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Solid-phase synthesis of oligodeoxynucleotides using nucleobase *N*-unprotected oxazaphospholidine derivatives bearing a long alkyl chain†

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In this study, we developed a new approach for the solid-phase synthesis of oligodeoxynucleotides (ODNs) using nucleobase-unprotected oxazaphospholidine derivatives. We tackled the problem of the difficult purification of *N*-unprotected monomers due to their high affinity to silica gel by introducing a tetrahydrogeranyl group into the oxazaphospholidine monomers, thereby enhancing the lipophilicity and facilitating the isolation. In addition, the cyclic structure of oxazaphospholidine enabled a hydroxy-group-selective condensation with sufficient efficiency. Unmodified and boranophosphate/phosphate chimeric ODNs were successfully synthesized using this strategy. This synthetic method can be expected to afford ODNs containing base-labile functional groups.

Introduction

The phosphoramidite approach is considered the definitive means for the synthesis of oligonucleotides.[1] In this method, chain elongation is conducted *via* condensation of a phosphoramidite monomer and a 5′-hydroxy group of a nucleotide under mild acidic conditions. The amino groups of nucleobases are generally protected by acyl groups to prevent undesired nucleobase phosphitylation during the condensation.[2] However, avoiding the nucleobase protection is highly desirable because it has three advantages. First, skipping nucleobase protection and deprotection processes allows reducing the number of steps in the synthesis of the phosphoramidite monomer.[3] Second, the synthesis of the oligomer without nucleobase protection considerably diminishes the risk of depurination of deoxyadenosine derivatives in the process of acid treatment, which has been reported to occur under the acidic conditions applied for the removal of the DMTr group in *N*[6]-acyl-protected derivatives.[4] Third, the synthesis without nucleobase protection allows obtaining chemically modified oligodeoxynucleotides (ODNs), which are unstable under basic conditions, such as alkylphosphonate,[5] because the base de-

protection process usually conducted by treating with aqueous ammonia is unnecessary.

Hayakawa *et al.* reported the synthesis of oligonucleotides using nucleobase-unprotected phosphoramidite derivatives.[6,7] They found that using imidazolium triflate as an acidic activator can reduce the nucleobase phosphitylation to some extent. However, a part of the amino groups is still phosphitylated during the condensation reaction, and treatment with benzimidazolium triflate–methanol to cleave the P–N bond(s) is necessary after each condensation.[6,7] An improved version of the nucleobase-unprotected approach was the proton-block method reported by Sekine *et al.*[8] In this method, the amino groups of nucleobases are temporary protected as unreactive protonated forms during condensation to prevent the reaction with an activated monomer using an acidic activator having relatively low pK_a values, affording 20-mer ODNs.

Meanwhile, we previously reported that the condensation reaction using nucleobase-unprotected oxazaphospholidine derivatives proceeded with a hydroxy group in a chemoselective manner even under mild acidic conditions.[9–11] Oxazaphospholidines are cyclic phosphoramidite derivatives that were originally developed to synthesize *P*-stereodefined oligonucleotides.[12] The chemoselectivity brought by the oxazaphospholidine derivatives can be explained in terms of an intramolecular recyclization that regenerates the oxazaphospholidine derivatives and the nucleobase having a free amino[9,10] or imide[11] group (Scheme 1).
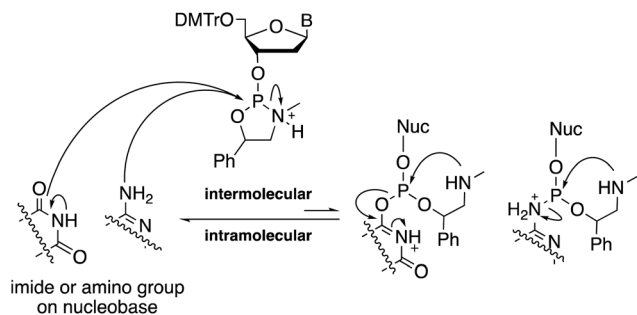
In such reports, we synthesized 5-H/*N*-Me- and 5-Ph/*N*-Me-substituted oxazaphospholidine derivatives with unprotected nucleobases (Scheme 2) and obtained unmodified ODNs up to

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† Electronic supplementary information (ESI) available: HPLC profiles, copies of ^{1}H, ^{13}C, ^{31}P NMR, COSY, HMQC and HMBC spectra. See DOI: https://doi.org/10.1039/d3ob01255g

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Scheme 1 Mechanism of the intermolecular nucleophilic addition.

tetramers and boranophosphate (PB) DNAs up to dimers using the former and the latter monomers, respectively. PB DNAs, in which one of the nonbridging oxygen atoms of the phosphodiester is substituted with a borano group (BH_3), have attractive properties as antisense oligonucleotides, such as high nuclease resistance and lipophilicity and low cytotoxicity.[13,14] When acyl-type amino protecting groups, which are widely used for nucleobase protection, are employed for the synthesis of PB DNAs, irreversible reduction of amide groups to alkylamino groups occurs as a serious side reaction during the boronation reaction.[15] This problem can be overcome by using synthetic strategies without nucleobase protection.[9,16] However, when using 5-H/N-Me- or 5-Ph/N-Me-substituted monomers, the synthesis of longer oligomers was unsuccessful, which was attributed to insufficient condensation efficiency, especially in the case of the deoxyguanosine monomer. Moreover, the purity of the isolated unprotected deoxyguanosine monomer

was not satisfactory (*ca.* 81% for the 5-H/N-Me-substituted derivative and *ca.* 71% for the 5-Ph/N-Me-substituted derivative according to a ^{31}P NMR spectroscopic analysis), most likely due to the high affinity of the monomer to silica gel, which results in a longer elution and, consequently, in the decomposition of the monomer.

To address this issue, in this study, we introduced a long alkyl chain into the oxazaphospholidine monomer to endow it with lipophilicity. The tetrahydrogeranyl (Thg) group, which has a branched structure, was selected as the long alkyl group because it would also improve the monomer solubility in reaction solvents compared with linear alkyl groups due to diminished interactions between branched alkyl chains.[17] Herein, we demonstrate that the monomers were applicable to the synthesis of ODNs up to dodecamers and oligonucleotides contain PB linkages.

Results and discussion

Synthesis of oxazaphospholidine monomers

First, an amino alcohol bearing an *N*-Thg group was synthesized using 2-amino-1-phenyl ethanol **1** as a substrate (Scheme 3). The hydroxy group of **1** was protected using trimethylsilyl chloride (TMSCl) followed by the introduction of a 2-nitrobenzenesulfonyl (Ns) group into the amino group. The Thg group was introduced into the sulfonamide using a Mitsunobu reaction with tetrahydrogeraniol.[18] Then, the TMS group was removed by treating with trifluoracetic acid (TFA), and the resulting crude product was purified by silica gel
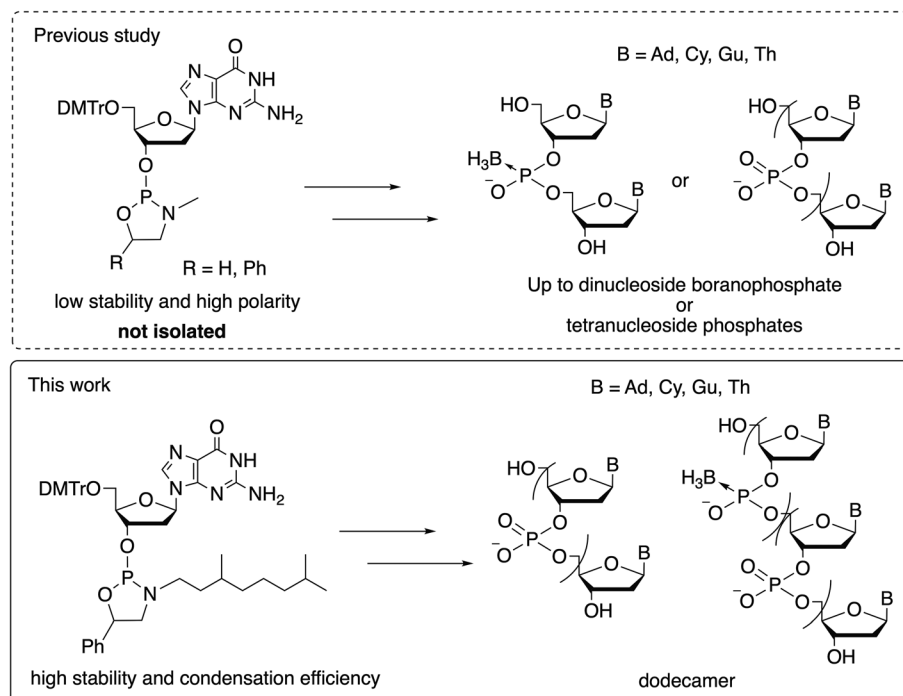
Scheme 2 Comparison of the monomer unit structure used in our previous study and this work.
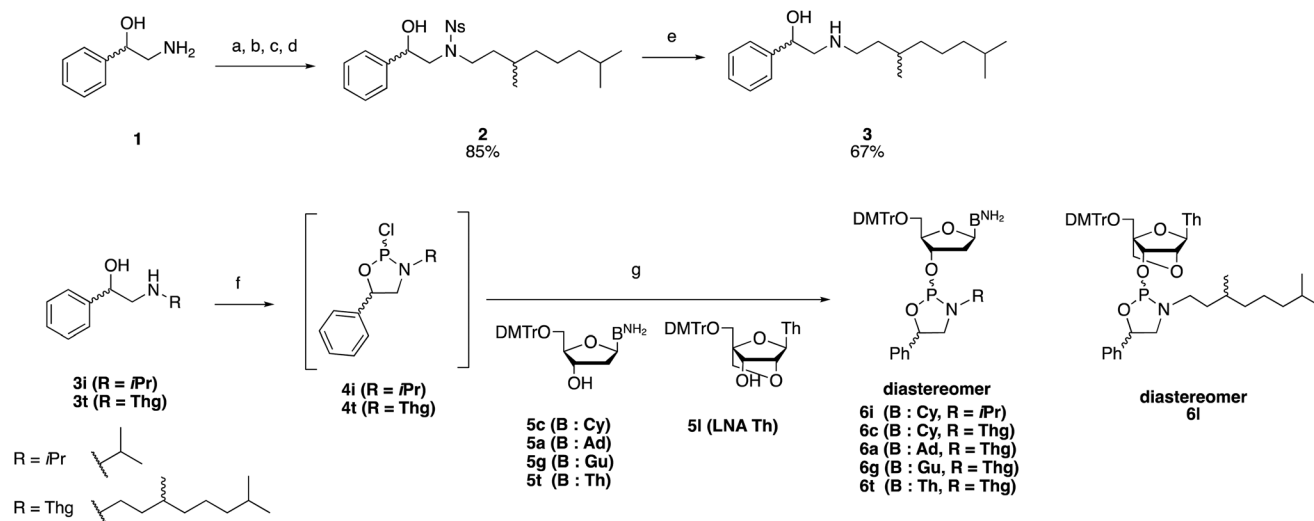
Scheme 3 Synthesis of N-Thg and nucleobase-unprotected oxazaphospholidine derivatives **6a–l**. Reagents and conditions: (a) TMSCl (1.5 equiv.), Et₃N (1.5 equiv.), THF, room temperature (rt), 2 h; (b) NsCl (1.05 equiv.), Et₃N (2.0 equiv.), CH₂Cl₂, 0 °C to rt, 2 h; (c) tetrahydrogeraniol (1.4 equiv.), PPh₃ (2.0 equiv.), DIAD (2.0 equiv.), THF, 0 °C to rt, 24 h; (d) 3% TFA, CH₂Cl₂, rt, 18 h, 85% over four steps; (e) 4-mercaptobenzoic acid (2.0 equiv.), K₂CO₃ (4.0 equiv.), DMF, 40 °C, 12 h, 67%; (f) PCl₃ (1.1 equiv.), N-methylmorpholine (2.1 equiv.), toluene, 0 °C to rt, 2 h; (g) 4 (2.5 equiv.), Et₃N (7.0 equiv.), THF, −78 °C (**6i**, **6a**, **6c**), −40 °C (**6g**), −78 °C to rt (**6t**, **6l**), 2 h, 27% (**6i**), 34% (**6a**), 39% (**6c**), 30% (**6g**), 55% (**6t**), 32% (**6l**).

