*^{6}-diphenylcarbamoyl-*N*^{2}-phenylacetyl-2′-deoxyguanosin-3′-yl Methyl 2-(Trimethylsilyl)ethyl Boranophosphate (19, B^1 = *O*^{6}-Diphenylcarbamoyl-*N*^{2}-phenylacetylguanin-9-yl).[23] This compound was obtained from **1** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) as a light yellow foam (80% yield) in a similar manner as **19** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl).

Triethylammonium 5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl Methyl Boranophosphate (4, B^1 = *N*^{6}-Benzoyladenin-9-yl). To a solution of **3** (B^1 = N^6-benzoyladenin-9-yl) (764 mg, 1.00 mmol) in THF (10 mL) were successively added Et_3N (5.54 mL, 40.0 mmol) and PhSH (4.08 mL, 40.0 mmol). After being stirred at rt for 2 h, the mixture was concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (100 g) by using a gradient of MeOH (0−4%) in CH_2Cl_2 with 0.5% Et_3N as an eluent. The fractions containing triethylammomium 5′-*O*-dimethoxytrityl-N^6-benzoyl-2′-deoxyadenosin-3′-yl methyl boranophosphate (**4**, B^1 = N^6-benzoyladenin-9-yl) were combined

(23) Characterization data are provided in the Supporting Information.

and concentrated to dryness. Excess Et_3N was removed by repeated coevaporation with toluene, and the residue was dissolved in $CHCl_3$ (10 mL). The solution was washed with satd NaCl aq (4 × 20 mL), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 10 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to obtain **4** (B^1 = N^6-benzoyladenin-9-yl) (791 mg, 93%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 9.18 (1H, bs), 8.71 (1H, s), 8.20, 8.19 (1H, 2s), 8.02, 8.05 (2H, 2m), 7.65−7.15 (12H, m), 6.79 (4H, d, J = 9.0 Hz), 6.58 (1H, m), 5.14 (1H, m), 4.43 (1H, m), 3.77 (6H, s), 3.55, 3.49 (3H, 2d, J_{POCH} = 10.8, 11.1 Hz), 3.41 (2H, m), 3.04 (6H, q, J = 7.2 Hz), 2.84 (2H, m), 1.31 (9H, t, J = 7.4 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 164.6, 158.4, 152.6, 152.5 151.5 149.3, 144.5, 141.4, 141.3 135.7 135.6, 133.8, 132.7, 130.1, 130.0, 128.8, 128.2, 127.8, 126.9, 123.1, 113.2, 113.0, 108.9, 86.5, 86.4, 85.8, 84.8, 84.7, 84.5, 74.0, 73.6, 73.4, 63.7, 55.3, 55.2, 55.1, 55.0, 50.2, 45.4, 40.2, 40.0, 8.6, 8.4; ^{31}P NMR ($CDCl_3$) δ 98.55−93.80 (m). IR (KBr) 3426, 2944, 2838, 2365, 1701, 1611, 1581, 1509, 1459, 1399, 1300, 1252, 1177, 1072, 1033, 946, 831, 794, 756, 710, 646 cm^{-1}. Anal. Calcd for $C_{45}H_{56}BN_6O_8P\cdot1.5H_2O$: C, 61.57; H, 6.77; N, 9.57. Found: C, 61.35; H, 6.51; N, 9.32.

Triethylammonium 5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl Methyl Boranophosphate (4, B^1 = *N*^{4}-Benzoylcytosin-1-yl).[23] This compound was obtained from **3** (B^1 = N^4-benzoylcytosin-1-yl) as a colorless foam (92% yield) in a similar manner as **4** (B^1 = N^6-benzoyladenin-9-yl).

Tributylammonium 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl Methyl Boranophosphate (20, B^1 = *N*^{3}-Benzoylthymin-1-yl). 19 (B^1 = N^3-benzoylthymin-1-yl) (168 mg, 0.200 mmol) was dried by repeated coevaporation with dry THF followed by dry pyridine and dry toluene and then dissolved in a 0.5 M solution of TBAF in THF (2.00 mL), which was predried over MS 4A for 12 h, and the mixture was stirred at rt for 5 min. The solution was diluted with $CHCl_3$ (10 mL) and washed with 1 M pH 7.0 phosphate buffer (3 × 10), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 10 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (30 g) using a gradient of MeOH (0−4%) in CH_2Cl_2 as an eluent. The fractions containg tributylammonium 5′-*O*-dimethoxytrityl-N^3-benzoylthymidin-3′-yl methyl boranophosphate (**20**, B^1 = N^3-benzoylthymin-1-yl) were combined and concentrated to dryness under reduced pressure to obtain **20** (B^1 = N^3-benzoylthymin-1-yl) (150 mg, 81%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 12.42 (1H, bs), 7.97−7.20 (16H, m), 6.46, 6.43 (1H, dd, $J_{1',2'}/J_{1',2''}$ = 5.7, 8.1 Hz), 5.21−5.10 (1H, m), 4.29 (1H, d, J = 12.0 Hz), 3.79 (6H, s), 3.52, 3.41 (3H, 2d, J_{POCH} = 12.0, 12.0 Hz), 3.45−3.42 (2H, m), 2.95−2.85 (6H, m), 2.69−2.56 (1H, m), 2.50−2.37 (1H, m), 1.73−1.60 (6H, m), 1.41−1.28 (6H, m), 1.35 (3H, s), 0.94 (9H, t, J = 6.0 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 169.2, 169.1, 162.9, 162.8, 158.6, 158.5, 149.2, 144.3, 144.2, 135.7, 135.6, 135.5, 135.4, 135.2, 134.8, 131.6, 130.5, 130.1, 129.0, 128.2, 128.0, 127.0, 113.3, 111.1, 111.0, 87.0, 86.9, 86.1, 86.0, 85.5, 85.4, 85.0, 84.8, 74.1, 73.9, 73.8, 63.7, 63.6, 55.2, 51.7, 50.0, 49.9, 49.8, 49.7, 45.8, 40.3, 40.2, 25.2, 20.1, 13.6, 11.5, 11.4, 8.5; ^{31}P NMR ($CDCl_3$) δ 98.31−93.59 (m); IR (KBr) 3448, 2961, 2874, 2838, 2362, 1749, 1703, 1661, 1608, 1509, 1460, 1445, 1392, 1279, 1253, 1178, 1114, 1066, 1034, 992, 829, 792, 763, 688, 648 cm^{-1}. Anal. Calcd. for $C_{51}H_{69}BN_3O_{10}P\cdot0.5H_2O$: C, 65.52; H, 7.55; N, 4.49. Found: C, 65.60; H, 7.42; N, 4.65.

Tributylammonium 5′-*O*-Dimethoxytrityl-*O*^{6}-diphenylcarbamoyl-*N*^{2}-phenylacetyl-2′-deoxyguanosin-3′-yl Methyl Boranophosphate (20, B^1 = *O*^{6}-Diphenylcarbamoyl-*N*^{2}-phenylacetylguanin-9-yl).[23] This compound was obtained from **19** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) as a light yellow foam (80% yield) in a similar manner as **20** (B^1 = N^3-benzoylthymin-1-yl).