column chromatography to obtain compound **2** (84% isolated yield over four steps). The Ns group was removed via treatment with 4-mercaptobenzoic acid, which is an odorless thiol,[19] to afford amino alcohol **3** in a 56% isolated yield from **1**.

Next, the synthesis of oxazaphospholidine monomers with a iPr or Thg group introduced into the N-atom of the oxazaphospholidine ring was carried out. These monomer units were synthesized using unprotected nucleosides including deoxyribose and 2′-O-4′-C-locked nucleoside (LNA)[20] derivatives and phosphitylating reagents **4i** (R = iPr) or **4t** (R = Thg), which was prepared from phosphorus trichloride and amino alcohols **3i** (R = iPr) or **3t** (R = Thg). The compounds **4** were not isolated considering the chemical instability and used after only removal of insoluble salts by filtration in the following reaction. For the synthesis of the deoxyadenosine and deoxycytidine derivatives, the reactions were conducted at −78 °C to prevent the phosphitylation of the unprotected amino groups. In the case of the deoxyguanosine derivative, the reaction was performed at −40 °C to enhance the conversion rate. Meanwhile, the phosphitylation of the thymidine and LNA thymidine derivatives was conducted initially at −78 °C and finally at room temperature (rt) through gradual warming. The deoxyadenosine, deoxycytidine, thymidine, and LNA thymidine oxazaphospholidine monomers were isolated with high purity and moderate yields (**6i**, 27%; **6a**, 34%; **6c**, 39%; **6t**, 55%; **6l**, 32%) via silica gel column chromatography. It is worth noting that t-butyl alcohol was effective as the eluent of silica gel chromatography in the case of the deoxycytidine and deoxyguanosine derivatives. When methanol was used as an eluent, the purified oxazaphospholidine monomer decomposed to phosphitetriester during the solvent removal process. Thus, an alcohol with low nucleophilicity was more

suitable as a polar eluent. In the case of the deoxyguanosine monomer, the purity was about 90% (determined via ³¹P NMR spectroscopy) and its separation from oxidized compounds via silica gel column chromatography was troublesome. Therefore, reversed-phase high-performance liquid chromatography (RP-HPLC) was used to purify the monomer after a silica gel column chromatography which roughly removed highly polar compounds and reduced the burden on HPLC columns. As a result, the deoxyguanosine monomer was isolated with a high purity and moderate yield (**6g**, 30%).

Although the isolated yields of N-Thg monomers were in the range of 30%–55%, these yields were considerably higher than those of 5-Ph/N-Me-substituted unprotected monomers, especially deoxycytidine and deoxyguanosine derivatives (17% and 3%, respectively). In addition to this, the isolated yield of N-Thg monomer was higher than that of N-iPr-substituted monomer (**6i**; 27% vs. **6c**; 39%). TLC monitoring of the reaction mixtures indicated that the conversion rates of **5c** and **5g** to **6c** and **6g** were about 50%, which were low compared with those of their N-Me-substituted counterparts probably due to the steric hindrance of the alkyl group. However, the higher isolation yields of the N-Thg derivatives compared with those of the N-Me derivatives can be attributed to their easier purification and higher chemical stability. Although a greater excess of compound **4t** could lead to the completion of the reaction, no optimization of equivalents of phosphitylating reagent was conducted.

Solid-phase synthesis of oligonucleotide phosphates

With the nucleobase-unprotected oxazaphospholidine monomers in hand, the solid-phase synthesis of the dinucleoside phosphates was conducted using highly crosslinked poly-

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styrene (HCP) as a solid support.[21] The 5′-hydroxy group of **7** was condensed with monomer **6** in the presence of *N*-(phenyl) imidazolium triflate (PhIMT)[22] as an acidic activator in CH₃CN–iPrCN (7 : 3). The resultant phosphite **8** was oxidized using *t*-butyl hydroperoxide (TBHP) to afford a phosphotriester. Moreover, the DMTr group on the 5′-hydroxy group was removed under acidic conditions with 3% dichloroacetic acid (DCA), affording **9**. The cleavage of the succinyl linker using an aqueous NH₃–EtOH (3 : 1, v/v) solution yielded dinucleoside phosphate derivatives **10a–l** (Scheme 4). It is worth noting that PhIMT was found to be effective for the activation of oxazaphosholidine monomers in the synthesis of oligoribonucleotide derivatives. Thus, PhIMT was chosen as an optimal activator in the current study.[23,24]

To begin with, the condensation efficiency was compared using *N*-Me-, *N*-iPr-, and *N*-Thg-substituted monomers. The HPLC yields of dinucleotide phosphates other than the deoxyguanosine derivative were 95%–97% when the *N*-Me-substituted monomers were used, whereas *N*-iPr- and *N*-Thg-substituted monomers afforded the corresponding products in HPLC yields higher than 99% (Table 1, entries 1–3 *vs.* 4–7), as confirmed by the area ratio defined by product/(product + Th). This result suggested that the *N*-iPr- and *N*-Thg-substituted monomers exhibited higher coupling efficiencies. In general, the phosphoramidite reactivity is known to be largely affected by steric hindrance.[25] However, in this case, the steric hindrance of the *N*-Me-substituted monomers around the N-atom are small, resulting in immediate hydrolysis during condensation. Thus, the introduction of the iPr and Thg groups
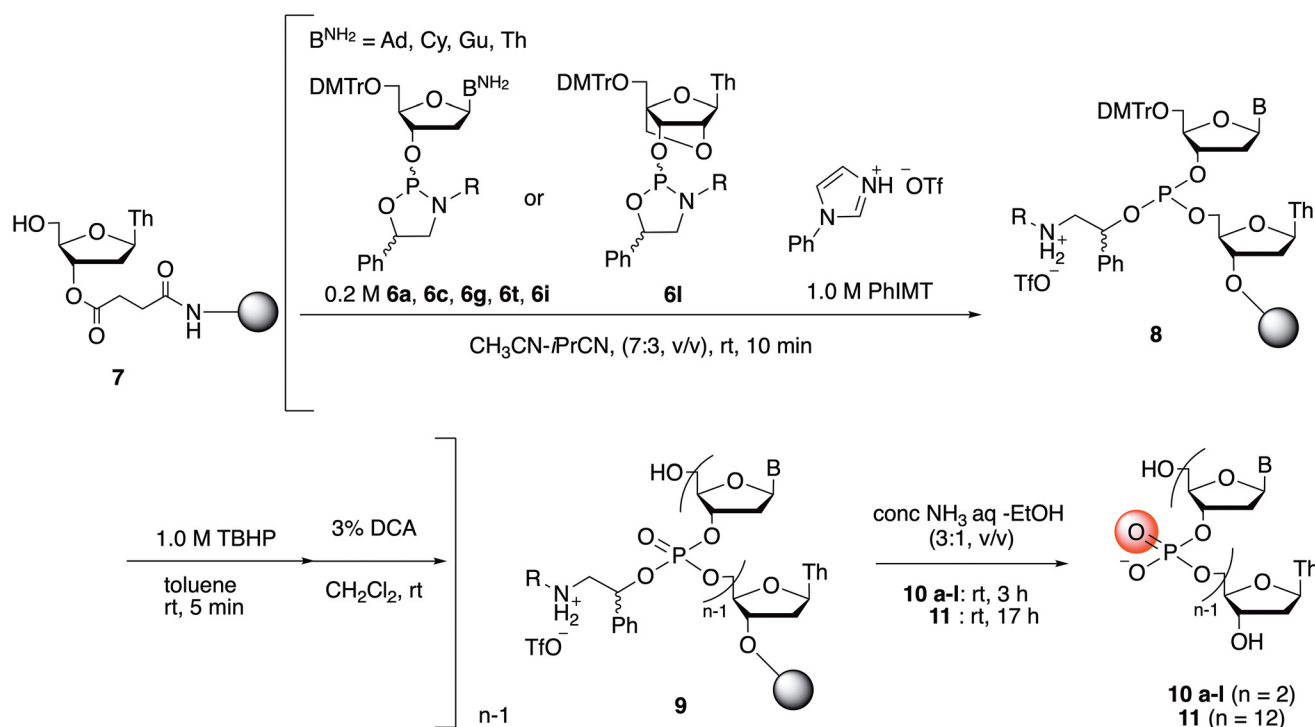
Table 1 Solid-phase synthesis of N$_{PO}$T dimers and oligonucleotide phosphate

Entry	Product[a]	R	Yield[b] (%)
1	dC$_{PO}$T **10c**	Me	97
2	dA$_{PO}$T **10a**	Me	95
3	T$_{PO}$T **10t**	Me	97
4	dC$_{PO}$T **10c**	iPr	>99
5	dC$_{PO}$T **10c**	Thg	>99
6	dA$_{PO}$T **10a**	Thg	>99
7	T$_{PO}$T **10t**	Thg	>99
8	dG$_{PO}$T **10g**	Thg	>99
9	^{L}T$_{PO}$T **10l**	Thg	>99
10	d(C$_{PO}$A$_{PO}$G$_{PO}$T$_{PO}$C$_{PO}$A$_{PO}$G$_{PO}$T$_{PO}$C$_{PO}$A$_{PO}$G$_{PO}$T) (**11**)	Thg	14[c]

[a] Subscript PO = phosphate, superscript L = LNA. [b] Determined *via* RP-HPLC. [c] Isolated yield, determined according to the UV absorbance at 260 nm.

improved the stability of the monomer while maintaining satisfactory condensation efficiency. The *N*-Thg-substituted monomer was chosen as an optimal monomer unit due to the higher isolated yield of the monomer.

Then, the deoxyguanosine monomer was examined for the synthesis of dimers (Table 1, entry 7). The dG$_{PO}$T dimer was obtained in more than 99% HPLC yield. No phosphitylation on the nucleobase amino groups was observed using oxazaphospholidine monomers bearing the *N*-Thg group. In addition, the synthesis was performed using LNA thymidine monomer **6l**, obtaining the ^{L}T$_{PO}$T dimer (the superscript L

Scheme 4 Solid-phase synthesis of N$_{PO}$T dimers and oligonucleotide phosphate.

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indicates LNA nucleoside) in more than 99% HPLC yield. The LNA monomer also exhibited high condensation efficiency, although the reactivity of LNA derivatives is typically low due to the steric hindrance caused by the 2′ and 4′ locked structure.

Next, the synthesis of a dodecamer of deoxyribonucleotide d($C_{PO}A_{PO}G_{PO}T_{PO}C_{PO}A_{PO}G_{PO}T_{PO}C_{PO}A_{PO}G_{PO}T$) **11** bearing four nucleobases was investigated. Fig. S2† shows the UPLC profile of crude **11**, which indicates that the desired product was obtained as the main product. The dodecamer was isolated in 14% yield, identified *via* mass spectrometry, and analyzed using ^1H and ^{31}P NMR spectroscopies (Fig. S7 and S8†). The ^1H NMR spectrum revealed the presence of signals stemming from the sugar backbone and the nucleobases. Meanwhile, in the ^{31}P NMR spectrum, only signals in the PO region were observed (Fig. S8†).

Finally, the purity of the synthesized oligomer was compared with that of a commercial one *via* RP-UPLC. The peaks of both products appeared at the same retention time, confirming the successful synthesis of the oligomer with similar purity to that of the commercial product and demonstrating that the newly designed monomers enable the synthesis of a PO ODN without nucleobase protection.
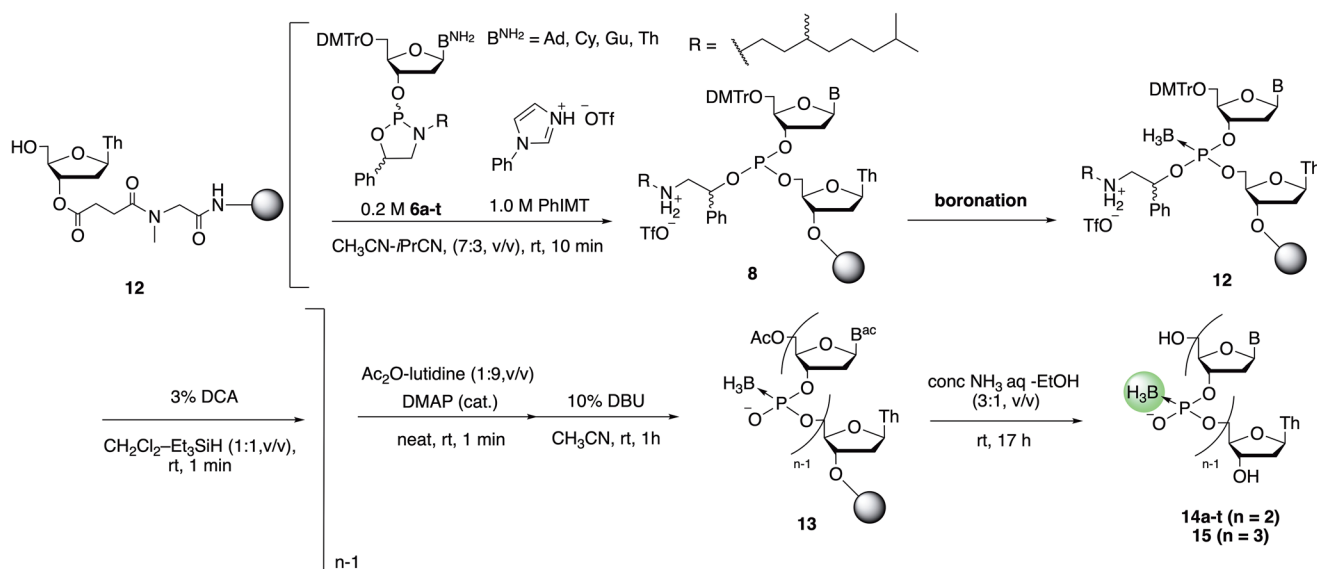
Solid-phase synthesis of dinucleoside and trinucleoside boranophosphates

Next, the synthesis of the dinucleoside boranophosphates was studied as an example of *P*-modifications using the nucleobase-unprotected approach. The succinyl sarcosyl linker[26] tethered between thymidine and HCP was selected to gain stability under the DBU treatment conditions required to remove the protecting group of the PO moiety.[27,28] The succinyl linker has been reported to be gradually cleaved by DBU to form a five-membered ring.[26] After the condensation reaction, the resul-

tant phosphite was boronated with 1.0 M $BH_3 \cdot SMe_2$/toluene for 15 min to afford boranophosphotriester **12**. Moreover, the DMTr group on the 5′-hydroxy group was cleaved under acidic conditions with DCA using Et_3SiH as a DMTr cation scavenger to prevent the side reaction on the borano group.[29] To avoid an intramolecular attack to the internucleotidic linkage of the boranophosphotriester by the 5′-hydroxy group,[30] the solid support was treated with DBU to afford the desired boranophosphodiester **13** after capping the 5′-hydroxy group with acetic anhydride (Ac_2O). Finally, the resulting dimers **14** were cleaved from the solid support after ammonia treatment (Scheme 5).

First, the dinucleoside boranophosphate was synthesized using the deoxycytidine monomer under boronation conditions with 1.0 M $BH_3 \cdot SMe_2$/toluene. The HPLC yield of the target compound was 97% as confirmed by the product/ product + Th area ratios. This result prompted us to synthesize the trinucleoside boranophosphate. Since our group found that the boronation reagent and/or its residue(s) inhibited the subsequent condensation reaction in the synthesis of tetrasaccharide boranophosphate,[31] a washing step with EtOH was conducted after the boronation reaction to remove the reagent. However, the coupling yield of the second condensation reaction was not satisfactory; specifically, $dC_{PB}T$ and $d(C_{PB}C_{PB}T)$ were obtained in 12% and 85% HPLC yields, respectively (Table 2, entry 2). To address this issue, the boronation conditions were investigated, finding that boronation using 0.05 M $BH_3 \cdot THF$/THF for 2 min were the optimal conditions (see ESI† for details), which provided $dC_{PB}T$ in a comparable yield to that obtained when using harsher conditions. With regard to the synthesis of trimers, the HPLC yield of $d(C_{PB}C_{PB}T)$ was improved to 94%.

When other nucleobase monomers were used for the synthesis of dimers, the $dA_{PB}T$, $dG_{PB}T$, and $T_{PB}T$ dimers were obtained in 97%–99% HPLC yields (Table 2, entries 5–7).

Scheme 5 Solid-phase synthesis of $N_{PB}T$ dimers and $N_{PB}N_{PB}T$ trimer.

Table 2 Solid-phase synthesis of dimers and trimers

Entry	Product[a]	Boronation conditions	T : dimer or T : dimer : trimer[b]
1	dC$_{PB}$T **14c**	1.0 M BH$_3$·SMe$_2$/toluene	3 : 97
2	d(C$_{PB}$C$_{PB}$T) **15**	1.0 M BH$_3$·SMe$_2$/toluene	3 : 12 : 85
3	dC$_{PB}$T **14c**	0.05 M BH$_3$·THF/THF	3 : 97
4	d(C$_{PB}$C$_{PB}$T) **15**	0.05M BH$_3$·THF/THF	1 : 5 : 94
5	dA$_{PB}$T **14a**[c]	0.05 M BH$_3$·THF/THF	1 : 99
6	dG$_{PB}$T **14g**	0.05 M BH$_3$·THF/THF	1 : 99
7	T$_{PB}$T **14t**	0.05 M BH$_3$·THF/THF	2 : 98

[a] Subscript PB = boranophosphate. [b] Determined *via* RP-HPLC. [c] Ammonia treatment was conducted at rt, for 3 h and then at 50 °C for 17 h.

In the case of dA$_{PB}$T, the removal of the borane adducts on the N^1 and/or N^7 positions of an adenine[29] required ammonia treatment for longer time and higher temperature, whereas other nucleobases did not require high-temperature conditions.[32] As summarized in Table 2, the dinucleoside and tri-nucleoside boranophosphates were synthesized in satisfactory HPLC yields without considerable amounts of byproducts.

Solid-phase synthesis of oligonucleotides bearing boranophosphates

On the basis of these results, the synthesis of all-PB and PB/PO chimeric tetramers was attempted according to Scheme 6. The synthesis cycle consisted of condensation of **6** in the presence of PhIMT, boronation or oxidation, and removal of the DMTr group. Capping of the 5′-hydroxy group and deprotection of PO or PB moieties followed by ammonia treatment afforded the tetramers.

Fig. S5† shows the HPLC profiles of all-PB and PB/PO chimeric tetramers, *i.e.*, d(C$_{PB}$A$_{PB}$G$_{PB}$T) **16** and d(C$_{PB}$A$_{PO}$G$_{PB}$T) **17**, respectively, which indicate that the desired products were obtained as the main products. Tetramers **16** and **17** were isolated in 28% and 33% yields, respectively (Table 3, entries 1 and 2). The isolated compounds were analyzed *via* ^{1}H NMR spectroscopy. The corresponding NMR spectra showed the characteristic signals of nucleobases and the borano groups (Fig. S9 and S10†). The successful synthesis of the PB/PO chimeric tetramer indicated that the PB linkages were stable under the oxidation conditions using TBHP.