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl 3′-*O*,*N*^{3}-Dibenzoylthymidin-5′-yl Methyl Boranophosphate (6, B^1 = *N*^{6}-Benzoyladenin-9-yl, B^2 = *N*^{3}-Benzoylthymin-1-yl). 5 (B^2 = N^3-benzoylthymin-1-yl) (45.0 mg, 0.100 mmol) and **4** (B^1 = N^6-benzoyladenin-9-yl) (102 mg, 0.120 mol) were dried by repeated coevaporation with dry toluene followed by dry pyridine and finally dissolved in dry THF (1.00 mL). To the solution were successively added *i*-Pr_2NEt (170 μL, 1.00 mmol), NT (34.2 mg, 0.300 mmol), and Bop-Cl (76.4 mg, 0.300 mmol). After being stirred at rt for 20 min, the mixture was diluted with $CHCl_3$ (10 mL). The solution was washed with satd $NaHCO_3$ aq (3 × 10 mL), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 10 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (30 g) using a gradient of MeOH (0−4%) in CH_2Cl_2 as an eluent. The fractions containg 5′-*O*-dimethoxytrityl-N^6-benzoyl-2′-deoxyadenosin-3′-yl 3′-*O*,N^3-dibenzoylthymidin-5′-yl methyl boranophosphate (**6**, B^1 = N^6-benzoyladenin-9-yl, B^2 = N^3-benzoylthymin-1-yl) were combined and concentrated to dryness under reduced pressure to obtain **6** (B^1 = N^6-benzoyladenin-9-yl, B^2 = N^3-benzoylthymin-1-yl) (100 mg, 85%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 8.99 (1H, bs), 8.65 (1H, m), 8.21, 8.18 (1H, 2s), 8.06−7.16 (25H, m), 6.86−6.74 (4H, m), 6.61−6.46 (2H, m), 5.57−5.46 (1H, m), 5.37−5.29 (1H, m), 4.57−4.26 (4H, m), 3.91−3.70 (9H, m), 3.55−3.39 (2H, m), 3.27−3.12 (1H, m), 2.84−2.71 (1H, m), 2.65−2.53 (1H, m), 2.40−2.25 (1H, m), 2.05, 2.02 (3H, 2s), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 168.7, 166.1, 164.5, 162.6, 158.5, 152.5, 151.5, 149.4, 144.2, 141.7, 141.5, 141.3, 135.3, 135.2, 135.1, 134.5, 133.8, 133.5, 132.8, 131.4, 130.4, 130.0, 129.7, 129.1, 128.9, 128.6, 128.0, 127.9, 127.8, 127.0, 123.5, 113.2, 112.3, 112.1, 86.9, 85.5, 85.3, 84.7, 84.6, 83.1, 79.0, 78.7, 74.9, 74.8, 66.0, 65.6, 63.1, 55.2, 54.4, 54.3, 45.5, 41.4, 38.5, 37.3, 37.0, 12.6; ^{31}P NMR ($CDCl_3$) δ 121.22−117.23 (m); IR (KBr) 3426, 2954, 2839, 2399, 1750, 1705, 1663, 1609, 1582, 1509, 1451, 1297, 1253, 1178, 1098, 1072, 1032, 952, 830, 762, 714, 646 cm^{-1}. Anal. Calcd for $C_{63}H_{61}BN_7O_{14}P\cdot{}^2/_3CHCl_3$: C, 60.61; H, 4.93; N, 7.77. Found: C, 60.56; H, 5.28; N, 7.97.

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl 3′-*O*,*N*^{3}-Dibenzoylthymidin-5′-yl methyl Boranophosphate (6, B^1 = *N*^{4}-Benzoylcytosin-1-yl, B^2 = *N*^{3}-Benzoylthymin-1-yl).[23] This compound was obtained from **4** (B^1 = N^4-benzoylcytosin-1-yl) and **5** (B^2 = N^3-benzoylthymin-1-yl) as a colorless foam (quantitative) in a similar manner as **6** (B^1 = N^6-benzoyladenin-9-yl, B^2 = N^3-benzoylthymin-1-yl).

5′-*O*-Dimethoxytrityl-*O*^{6}-diphenylcarbamoyl-*N*^{2}-phenylacetyl-2′-deoxyguanosin-3′-yl 3′-*O*,*N*^{3}-Dibenzoylthymidin-5′-yl Methyl Boranophosphate (6, B^1 = *O*^{6}-Diphenylcarbamoyl-*N*^{2}-phenylacetylguanin-9-yl, B^2 = *N*^{3}-Benzoylthymin-1-yl).[23] This compound was obtained from **4** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) and **5** (B^2 = N^3-benzoylthymin-1-yl) as a colorless foam (83%) in a similar manner as **6** (B^1 = N^6-benzoyladenin-9-yl, B^2 = N^3-benzoylthymin-1-yl).