In a similar manner, PB/PO chimeric dodecamer **18** containing alternate PB moieties was synthesized. Considering the
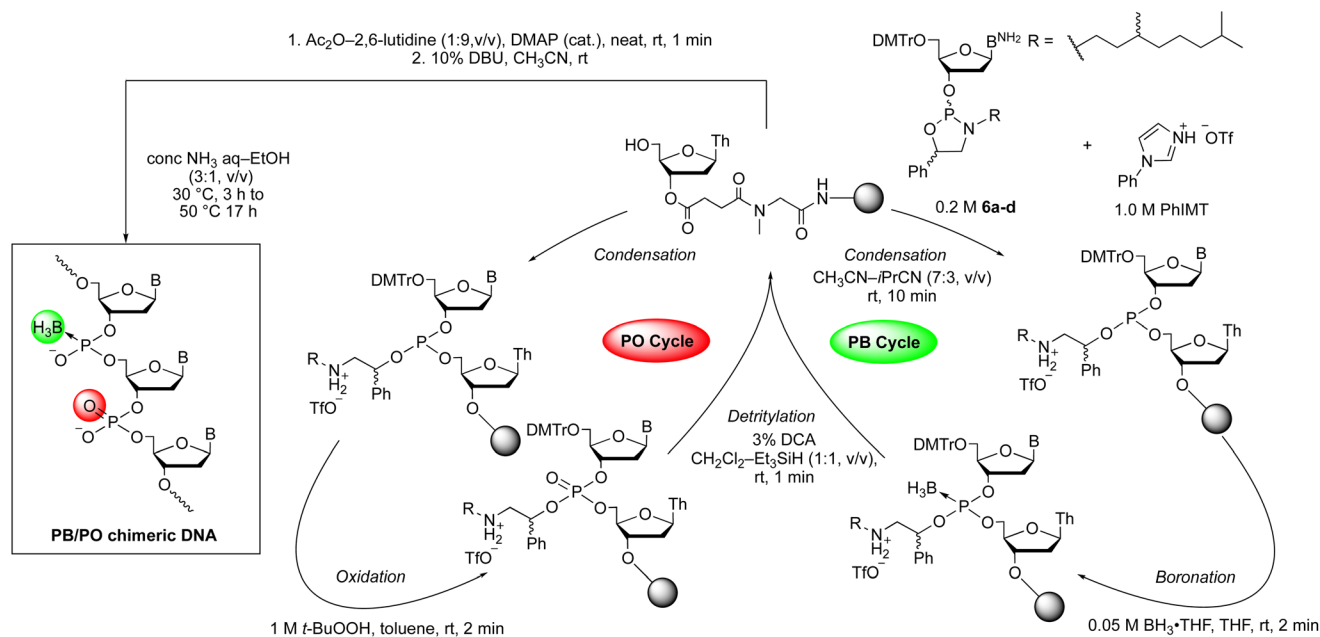
Scheme 6 Solid-phase synthesis of oligonucleotides bearing boranophosphates.

Table 3 Solid-phase synthesis of oligonucleotides bearing boranophosphates

Entry	Product[a]	DBU treatment	Isolated yield[b] (%)
1	d(C$_{PB}$A$_{PB}$G$_{PB}$T) **16**	1 h	28
2	d(C$_{PB}$A$_{PO}$G$_{PB}$T) **17**	1 h	33
3	d(C$_{PB}$A$_{PO}$G$_{PB}$T$_{PO}$C$_{PB}$A$_{PO}$G$_{PB}$T$_{PO}$C$_{PB}$A$_{PO}$G$_{PB}$T) **18**	3 h	3

[a] Subscript PB = boranophosphate, PO = phosphate. [b] Determined *via* UV absorbance at 260 nm.

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number of protecting groups on the PO and PB moieties, a longer DBU treatment was performed.[27,28] After RP-HPLC purification, dodecamer **18** was obtained in a 3% yield (Table 3, entry 3). The oligomer was identified *via* mass spectrometry. Peaks corresponding to deboronated and nucleotide deletion compounds were not detected. Unfortunately, the low yield of **18**, which can be attributed to the inferior coupling efficiency after boronation, prevented us from recording a ^{1}H NMR spectrum. Nevertheless, although there is room for improvement, the synthesis of chimeric oligonucleotides was achieved.

Conclusion

An efficient synthetic strategy for oligonucleotides was developed according to a nucleobase-unprotected approach using oxazaphospholidine monomers bearing a Thg group. One of the major obstacles of the nucleobase-unprotected synthesis is the difficult purification of the deoxyguanosine monomer. The introduction of a Thg group into the oxazaphospholidine ring facilitated the purification by increasing lipophilicity. Nucleobase phosphitylation during the condensation reaction was not observed, unlike the synthesis using nucleobase-unprotected phosphoramidite monomers.

This synthetic strategy could be also applicable to the synthesis of stereocontrolled PB ODNs because the oxazaphospholidine method was originally developed to synthesize stereopure *P*-modified oligonucleotides. Further investigation on the synthesis of stereopure oligonucleotides containing PB, PS, and PO linkages based on this concept is now in progress.

Experimental section

General information

All the reactions were conducted under Ar atmosphere. Organic solvents were dried according to the relevant procedures. ^{1}H NMR spectra were recorded at 400 MHz using tetramethylsilane (δ 0.0 ppm) as the internal standard in CDCl$_3$ or at 500 MHz or 600 MHz with CH$_3$CN (δ 2.06 ppm) as the internal standard in D$_2$O. ^{13}C NMR spectra were recorded at 101 MHz in CDCl$_3$, which was used as the internal standard (δ 77.0 ppm). COSY, HMQC, and HMBC spectra were recorded on a 400 MHz spectrometer. The ^{31}P NMR spectra were recorded at 162 MHz using H$_3$PO$_4$ (δ 0.0 ppm) as the external standard in CDCl$_3$ or at 201 MHz using H$_3$PO$_4$ (δ 0.0 ppm) as the external standard in D$_2$O. Analytical TLC was performed on commercial glass-coated 0.25 mm-thick silica gel plates. Silica gel column chromatography was performed using spherical, neutral, 63–210 μm silica gel unless otherwise noted. The solid-phase synthesis was conducted manually using a glass filter (10 × 50 mm) with a stopper at the top and a stopcock at the bottom as a reaction vessel, and the obtained compounds were analyzed and purified *via* RP-HPLC or RP-UPLC and identified *via* ESI MS.

The RP-HPLC analysis was performed at 260 nm, 30 °C, and a flow rate of 0.5 mL min^{-1} using a C18 column (100 Å, 3.9 mm × 150 mm) unless otherwise noted. The RP-UPLC measurements were performed at 260 nm, 50 °C, and a flow rate of 0.5 mL min^{-1} using a C18 column 1.7 μm (2.1 mm × 50 mm) unless otherwise noted.

The amount of a loaded nucleoside on solid-support was estimated by the calculation of released 4,4′-dimethoxytrityl cation by a solution of 0.1 M TsOH/CH$_3$CN using a molar absorption constant at 498 nm (ε = 76 000 L mol^{-1} cm^{-1}).[33]

The isolated yields of the synthesized oligomers were estimated *via* UV–vis spectroscopy. The PB/PO chimeric dodecamer was only characterized *via* ESI MS owing to the small quantities obtained. The isolation of the oxazaphospholidine monomer *via* RP-HPLC was conducted with detection at 260 nm, at rt, and a flow rate of 10 mL min^{-1} using a C18 column (120 Å) unless otherwise noted.

N-(3,7-Dimethyloctyl)-*N*-(2-hydroxy-2-phenylethyl)-2-nitrobenzenesulfonamide (2)

2-Amino-1-phenyl ethanol **1** (11.3 g, 83 mmol) was dried by performing repeated coevaporations with pyridine and toluene and then dissolved in dry THF (500 mL). Triethylamine (17.5 mL, 125 mmol) and TMSCl (15.7 mL, 124 mmol) were added to the mixture, which was then stirred for 2 h. Subsequently, the reaction was quenched by adding MeOH (5.5 mL), and the mixture was concentrated under reduced pressure to give a mixture of colorless crystals. The residue was dissolved in dry CH$_2$Cl$_2$ (160 mL) and cooled to 0 °C. After the addition of triethylamine (29.0 mL, 209 mmol), NsCl (19.3 g, 87.1 mmol) was added in ten installments, and the mixture was stirred for 2 h. The reaction was then quenched by adding MeOH (5 mL), and the mixture was concentrated under reduced pressure. CH$_2$Cl$_2$ (150 mL) was added to the residue, and the organic layer was washed with saturated aqueous solutions of NaHCO$_3$ (3 × 300 mL). The aqueous layers were combined and extracted with CH$_2$Cl$_2$ (2 × 100 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to give a mixture of colorless crystals.

The crude products were dissolved in dry THF (260 mL), and tetrahydrogeraniol (22.3 mL, 116 mmol) and PPh$_3$ (44.0 g, 168 mmol) were then added. To this solution, a diisopropyl azodicarboxylate solution in dry THF (33.0 mL, 168 mmol per 150 mL) was added at 0 °C, and the mixture was then warmed to rt. After 24 h, the reaction was quenched by adding MeOH (7 mL) and concentrated under reduced pressure. A mixed solution of hexane–EtOAc (6 : 1, v/v 350 mL) was added to the residue and then cooled at 0 °C. Triphenyl phosphine oxide was removed by means of repeated precipitation, filtration, evaporation, dilution, and cooling. These operations were conducted three times. EtOAc (500 mL) was added to the residue, and the organic layer was washed with saturated aqueous solutions of NaCl (3 × 200 mL). The aqueous layers were combined and extracted with EtOAc (2 × 200 mL), and the combined

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organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated to give a light-yellow oil.

MeOH (41.5 mL) and a 3% TFA/CH$_2$Cl$_2$ solution (360 mL) were added to the crude product at rt. After 18 h, the mixture was washed with saturated aqueous solutions of NaHCO$_3$ (3 × 200 mL). The aqueous layers were combined and extracted with CH$_2$Cl$_2$ (2 × 200 mL). The organic layers were combined and dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude product was purified *via* silica gel column chromatography (neutral silica gel) using CHCl$_3$–hexane (5 : 5–10 : 0, v/v) as an eluent to afford **2** as light yellow oil (32.4 g, 70 mmol, 85% from **1**).