5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl 3′-*O*,*N*^{3}-Dibenzoylthymidin-5′-yl Methyl Boranophosphate (6, B^1 = B^2 = *N*^{3}-Benzoylthymin-1-yl).[23] This compound was obtained from **4** (B^1 = N^3-benzoylthymin-1-yl) and **5** (B^2 = N^3-benzoylthymin-1-yl) as a colorless foam (quantitative) in a similar manner as **6** (B^1 = N^6-benzoyladenin-9-yl, B^2 = N^3-benzoylthymin-1-yl).

5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl 3′-*O*,*N*^{6}-Dibenzoyl-2′-deoxyadenosin-5′-yl Methyl Boranophosphate (6, B^1 = *N*^{3}-Benzoylthymin-1-yl, B^2 = *N*^{6}-Benzoyladenin-9-yl). By repeated coevaporation with dry toluene followed by dry pyridine, **5** (B^2 = N^6-benzoyladenin-9-yl) (45.9 mg, 0.100 mmol) and **20** (B^1 = N^3-benzoylthymin-1-yl) (111 mg, 0.120 mol) were dried and finally dissolved in dry CH_3CN (1.00 mL). To the solution were successively added *i*-Pr_2NEt (170 μL, 1.00 mmol) and PyNTP (150 mg, 0.300 mmol). After being stirred at rt for 20 min, the mixture was diluted

with $CHCl_3$ (10 mL). The solution was washed with 1 M pH 7.0 phosphate buffer (3 × 10 mL), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 10 mL). The organic layer and washings were combined, washed with satd $NaHCO_3$ aq (3 × 50 mL), and the aqueous layer was back-extracted with $CHCl_3$ (3 × 100 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (30 g) using a gradient of MeOH (0−4%) in CH_2Cl_2 as an eluent. The fractions containing 5′-*O*-dimethoxytrityl-N^3-benzoylthymidin-3′-yl 3′-*O*,N^6-dibenzoyl-2′-deoxyadenosin-5′-yl methyl boranophosphate (**6**, B^1 = N^3-benzoylthymin-1-yl, B^2 = N^6-benzoyladenin-9-yl) were combined and concentrated to dryness under reduced pressure to obtain **6** (B^1 = N^3-benzoylthymin-1-yl, B^2 = N^6-benzoyladenin-9-yl) (109 mg, 92%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 8.83−8.79 (1H, bs), 8.32, 8.27 (1H, 2s), 8.10−7.15 (26H, m), 6.90−6.81 (4H, m), 6.67−6.49 (1H, m), 6.48−6.38 (1H, m), 5.77−5.59 (1H, m), 5.32−5.20 (1H, m), 4.49−4.26 (4H, m), 3.80−3.74 (6H, m), 3.68, 3.64 (3H, 2d, J_{POCH} = 12.0, 11.4 Hz), 3.51−3.41 (2H, m), 3.15−2.97 (1H, m), 2.86−2.41 (3H, m), 1.47, 1.44 (3H, 2s), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 168.8, 165.8, 164.5, 162.6, 159.1, 158.7, 152.9, 152.6, 151.5, 151.3, 149.6, 149.2, 143.9, 141.2, 141.0, 135.2, 134.9, 133.6, 133.2, 132.6, 131.4, 130.9, 130.3, 129.9, 129.6, 129.0, 128.6, 128.5, 127.9, 127.7, 127.5, 127.1, 125.3, 123.4, 113.3, 111.5, 87.2, 84.9, 84.6, 84.4, 84.2, 83.3, 78.2, 74.8, 65.9, 65.7, 63.1, 55.1, 53.7, 39.5, 39.3, 37.3, 37.0, 29.5, 14.0, 11.7; ^{31}P NMR ($CDCl_3$) δ 121.33−117.45 (m). Anal. Calcd for $C_{63}H_{61}BN_7O_{14}P\cdot CHCl_3$: C, 59.07; H, 4.80; N, 7.53. Found: C, 59.05; H, 4.92; N, 7.48.

5′-*O*-Dimethoxytrityl-N^3-benzoylthymidin-3′-yl 3′-*O*-*tert*-Butyldimethylsilyl-N^4-benzoylcytidin-5′-yl Methyl Boranophosphate (6, B^1 = N^3-Benzoylthymin-1-yl, B^2 = N^4-Benzoylcytosin-1-yl).[23] This compound was obtained from **20** (B^1 = N^3-benzoylthymin-1-yl) and **5** (B^2 = N^4-benzoylcytosin-1-yl) as a colorless foam (99%) in a similar manner as **6** (B^1 = N^3-benzoylthymin-1-yl, B^2 = N^6-benzoyladenin-9-yl).

5′-*O*-Dimethoxytrityl-N^3-benzoylthymidin-3′-yl 3′-*O*-benzoyl-N^6-diphenylcarbamoyl-N^2-phenylacetyl-2′-deoxyguanosin-5′-yl Methyl Boranophosphate (6, B^1 = N^3-Benzoylthymin-1-yl, B^2 = N^6-Diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl).[23] This compound was obtained from **20** (B^1 = N^3-benzoylthymin-1-yl) and **5** (B^2 = N^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl) as a colorless foam (94%) in a similar manner as **6** (B^1 = N^3-benzoylthymin-1-yl, B^2 = N^6-benzoyladenin-9-yl).

Triethylammonium Thymidin-5′-yl Thymidin-3′-yl Boranophosphate (27, B^1 = B^2 = Thymin-1-yl). **6** (B^1 = B^2 = N^3-benzoylthymin-1-yl) (58.6 mg, 50 μmol) were added to a solution of 3% DCA in $Et_3SiH-CH_2Cl_2$ (1:1 v/v, 5 mL), and the solution was stirred at rt for 1 min. The mixture was diluted with CH_2Cl_2 and washed with satd $NaHCO_3$ aq (3 × 10 mL), and the aqueous layer was back-extracted with $CHCl_3$ (1 × 10 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. To a solution of the residue in THF (2 mL) was added a mixture of Et_3N and PhSH (1:1 v/v; 2 mL). After being stirred at rt for 2 h, the mixture was concentrated to dryness under reduced pressure. Then, the residue was dissolved in concentrated NH_3/MeOH (50 mL) and heated at 55 °C for 12 h. The reaction mixture was then allowed to cool to rt and concentrated to dryness under reduced pressure. The residue was purified by reversed-phase column chromatography [a linear gradient of acetonitrile 0−15% in 0.1 M triethylammonium acetate buffer (pH 7.0)] to afford **27** (B^1 = B^2 = thymin-1-yl) (27.7 mg, 86%) as a colorless foam: ^{1}H NMR (D_2O) δ 7.75−7.64 (2H, m), 6.36−6.20 (2H, m), 4.93−4.79 (1H, m), 4.61−4.53 (1H, m), 4.20−4.12 (2H, m), 4.14−4.05 (1H, m), 3.89−3.74 (2H, m), 3.57 (1H, m), 3.20 (6H, q, J = 8.0 Hz), 2.55−2.29 (4H, m), 1.94−1.86 (6H, m), 1.28 (9H, t, J = 6.0 Hz), 1−0 (3H, bq); ^{13}C NMR (D_2O) δ 169.2, 169.1, 169.0, 168.9, 154.3, 154.2, 140.2, 140.0, 114.2, 114.1, 88.5, 88.1, 88.0, 87.9, 87.5, 75.6, 75.3, 75.1, 73.5, 65.4, 64.3, 63.7, 63.6, 52.8, 49.4, 41.6, 41.5, 40.9, 40.7, 14.5, 14.4, 14.3, 10.9; ^{31}P NMR (D_2O) δ 95.78−90.60 (m); FAB$^+$ *m*/*z* calcd for $C_{20}H_{31}BN_4O_{11}P$ [M + H]$^+$ 545.1820, found 545.1814; UV (H_2O) λ_{max} 267 nm; ϵ_{267} 17800.