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.05–8.02 (m, 1H, Ar), 7.72–7.60 (m, 3H, Ar), 7.36–7.26 (m, 5H, Ar), 4.90 (dt, *J* = 8.5, 3.5 Hz, 1H, Ph–CH̲), 3.56–3.29 (m, 4H, H-2, NCH̲$_2$), 2.77–2.75 (m, 1H, OH̲, mixtures of diastereomers), 1.60–1.03 (m, 10H, CH̲$_2$ × 4, CH̲ × 2), 0.90–0.81 (m, 9H, CH̲$_3$ × 3). ^{13}C {^{1}H} NMR (101 MHz, CDCl$_3$) δ 148.0, 141.1, 141.1, 133.6, 133.2, 131.6, 130.9, 128.6, 128.1, 125.8, 124.2, 79.0, 72.5, 72.3, 54.8, 54.7, 47.2, 47.0, 39.1, 36.9, 36.9, 34.8, 34.7, 30.6, 30.5, 28.0, 27.9, 24.5, 22.6, 22.5, 19.4, 19.3.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{24}$H$_{35}$N$_2$O$_5$S$^+$, 463.2262; found 463.2262.

2-((3,7-Dimethyloctyl)amino)-1-phenylethan-1-ol (3)

Compound **2** (32.4 g, 70.0 mmol) was dissolved in dry DMF (350 mL), and 18-crown 6-ether (37.0 g, 140 mmol), K$_2$CO$_3$ (37.9 g, 274 mmol), and 4-mercaptobenzoic acid (19.0 g, 126 mmol) were added at rt. After 12 h, half of the solvent was removed under reduced pressure. The mixture was diluted with hexane–EtOAc (4 : 1, v/v, 300 mL) and then washed with 1.0 M NaOH aqueous solutions (5 × 300 mL). The aqueous layers were combined and extracted with hexane–EtOAc (4 : 1, v/v, 1 × 300 mL), and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude was purified *via* silica gel column chromatography (neutral silica gel) using CHCl$_3$–MeOH (100 : 0–97 : 3, v/v) as an eluent to afford **3** as a light-yellow oil (12.99 g, 46.8 mmol, 67% from **2**).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39–7.25 (m, 5H, Ar), 4.75 (dd, *J* = 9.1, 3.7 Hz, 1H, H-1), 2.93 (dt, *J* = 11.9, 3.2 Hz, 1H, H-2), 2.78–2.60 (m, 3H, H-2, NCH̲$_2$), 2.6–2.1 (br, 2H, OH̲, NH̲), 1.58–1.45 (m, 3H, CH̲$_2$ × 0.5, CH̲ × 2), 1.37–1.19 (m, 4H, CH̲$_2$ × 2), 1.18–1.05 (m, 3H, CH̲$_2$ × 1.5), 0.87 (m, 9H, CH̲$_3$ × 3). ^{13}C {^{1}H} NMR (101 MHz, CDCl$_3$) δ 142.5, 128.4, 127.5, 125.8, 71.4, 71.4, 57.1, 57.0, 47.4, 47.3, 39.2, 37.3, 37.2, 30.8, 28.0, 24.7, 22.7, 22.6, 19.7.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{18}$H$_{32}$NO$^+$, 278.2479; found 278.2474.

General procedure for the synthesis of the 2-chloro-1,3,2-oxazaphospholidine derivative 4i and 4t

Compound **3i** (0.67 g, 3.8 mmol) or compound **3t** (13.9 g, 50 mmol) was dried by performing repeated coevaporations with toluene and dissolved in toluene (4.0 mL for the synthesis of **4i**, 40.0 mL for **4t**). *N*-Methylmorpholine (0.9 mL, 8.2 mmol

for **4i**, 11.5 mL, 105 mmol for **4t**) was added to the solution, and the resulting solution was then added dropwise to a solution of phosphorus trichloride (0.9 mL, 3.9 mmol for **4i**, 4.8 mL, 55 mmol for **4t**) in toluene (2.5 mL for **4i**, 40 mL for **4t**) at 0 °C over 10 min. The mixture was warmed to rt, stirred for 2 h, and then filtered under Ar atmosphere at −78 °C. The filtrate was then concentrated to afford compound (**4i**; 1.18 g, **4t**; 18.2 g) as a light-yellow oil, which was used for the phosphitylation without further purification.

General procedure for the synthesis of oxazaphospholidine monomers 6a, 6c, 6i and 6g

5′-*O*-DMTr-deoxyadenosine (2.2 g, 4.0 mmol for the synthesis of **6a**), 5′-*O*-DMTr-deoxycytidine (3.2 g, 6.0 mmol for the synthesis of **6c**, 0.82 g, 1.5 mmol for **6i**), or 5′-*O*-DMTr-deoxyguanosine (2.6 g, 4.5 mmol for the synthesis of **6c**) was dried by performing repeated coevaporations with pyridine, toluene, and THF and then dissolved in dry THF (10 mL for the synthesis of **6a**, 15 mL for **6c**, 3.8 mL for **6i**, and 10 mL for **6g**). Triethylamine (3.9 mL, 28 mmol for **6a**, 5.8 mL, 42 mmol for **6c**, 1.5 mL, 10.8 mmol for **6i**, and 4.4 mL, 32 mmol for **6g**) was added, and the mixture was cooled to −78 °C for **6a** and **6c**, and **6i** and to −40 °C for **6g**. A 0.6 M solution of compound **4t** in dry THF (17 mL for **6a**, 25 mL for **6c**, and 20 mL for **6g**) or a 0.6 M solution of compound **4i** in dry THF (6.3 mL for **6i**) was added dropwise over 5 min and the mixture was stirred at −78 °C for **6a** and **6c**, and **6i** and at −40 °C for **6g**. After 2 h, the mixture was diluted with CHCl$_3$ (20 mL) and the reaction was quenched by adding a saturated aqueous solution of NaHCO$_3$ (5 mL). CHCl$_3$ and a saturated aqueous of NaHCO$_3$ were added to the mixture, and the organic layer was collected and washed with a saturated aqueous solution of NaHCO$_3$ (3 × 50 mL). The aqueous layers were combined and extracted with CHCl$_3$ (3 × 50 mL), and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated. The residue was dried by performing coevaporations with toluene.

Seven tenths of the crude product (2.75 g out of 3.92 g) obtained in the synthesis of **6a** was purified *via* silica gel column chromatography (NH-silica gel) using toluene–EtOAc (10 : 0–8 : 2, v/v, containing 0.5% triethylamine) as an eluent to afford **6a** as a colorless foam (0.82 g, 0.95 mmol, 34%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.31, 8.30 (s, s, 1H, H-2, diastereomers), 7.99 (s, 1H, H-8), 7.41–7.16 (m, 14H, Ar), 6.80–6.74 (m, 4H, Ar), 6.48–6.44 (m, 1H, H-1′), 5.67 (brs, 2H, NH̲$_2$), 5.59–5.54 (m, 1H, 5-position of oxazaphospholidine), 5.01 (qd, *J* = 5.8, 2.9 Hz, 1H, H-3′), 4.23 (q, *J* = 3.4 Hz, 1H, H-4′), 3.77, 3.77, 3.74 (s, s, s, 6H, OCH̲$_3$ × 2, diastereomers), 3.58–3.34 (m, 3H, H-5′, 5″, 4-position of oxazaphospholidine), 3.09–2.97 (m, 2H, NCH̲$_2$), 2.95–2.85 (m, 2H, 4-position of oxazaphospholidine, H-2′), 2.61–2.54 (m, 1H, H-2″), 1.62–1.04 (m, 10H, CH̲$_2$ × 4, CH̲ × 2), 0.88–0.82 (m, 9H, CH̲$_3$ × 3). ^{13}C {^{1}H} NMR (101 MHz, CDCl$_3$) δ 158.5, 158.5, 155.4, 153.0, 149.7, 149.7, 144.5, 144.5, 140.5, 139.0, 135.7, 130.0, 128.5, 128.2, 128.0, 127.8, 127.8, 126.9, 126.8, 125.8, 125.8, 120.1, 113.1, 86.5, 86.0 (d, $^3J_{PC}$ = 4.8 Hz), 85.8, 84.3, 81.5, 81.4, 81.2, 72.9 (d, $^2J_{PC}$ = 10.6 Hz), 72.8 (d, $^2J_{PC}$ = 9.6 Hz), 63.3, 54.5 (d, $^2J_{PC}$ = 4.8

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Hz), 43.7 (d, $^2J_{PC}$ = 22.2 Hz), 43.3 (d, $^2J_{PC}$ = 21.2 Hz) 40.1, 39.8, 39.2, 37.3, 37.1, 37.0, 36.8, 36.7, 36.7, 36.6, 36.4, 36.4, 30.5, 30.5, 30.4, 27.9, 24.7, 22.7, 22.6, 19.6, 19.5, 19.4. ^{31}P {^{1}H} NMR (162 MHz, CDCl$_3$) δ 142.7, 142.1, 141.9, 141.5.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{49}$H$_{60}$N$_6$O$_6$P$^+$, 859.4306; found 859.4292.

For **6c**, forty-one hundredths of the crude product (2.50 g out of 6.11 g) was purified *via* silica gel column chromatography (NH-silica gel) using toluene–EtOAc–*t*BuOH (99 : 1, v/v, containing 0.5% triethylamine) as an eluent to afford **6c** as a colorless foam (0.80 g, 0.96 mmol, 39%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.92, 7.92, 7.92, 7.92 (d, d, d, d, *J* = 7.3 Hz, 1H, H-6, mixtures of diastereomers), 7.43–7.17 (m, 14H, Ar), 6.84–6.77 (m, 4H, Ar), 6.33–6.28 (m, 1H, H-1′), 5.51 (qd, *J* = 7.3, 2.4 Hz, 1H, (5-position of oxazaphospholidine), 5.42–5.34 (m, 1H, H-5, diastereomers), 4.90–4.81 (m, 1H, H-3′), 4.07–4.05 (m, H-4′), 3.77, 3.73, 3.71 (s, s, s, 6H, OC<u>H</u>$_3$ × 2, diastereomers), 3.50–3.36 (m, 3H, H-5′, 5″, 4-position of oxazaphospholidine), 3.09–2.84 (m, 3H, 4-position of oxazaphospholidine, NC<u>H</u>$_2$), 2.60–2.53 (m, 1H, H-2′), 2.33–2.21 (m, 1H, H-2″), 1.58–1.02 (m, 10H, C<u>H</u>$_2$ × 4, C<u>H</u> × 2), 0.88–0.80 (m, 9H, C<u>H</u>$_3$ × 3). ^{13}C {^{1}H} NMR (101 MHz, CDCl$_3$) δ 165.6, 158.5, 158.5, 158.5, 155.8, 155.7, 144.5, 144.4, 141.3, 141.2, 140.7 (d, $^3J_{PC}$ = 8.7 Hz), 140.6 (d, $^3J_{PC}$ = 9.6 Hz), 140.5 (d, $^3J_{PC}$ = 9.6 Hz), 140.4 (d, $^3J_{PC}$ = 9.6 Hz), 135.5, 135.4, 135.4, 130.1, 130.1, 130.0, 128.4, 128.3, 128.1, 127.9, 126.9, 126.9, 125.8, 125.8, 113.2, 94.0, 93.8, 86.7, 85.7, 85.2, 85.1, 85.0, 81.3 (d, $^2J_{PC}$ = 9.6 Hz), 81.2 (d, $^2J_{PC}$ = 9.6 Hz), 81.1 (d, $^2J_{PC}$ = 9.6 Hz), 81.0 (d, $^2J_{PC}$ = 9.6 Hz), 71.3 (d, $^2J_{PC}$ = 17.3 Hz), 71.2 (d, $^2J_{PC}$ = 18.3 Hz), 71.0, 70.9, 62.1, 61.9, 61.9, 55.2, 55.1, 54.6 (d, $^2J_{PC}$ = 3.9 Hz), 54.5 (d, $^2J_{PC}$ = 4.8 Hz), 54.3 (d, $^2J_{PC}$ = 4.8 Hz), 54.2 (d, $^2J_{PC}$ = 4.8 Hz), 43.6 (d, $^2J_{PC}$ = 22.2 Hz), 43.5 (d, $^2J_{PC}$ = 20.2 Hz), 43.1 (d, $^2J_{PC}$ = 20.2 Hz), 43.1 (d, $^2J_{PC}$ = 19.3 Hz), 39.2, 39.1, 37.3, 37.0, 37.0, 36.7, 36.6, 36.6, 36.4, 36.3, 36.3, 30.5, 30.4, 30.3, 30.3, 27.9, 24.6, 24.6, 22.7, 22.6, 19.7, 19.6, 19.5, 19.4. ^{31}P {^{1}H} NMR (162 MHz, CDCl$_3$) δ 144.6, 144.0, 142.1, 141.6.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{48}$H$_{60}$N$_4$O$_7$P$^+$, 835.4194; found 835.4200.

All the crude product obtained in the synthesis of **6i** was purified *via* silica gel column chromatography (NH-silica gel) using CHCl$_3$–*t*BuOH (100 : 0–89 : 11, v/v, containing 0.5% triethylamine) as an eluent to afford **6i** as a colorless foam (0.30 g, 0.41 mmol, 27%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.04, 8.02 (d, d, *J* = 7.3 Hz, 1H, H-6, mixtures of diastereomers), 7.43–7.17 (m, 14H, Ar), 6.85–6.75 (m, 4H, Ar), 6.31, 6.28 (dd, *J* = 5.0 Hz, H-1′, 1H, mixtures of diastereomers) 5.49 (q, *J* = 7.0 Hz, 1H, 5-position of oxazaphospholidine), 5.30, 5.30 (d, *J* = 7.3 Hz, 1H, H-5, mixtures of diastereomers), 4.93–4.84 (m, 1H, H-3′), 4.07–4.05 (m, 1H, H-4′), 3.79, 3.74, 3.71 (s, s, s, 6H, OC<u>H</u>$_3$ × 2, diastereomers), 3.54–3.31 (m, 4H, H-5′, H-5″ NC<u>H</u>(CH$_3$)$_2$, 4-position of oxazaphospholidine), 2.89–2.82 (m, 1H, 4-position of oxazaphospholidine), 2.65–2.55 (m, 1H, H-2′), 2.38–2.26 (m, 1H, H-2″), 1.21 (dd, *J* = 6.4, 4.1 Hz, 3H, NCH(C<u>H</u>$_3$)$_2$), 1.14 (t, *J* = 6.9 Hz, 3H, NCH(C<u>H</u>$_3$)$_2$). ^{13}C {^{1}H} NMR (101 MHz, CDCl$_3$) δ 165.6, 165.5, 158.6, 158.5, 158.5, 155.8, 155.7, 144.5, 144.4, 141.4,

141.3, 140.5, 140.5, 140.3, 140.2, 135.5, 135.4, 135.4, 130.1, 130.0, 128.4, 128.2, 128.2, 128.0, 127.9, 127.9, 127.0, 126.9, 125.9, 113.2, 93.8, 93.7, 86.7, 86.7, 85.8, 85.7, 85.0, 84.9, 80.7 (d, $^2J_{PC}$ = 9.6 Hz), 80.6 (d, $^2J_{PC}$ = 8.7 Hz), 71.1, 71.0, 70.6, 70.4, 61.8, 55.2, 55.2, 51.2 (d, $^2J_{PC}$ = 4.8 Hz), 51.1 (d, $^2J_{PC}$ = 4.8 Hz), 46.5 (d, $^2J_{PC}$ = 9.6 Hz), 46.3 (d, $^2J_{PC}$ = 9.6 Hz), 41.3, 41.2, 41.0, 22.8 (d, $^2J_{PC}$ = 8.7 Hz), 22.7 (d, $^2J_{PC}$ = 8.7 Hz), 22.6 (d, $^2J_{PC}$ = 6.7 Hz), 22.5 (d, $^2J_{PC}$ = 6.7 Hz). ^{31}P {^{1}H} NMR (162 MHz, CDCl$_3$) δ 143.8, 141.6.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{41}$H$_{46}$N$_4$O$_7$P$^+$, 737.3099; found 737.3100.

In the case of **6g**, all the crude product (4.2 g) was purified *via* silica gel column chromatography (NH-silica gel) using CHCl$_3$–*t*BuOH (100 : 0–93 : 7, v/v, containing 0.5% triethylamine) as an eluent to afford **6g** as a colorless foam (1.59 g) containing 9% oxide and 1% hydrolysate. Afterward, the obtained foam (0.82 g out of 1.59 g) was purified *via* RP-HPLC (ODS column) using CH$_3$CN–CHCl$_3$ (85 : 15, v/v) as an eluent to afford **6g** as a colorless foam (0.61 g, 0.70 mmol, 30%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (s, 1H, H-8), 7.43–7.15 (m, 14H, Ar), 6.81–6.75 (m, 4H, Ar), 6.27–6.23 (m, 1H, H-1′), 6.2–6.1 (br, 2H, NH$_2$), 5.57–5.54 (m, 1H, 5-position of oxaza-phospholidine), 5.02–4.95 (m, 1H, H-3′), 4.21 (q, *J* = 3.5 Hz, 1H, H-4′), 3.74, 3.73, 3.71 (s, s, s, 6H, OC<u>H</u>$_3$ × 2, diastereomers), 3.55–3.31 (m, 3H, H-5′, 5″, 4-position of oxazaphospholidine), 3.07–2.98 (m, 2H, NC<u>H</u>$_2$), 2.96–2.89 (m, 1H, 4-position of oxazaphospholidine), 2.86–2.77 (m, 1H, H-2′), 2.52–2.45 (m, 1H, H-2″), 1.61–1.08 (m, 10H, C<u>H</u>$_2$ × 4, C<u>H</u> × 2), 0.88–0.80 (m, 9H, C<u>H</u>$_3$ × 3). ^{13}C {^{1}H} NMR (101 MHz, CDCl$_3$) δ 159.2, 158.5, 158.4, 153.6, 151.5, 144.5, 144.5, 140.6, 140.5, 135.6, 135.6, 130.1, 128.7, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 126.9, 126.9, 126.7, 125.8, 125.8, 117.6, 113.1, 86.4, 85.8 (d, $^3J_{PC}$ = 3.9 Hz), 85.6, 83.9, 81.4, 81.3 (d, $^2J_{PC}$ = 10.6 Hz), 73.0 (d, $^2J_{PC}$ = 12.5 Hz), 63.2, 55.2, 54.8, 54.5 (d, $^2J_{PC}$ = 5.8 Hz), 54.5 (d, $^2J_{PC}$ = 4.8 Hz), 43.7 (d, $^2J_{PC}$ = 22.2 Hz), 43.3 (d, $^2J_{PC}$ = 20.2 Hz), 43.3 (d, $^2J_{PC}$ = 20.2 Hz), 39.6, 39.4, 39.2, 37.3, 37.1, 37.0, 36.8, 36.7, 36.6, 36.5, 36.4, 36.3, 30.7, 30.5, 30.5, 30.3, 27.9, 24.7, 22.7, 22.6, 22.6, 19.6, 19.5, 19.4, 19.4. ^{31}P {^{1}H} NMR (162 MHz, CDCl$_3$) δ 142.8, 142.3, 142.2, 141.8.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{49}$H$_{60}$N$_6$O$_7$P$^+$, 875.4256; found 875.4256.

General procedure for the synthesis of oxazaphospholidine monomers 6t and 6l

5′-*O*-DMTr-thymidine (1.4 g, 2.5 mmol) or 5′-*O*-DMTr-2′-*O*-4′-*C*-locked-thymidine (1.7 g, 3.0 mmol) was dried by performing repeated coevaporations with pyridine, toluene, and THF and then dissolved in dry THF (6.3 mL for the synthesis of **6t** and 7.5 mL for **6l**). Triethylamine (2.4 mL, 28 mmol for **6t** and 2.9 mL, 21 mmol for **6l**) was added, and the mixture was cooled to −78 °C. A 0.6 M solution of compound **4t** in dry THF (10 mL for **6t** and 13 mL for **6l**) was added dropwise over 5 min, and the mixture was stirred at rt. After 2 h, the mixture was diluted with CHCl$_3$ (20 mL) and the reaction was quenched by adding a saturated aqueous solution of NaHCO$_3$ (5 mL). CHCl$_3$ and a saturated aqueous of NaHCO$_3$ were added

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to the mixture, and the organic layer was collected and washed with a saturated aqueous solution of NaHCO$_3$ (3 × 50 mL). The aqueous layers were combined and extracted with CHCl$_3$ (3 × 50 mL), and the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated. The residue was dried by performing coevaporations with toluene.