Triethylammonium 2′-Deoxyadenosin-3′-yl Thymidin-5′-yl Boranophosphate (27, B^1 = Adenin-9-yl, B^2 = Thymin-1-yl).[23] This compound was obtained from **6** (B^1 = N^6-benzoyladenin-9-yl, B^2 = N^3-benzoylthymin-1-yl) as a colorless foam (75% yield) in a similar manner as **27** (B^1 = B^2 = thymin-1-yl).

Triethylammonium 2′-Deoxycytidin-3′-yl thymidin-5′-yl Boranophosphate (27, B^1 = Cytosin-1-yl, B^2 = Thymin-1-yl).[23] This compound was obtained from **6c't'** (B^1 = N^4-benzoylcytosin-1-yl, B^2 = N^3-benzoylthymin-1-yl) as a colorless foam (89% yield) in a similar manner as **27** (B^1 = B^2 = thymin-1-yl).

Triethylammonium 2′-Deoxyguanosin-3′-yl Thymidin-5′-yl Boranophosphate (27, B^1 = Guanin-9-yl, B^2 = Thymin-1-yl). **6** (B^1 = O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl, B^2 = N^3-benzoylthymin-1-yl) (28.1 mg, 20.0 μmol) were added to a solution of 3% DCA in $Et_3SiH-CH_2Cl_2$ (1:1 v/v, 2 mL), and the solution was stirred at rt for 1 min. The mixture was diluted with CH_2Cl_2 and washed with satd $NaHCO_3$ aq (3 × 5 mL), and the aqueous layer was back-extracted with $CHCl_3$ (1 × 5 mL). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in concentrated NH_3/MeOH (2 mL) and allowed to cool to 0 °C. After being kept at 0 °C for 2 h, the mixture was concentrated to dryness under reduced pressure. To a solution of the residue in THF (1 mL) was added a mixture of Et_3N and PhSH (1:1 v/v; 1 mL). After being stirred at rt for 2 h, the mixture was concentrated to dryness under reduced pressure. Then, the residue was dissolved in concentrated NH_3/MeOH (20 mL) and heated at 55 °C for 12 h. The reaction mixture was then allowed to cool to rt and concentrated to dryness under reduced pressure. The residue was purified by reversed-phase column chromatography [a linear gradient of acetonitrile 0−15% in 0.1 M triethylammonium acetate buffer (pH 7.0)] to afford **27** (B^1 = guanin-9-yl, B^2 = thymin-1-yl) (12.4 mg, 92%) as a colorless foam: ^{1}H NMR (D_2O) δ 7.88 (1H, s), 7.61, 7.54 (1H, 2s), 6.33−6.12 (2H, m), 5.02−4.93 (1H, m), 4.57−4.49 (1H, m), 4.30−3.47 (6H, m), 3.26 (6H, q, J = 8.0 Hz), 2.82−2.53 (2H, m), 2.36−2.24 (2H, m), 1.89, 1.76 (3H, 2s), 1.24 (9H, t, J = 7.5 Hz), 1−0 (3H, bq); ^{13}C NMR (D_2O) δ 184.2, 169.1, 169.0, 162.2, 157.0, 154.4, 153.5, 140.4, 140.2, 140.0, 139.8, 139.7, 119.5, 114.3, 114.1, 114.0, 89.2, 88.4, 87.9, 87.8, 87.6, 87.3, 87.0, 76.4, 76.3, 74.0, 73.2, 65.5, 64.3, 64.2, 63.9, 52.8, 49.4, 41.7, 41.5, 41.2, 40.9, 26.0, 14.5, 14.4, 11.0; ^{31}P NMR (D_2O) 96.07−90.74 (m); FAB$^+$ *m*/*z* calcd for $C_{20}H_{30}BN_7O_{10}P$ [M + H]$^+$ 570.1885, found 570.1891; UV (H_2O) λ_{max} 256.4 nm; $\epsilon_{256.4}$ 16500.

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra of **2**−**4**, **6**, **17**, **19**, **20**, and **27**. FAB-MS spectra of **27**. Experimental details and characterization data for O^6-diphenylcarbamoyl-N^2-phenylacetyl-2′-deoxyguanosine and **1**. Characterization data for **3**, **19**, **4**, **20**, **6**, and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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