All the crude product (2.32 g) obtained in the synthesis of **6t** was purified *via* silica gel column chromatography (NH-silica gel) using toluene–EtOAc (10 : 0–8 : 2, v/v, containing 0.5% triethylamine) as an eluent to afford **6t** as a colorless foam (1.02 g, 1.20 mmol, 55%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.7–8.3 (br, 1H, N$\underline{\text{H}}$-3), 7.63–7.61 (m, 1H, H-6), 7.42–7.19 (m, 14H, Ar), 6.85–6.79 (m, 4H, Ar), 6. 42 (dd, J = 7.5, 6.2 Hz, 1H, H-1′), 5.51–5.45 (m, 1H, 5-position of oxazaphospholidine), 4.92–4.86 (m, 1H, H-3′), 4.11–4.07 (m, 1H, H-4′), 3.77, 3.75, 3.74 (s, s, s, 6H, O$\text{C}\underline{\text{H}}_3$ × 2, diastereomers), 3.53–3.33 (m, 3H, H-5′, 5″, 4-position of oxazaphospholidine), 3.07–2.95 (m, 2H, N$\text{C}\underline{\text{H}}_2$), 2.92–2.83 (m, 1H, 4-position of oxazaphospholidine), 2.47–2.29 (m, 2H, H-2′, 2″), 1.60–1.01 (m, 13H, C$\underline{\text{H}}_2$ × 4, C$\underline{\text{H}}$ × 2, thymine-5-C$\underline{\text{H}}_3$), 0.88–0.80 (m, 9H, C$\underline{\text{H}}_3$ × 3). ^{13}C $\{^1$H$\}$ NMR (101 MHz, CDCl$_3$) δ 163.6, 158.7, 150.3, 144.3, 144.2, 140.4, 135.6, 135.3, 135.3, 130.1, 130.1, 128.5, 128.2, 128.0, 128.0, 127.2, 127.1, 125.9, 113.2, 111.2, 86.9, 85.8 (d, $^3J_{PC}$ = 4.8 Hz), 85.7, 84.6, 81.3 (d, $^2J_{PC}$ = 9.6 Hz), 81.3 (d, $^2J_{PC}$ = 10.1 Hz), 72.9 (d, $^2J_{PC}$ = 15.4 Hz), 72.6 (d, $^2J_{PC}$ = 13.5 Hz), 63.1, 63.0, 54.7 (d, $^2J_{PC}$ = 4.8 Hz), 54.6 (d, $^2J_{PC}$ = 4.8 Hz), 54.4 (d, $^2J_{PC}$ = 4.8 Hz), 54.3 (d, $^2J_{PC}$ = 5.8 Hz), 43.6 (d, $^2J_{PC}$ = 21.2 Hz), 43.3 (d, $^2J_{PC}$ = 20.2 Hz), 40.5, 40.3, 39.2, 37.3, 37.1, 37.0, 36.7, 36.4, 30.5, 30.4, 30.4, 30.4, 27.9, 24.6, 22.7, 22.6, 19.6, 19.5, 19.4, 11.7, 11.7. ^{31}P $\{^1$H$\}$ NMR (162 MHz, CDCl$_3$) δ 143.9, 143.4, 142.5, 142.1.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{49}$H$_{61}$N$_3$O$_8$P$^+$, 850.4191; found 850.4203.

For **6l**, ninety-five hundredths of the crude product (2.95 g out of 3.10 g) was purified *via* silica gel column chromatography (NH-silica gel) using toluene–EtOAc (10 : 0–8 : 2, v/v, containing 0.5% triethylamine) as an eluent to afford **6l** as a colorless foam (0.82 g, 0.93 mmol, 32%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.1–8.7 (br, 1H, N$\underline{\text{H}}$-3), 7.74–7.73 (m, 1H, H-6), 7.48–7.45 (m, 2H, Ar), 7.37–7.17 (m, 12H, Ar), 6.85–6.82 (m, 2H, Ar), 6.78–6.73 (m, 2H, Ar), 5.66 (d, J = 9.6 Hz, 1H, H-1′), 5.54 (t, J = 7.3 Hz, 0.5 H, 5-position of oxazaphospholidine), 5.40 (t, J = 7.3 Hz, 0.5 H, 5-position of oxazaphospholidine), 4.61–4.58 (m, 1H, H-3′), 4.53, 4.47 (s, s, 1H, H-2′, diastereomers), 3.86–3.68 (m, 8H, O$\text{C}\underline{\text{H}}_3$ × 2, LNA–C$\underline{\text{H}}_2$), 3.61–3.49 (m, 1.5H, H-5′, 5″), 3.41–3.38 (m, 1.5H, H-5″, 4-position of oxazaphospholidine), 3.12–2.79 (m, 3H, 4-position of oxazaphospholidine, N$\text{C}\underline{\text{H}}_2$), 1.59–1.00 (m, 13H, C$\underline{\text{H}}_2$ × 4, C$\underline{\text{H}}$ × 2, thymine-5-C$\underline{\text{H}}_3$), 0.89–0.73 (m, 9H, C$\underline{\text{H}}_3$ × 3). ^{13}C $\{^1$H$\}$ NMR (101 MHz, CDCl$_3$) δ 163.9, 163.8, 158.7, 158.6, 158.5, 149.8, 149.7, 144.4, 144.1, 140.5 (d, $^3J_{PC}$ = 2.9 Hz), 140.4 (d, $^3J_{PC}$ = 2.9 Hz), 140.1 (d, $^3J_{PC}$ = 2.9 Hz), 139.9 (d, $^3J_{PC}$ = 2.9 Hz), 135.4, 135.3, 135.2, 134.6, 134.5, 130.2, 130.1, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.1, 127.0, 126.8, 125.9, 125.9, 125.7, 113.2, 113.2, 113.2, 113.1, 110.5, 110.5, 88.0, 87.9 (d, $^3J_{PC}$ = 2.9 Hz), 87.2, 86.8, 86.7, 81.7 (d, $^2J_{PC}$ = 9.6 Hz), 81.6 (d, $^2J_{PC}$ = 10.6

Hz), 81.2 (d, $^2J_{PC}$ = 9.6 Hz), 81.2 (d, $^2J_{PC}$ = 9.6 Hz), 78.9, 78.8, 78.8, 78.7, 71.9, 70.8, 70.7, 69.9, 69.8, 69.8, 69.7, 58.0, 57.8, 55.1, 55.1, 54.3 (d, $^2J_{PC}$ = 5.8 Hz), 54.2 (d, $^2J_{PC}$ = 3.9 Hz), 53.9 (d, $^2J_{PC}$ = 4.8 Hz), 53.9 (d, $^2J_{PC}$ = 4.8 Hz), 43.3 (d, $^2J_{PC}$ = 19.3 Hz), 43.1 (d, $^2J_{PC}$ = 21.1 Hz), 42.9 (d, $^2J_{PC}$ = 19.3 Hz), 42.8 (d, $^2J_{PC}$ = 20.3 Hz), 39.1, 37.4, 37.3, 37.1, 37.0, 36.6, 36.5, 36.4, 36.3, 36.3, 36.2, 30.4, 30.3, 30.2, 27.9, 24.6, 24.6, 22.7, 22.6, 22.5, 19.5, 19.4, 19.3, 12.4, 12.3. ^{31}P $\{^1$H$\}$ NMR (162 MHz, CDCl$_3$) δ 145.3, 144.9, 142.3, 141.7.

HRMS (ESI-QTOF) *m/z*: [M + H]$^+$ calcd for C$_{50}$H$_{61}$N$_3$O$_9$P$^+$, 878.4140; found 878.4141.

General procedure for the synthesis of dinucleoside phosphates 10a–l

The HCP-loaded 5′-*O*-DMTr-Th (30.3 µmol g^{-1}, 0.50 µmol), *via* a succinyl linker, was treated in a reaction vessel with 3% DCA in dry CH$_2$Cl$_2$ (5 × 12 s, 1 mL each) and washed with dry CH$_2$Cl$_2$ (3 × 1 mL) and CH$_3$CN (3 × 1 mL). The product was dried *in vacuo* for 5 min. Then, the oxazaphospholidine monomer (**6a**, **6c**, **6g**, **6t**, **6i**, or **6l**, 30 µmol), which was dried *in vacuo* overnight, was added to the reaction vessel and dried *in vacuo* for 5 min. A 1.0 M solution of PhIMT (44.1 mg, 150 µmol) in dry CH$_3$CN–iPrCN (7 : 3, v/v, 150 µL), which was dried over MS 3 Å overnight, was added under Ar atmosphere to the reaction vessel. After 10 min, the HCP was washed with dry CH$_3$CN (3 × 1 mL) and dry CH$_2$Cl$_2$ (3 × 1 mL) and dried *in vacuo* for 5 min. The resultant phosphite was oxidized *via* treatment with a 1.0 M solution of TBHP (500 µL, 500 µmol) in dry toluene, and the reaction vessel was shaken for 5 min. Then, the HCP was washed with dry CH$_2$Cl$_2$ (6 × 1 mL) and the detritylation reaction was conducted using 3% DCA in dry CH$_2$Cl$_2$ (5 × 12 s, 1 mL each). The HCP was then washed with dry CH$_2$Cl$_2$ (3 × 1 mL) and dry CH$_3$CN (3 × 1 mL), treated with a 25% NH$_3$ aqueous solution–EtOH (3 : 1, v/v, 5 mL) at rt for 3 h, filtered, and washed with CH$_3$CN. The filtrate and the washings were combined, concentrated under reduced pressure, and the obtained residue was analyzed *via* RP-HPLC, which was performed with a linear gradient of 0%–20% CH$_3$CN for 60 min in a 0.1 M TEAA buffer (pH 7.0).

General procedure for the synthesis of oligonucleotide 11 bearing phosphate linkages

The HCP-loaded 5′-*O*-DMTr-Th (30.3 µmol g^{-1}, 0.50 µmol), *via* a succinyl linker, was treated in a reaction vessel with 3% DCA in dry CH$_2$Cl$_2$ (5 × 12 s, 1 mL each), washed with dry CH$_2$Cl$_2$ (3 × 1 mL) and CH$_3$CN (3 × 1 mL), and dried *in vacuo* for 5 min. Then, the oxazaphospholidine monomer (**6a**, **6c**, **6g**, or **6t**, 30 µmol), which was dried *in vacuo* overnight, was added to the reaction vessel and dried *in vacuo* for 5 min. A 1.0 M solution of PhIMT (44.1 mg, 150 µmol) in dry CH$_3$CN–iPrCN (7 : 3, v/v, 150 µL), which was dried over MS 3 Å overnight, was added under Ar atmosphere to the reaction vessel. After 10 min, the HCP was washed with dry CH$_3$CN (3 × 1 mL) and dry CH$_2$Cl$_2$ (3 × 1 mL) and dried *in vacuo* for 5 min. The resultant phosphite was oxidized with a 1.0 M solution of TBHP (500 µL, 1000 µmol) in dry toluene, and the reaction vessel was shaken

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for 5 min. After washing the HCP with dry CH_2Cl_2 (6 × 1 mL), the detritylation reaction was conducted using 3% DCA in dry CH_2Cl_2 (5 × 12 s, 1 mL each). Then, the HCP was washed with dry CH_2Cl_2 (3 × 1 mL) and dry CH_3CN (3 × 1 mL).

The cycles of detritylation, condensation, and oxidation were repeated. After extending the chain length, the HCP was treated with a 25% NH_3 aqueous solution–EtOH (3 : 1, v/v, 5 mL) at rt for 17 h, filtered, and washed with CH_3CN. The filtrate and the washings were combined and concentrated under reduced pressure, and the obtained residue was analyzed *via* RP-UPLC, which was performed with a linear gradient of 5%–25% MeOH for 10 min in 0.4 M 1,1,1,3,3,3-hexafluoro-2-propanol and 16 mM triethylamine at 50 °C. The purification was conducted with four-fifths of the crude mixture, and the quantity of purified **11** was estimated by measuring the UV absorption at 260 nm. Isolated yield: 14% (**11**, 57 nmol).

11: HRMS (ESI-QTOF) *m/z*: $[M - 4H]^{4-}$ calcd for $C_{117}H_{143}N_{45}O_{70}P_{11}{}^{4-}$, 727.9238; found 727.9209.

^{1}H NMR (500 MHz, D_2O) δ 8.19–7.41 (m, 15H, nucleobase), 6.20–5.89 (m, 15H, H-1′, nucleobase), 4.98–3.61 (m, 48H, H-3′, H-4′, H-5′), 3.22–3.18 (q, 66H, TEA), 2.69–2.63 (m, 12H, H-2′), 2.35–2.11 (m, 12H, H-2′), 1.72–1.67 (s, s, 9H, nucleobase), 1.29–1.26 (q, 99H, TEA). ^{31}P $\{^1$H$\}$ NMR (201 MHz, D_2O) −0.48 to −0.61.

General procedure for the synthesis of dinucleoside boranophosphates 14a–t

The HCP-loaded 5′-*O*-DMTr-Th (29.6 μmol g^{-1}, 0.50 μmol), *via* a succinyl sarcosinyl linker, was treated in a reaction vessel with 3% DCA in dry CH_2Cl_2 (5 × 12 s, 1 mL each), washed with dry CH_2Cl_2 (3 × 1 mL) and CH_3CN (3 × 1 mL), and dried *in vacuo* for 5 min. Then, the oxazaphospholidine monomer (**6a**, **6c**, **6g**, or **6t**, 30 μmol), which was dried *in vacuo* overnight, was added to the reaction vessel and dried *in vacuo* for 5 min. A 1.0 M solution of PhIMT (44.1 mg, 150 μmol) in dry CH_3CN–iPrCN (7 : 3, v/v, 150 μL), which was dried over MS 3 Å overnight, was added under Ar atmosphere to the reaction vessel. After 10 min, the HCP was washed with dry CH_3CN (3 × 1 mL) and dry CH_2Cl_2 (3 × 1 mL) and dried *in vacuo* for 5 min.

The resultant phosphite was boronated using a 1.0 M solution of BH_3·THF (50 μL, 50 μmol) and dry THF (950 μL), and the reaction vessel was shaken for 2 min. Afterward, the HCP was washed with dry THF (3 × 1 mL) and dry CH_2Cl_2 (3 × 1 mL) and the detritylation was performed using 3% DCA in dry CH_2Cl_2–Et$_3$SiH (1 : 1, v/v) (4 × 5 s and 1 × 40 s, 1 mL each). After washing the HCP with dry CH_2Cl_2 (3 × 1 mL) and CH_3CN (3 × 1 mL) and drying *in vacuo* for 5 min, DMAP (10 mg, 82 μmol), dry 2,6-lutidine (450 μL), and Ac_2O (50 μL, 529 μmol) were sequentially added, and the mixture was shaken for 1 min. Subsequently, the HCP was washed with dry CH_2Cl_2 (3 × 1 mL) and dry CH_3CN (3 × 1 mL) and dried *in vacuo* for 5 min. Dry CH_3CN (450 μL) and DBU (50 μL, 335 μmol) were added, and the reaction vessel was shaken for 1 h. The HCP was washed with dry CH_3CN (6 × 1 mL), treated with a 25% NH_3 aqueous solution–EtOH (3 : 1, v/v, 5 mL) at rt for 17 h (or at rt for 3 h and then 50 °C for 17 h for the synthesis of

$dA_{PB}T$), filtered, and washed with CH_3CN. The filtrate and the washings were combined and concentrated under reduced pressure, and the obtained residue was analyzed *via* RP-HPLC with a linear gradient of 0%–20% CH_3CN for 60 min in a 0.1 M TEAA buffer (pH 7.0).

General procedure for the synthesis of oligonucleotides bearing PB linkages: d($C_{PB}C_{PB}T$) 15, d($C_{PB}A_{PB}G_{PB}T$) 16, d($C_{PB}A_{PO}G_{PB}T$) 17, and d($C_{PB}A_{PO}G_{PB}T_{PO}C_{PB}A_{PO}G_{PB}T_{PO}C_{PB}A_{PO}G_{PB}T$) 18

The HCP-loaded 5′-*O*-DMTr-Th (29.6 μmol g^{-1}, 0.50 μmol), *via* a succinyl sarcosinyl linker, was treated in a reaction vessel with 3% DCA in dry CH_2Cl_2 (5 × 12 s, 1 mL each), washed with dry CH_2Cl_2 (3 × 1 mL) and CH_3CN (3 × 1 mL), and dried *in vacuo* for 5 min. Then, the oxazaphospholidine monomer (**6a**, **6c**, **6g**, or **6t**, 30 μmol), which was dried *in vacuo* overnight, was added to the reaction vessel and dried *in vacuo* for 5 min. A 1.0 M solution of PhIMT (44.1 mg, 150 μmol) in dry CH_3CN–iPrCN (7 : 3, v/v, 150 μL), which was dried over MS 3 Å overnight, was added under Ar atmosphere to the reaction vessel. After 10 min, the HCP was washed with dry CH_3CN (3 × 1 mL) and dry CH_2Cl_2 (3 × 1 mL) and dried *in vacuo* for 5 min. The resultant phosphite was boronated using a 1.0 M solution of BH_3·THF (50 μL, 50 μmol) and dry THF (950 μL), and the reaction vessel was shaken for 2 min. After washing the HCP with dry THF (3 × 1 mL), dry EtOH (3 × 1 mL), and dry CH_2Cl_2 (3 × 1 mL), the detritylation was conducted using 3% DCA in dry CH_2Cl_2–Et$_3$SiH (1 : 1, v/v) (4 × 5 s and 1 × 40 s, 1 mL each). The HCP was washed with dry CH_2Cl_2 (3 × 1 mL) and CH_3CN (3 × 1 mL) and then dried *in vacuo* for 5 min. The cycles of detritylation, condensation, and boronation or oxidation were repeated. After extending the chain length, DMAP (10 mg, 82 μmol), dry 2,6-lutidine (450 μL), and Ac_2O (50 μL, 529 μmol) were successively added and the vessel was shaken for 1 min. Subsequently, the HCP was washed with dry CH_2Cl_2 (3 × 1 mL) and dry CH_3CN (3 × 1 mL) and then dried *in vacuo* for 5 min. Dry CH_3CN (450 μL) and DBU (50 μL, 335 μmol) were added, and the reaction vessel was shaken for 1 h. The HCP was washed with dry CH_3CN (6 × 1 mL), treated with a 25% NH_3 aqueous solution–EtOH (3 : 1, v/v, 5 mL) at rt for 17 h (for the synthesis of **15**) or at rt for 3 h and then 50 °C for 17 h (for the synthesis of **16–18**), filtered, and washed with CH_3CN. The filtrate and the washings were combined and concentrated under reduced pressure, and the obtained residue of trimer **15**, tetramers **16** and **17**, or dodecamer **18** was analyzed *via* RP-HPLC using a linear gradient of 0%–20% CH_3CN for 60 min in a 0.1 M TEAA buffer (pH 7.0) for trimer **15** or 0%–60% CH_3CN for 60 min in a 0.1 M TEAA buffer (pH 7.0) for tetramers **16** and **17**. The purification of tetramers **16** and **17** was conducted with one-fifth of the crude mixture and the quantity of purified **16** and **17** was estimated by measuring the UV absorption at 260 nm. Isolated yield: 28% and 33% for **16** (25 nmol) and **17** (30 nmol), respectively.

16: HRMS (ESI-QTOF) *m/z*: $[M - 2H]^{2-}$ calcd for $C_{39}H_{57}B_3N_{15}O_{19}P_3{}^{2-}$, 582.6729; found 582.6742.

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17: HRMS (ESI-QTOF) m/z: $[M - 2H]^{2-}$ calcd for $C_{39}H_{59}B_2N_{15}O_{20}P_3^{2-}$, 583.6540; found 583.6559.

The residue obtained in the synthesis of dodecamer **18** was analyzed via RP-HPLC using a linear gradient of 5%–40% MeOH for 20 min in 0.4 M 1,1,1,3,3,3-hexafluoro-2-propanol and 8 mM triethylamine at 60 °C. The purification was conducted with one-twentieth of the crude mixture, and the quantity of purified **18** was estimated by measuring the UV absorption at 260 nm. Isolated yield: 3% (**18**, 0.8 nmol).

18: HRMS (ESI-QTOF) m/z: $[M - 4H]^{4-}$ calcd for $C_{117}H_6B_2N_{45}O_{64}P_{11}^{4-}$, 906.9642; found 906.9630.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

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