specificity and selectivity. However, the details of the synthesis, such as the coupling efficiency, are not described in this paper.

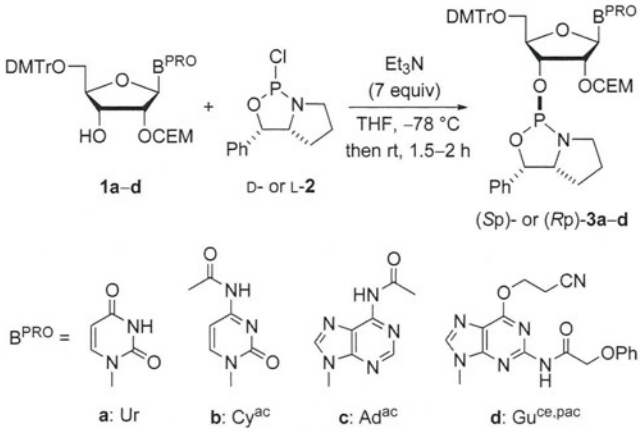
To overcome these limitations, we have recently developed a method for synthesizing *P*-stereodefined PS-ORNs using diastereopure 2′-*O*-TBS-protected nucleoside 3′-*O*-(1,3,2-oxazaphospholidine) monomer units.[13] Using this method, we have synthesized all-(*Rp*)- and all-(*Sp*)-PS-U_{10} with good coupling yields (97−99%) and stereoselectivity (≥96:4) and have shown that the duplex of the former with the complementary ORN (rA_{10}) was slightly more stable than the unmodified duplex (ΔT_m = ca. +0.4 °C per modification). On the other hand, hybridization was not observed above 4 °C for the all-(*Sp*)-PS-U_{10} and rA_{10}. However, attempts to synthesize *P*-stereodefined PS-ORNs of mixed sequence for a thermal denaturing study have revealed that the coupling efficiency was lower than that observed for the synthesis of PS-U_{10}, and only short oligomers up to 4mers were accessible in satisfactory yields. To solve this problem, we sought to develop new monomers with improved reactivity. Recently, significant efforts have been devoted to the development of new methods for synthesizing ORNs owing to their growing demand in biological and medical studies, and a variety of new protecting groups for the 2′-OH of ribonucleosides have been developed.[14−16] Among these new protecting groups, we selected the 2-cyanoethoxymethyl (CEM)[16] group because the replacement of the conventional 2′-*O*-TBS group by this new, less bulky protecting group has been demonstrated to greatly improve the reactivity of the phosphoramidite monomers, and very long ORNs (110−170mers) were efficiently synthesized.[16b,c] It is also notable that CEM groups can be removed under mild conditions using TBAF. In this paper, we describe the development of novel 2′-*O*-CEM-protected oxazaphospholidine monomers, their application to the stereocontrolled synthesis of PS-ORNs, and a thermal denaturing study on the duplexes of the resultant PS-ORNs.

■ RESULTS AND DISCUSSION

Stereoselective Synthesis of 2′-*O*-CEM-Protected 3′-*O*-Oxazaphospholidine Monomers 3a−d. Table 1 summarizes the synthesis of the 2′-*O*-CEM-protected nucleoside 3′-*O*-oxazaphospholidine monomers **3a−d**. The 2′-*O*-CEM-protected nucleosides having a free 3′-OH (**1a−d**)[16] were allowed to react with 2-chlorooxazaphospholidine derivatives D- and L-**2**, which were synthesized from D- and L-proline, respectively.[17] The reactions proceeded with complete stereoselectivity, and only the *trans*-isomers were generated as was the case for their 2′-deoxy and 2′-*O*-TBS counterparts.[13,17] Because the 2′-*O*-CEM-monomers were less stable on silica gel than the 2′-*O*-TBS-protected oxazaphospholidine monomers, 3-aminopropyl-functionalized silica gel was used for purification.[18] As a result, the complete set of diastereopure monomer units required for the synthesis of the *P*-stereodefined PS-ORNs of mixed sequence were successfully isolated in modest to good yields (Table 1), though partial decomposition of the monomers was observed even with the 3-aminopropyl-functionalized silica gel. The stability of monomers varied with the *P*-configuration and nucleobase; the (*Sp*)- and pyrimidine monomers were more stable than the (*Rp*)- and purine counterparts, respectively.

Stereocontrolled Solid-Phase Synthesis of PS-ORNs. Next, we investigated the manual solid-phase synthesis of PS-ORN 2mers by using the monomers described above (Table 2). Uridine anchored to a controlled-pore glass (CPG) support via a succinate linker (**4**) was condensed with each of the

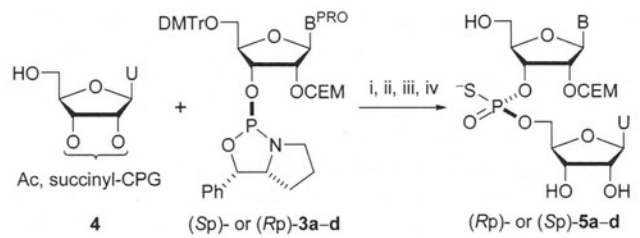
Table 1. Synthesis of 2′-*O*-CEM-Nucleoside 3′-*O*-Oxazaphospholidine Monomers 3a−d



entry	product	B^{PRO}	yield (%)	*trans*:*cis*[a]
1	(*Sp*)-**3a**	U	71	>99:1
2	(*Sp*)-**3b**	C^{ac}	75	>99:1
3	(*Sp*)-**3c**	A^{ac}	51	>99:1
4	(*Sp*)-**3d**	$G^{ce,pac}$	54	>99:1
5	(*Rp*)-**3a**	U	62	>99:1
6	(*Rp*)-**3b**	C^{ac}	53	>99:1
7	(*Rp*)-**3c**	A^{ac}	49	>99:1
8	(*Rp*)-**3d**	$G^{ce,pac}$	42	>99:1

[a]Determined by ^{31}P NMR.

Table 2. Stereocontrolled Solid-Phase Synthesis of 2′-*O*-CEM-Protected Dinucleoside Phosphorothioates N_SU 5a−d[a,b]



entry	monomer	product		yield (%)[c]	Rp:Sp[c]
1	(*Sp*)-**3a**	(*Rp*)-U_SU	(*Rp*)-**5a**	96	>99:1
2	(*Sp*)-**3b**	(*Rp*)-C_SU	(*Rp*)-**5b**	95	>99:1
3	(*Sp*)-**3c**	(*Rp*)-A_SU	(*Rp*)-**5c**	97	>99:1
4	(*Sp*)-**3d**	(*Rp*)-G_SU	(*Rp*)-**5d**	96	>99:1
5	(*Rp*)-**3a**	(*Sp*)-U_SU	(*Sp*)-**5a**	98	>1:99
6	(*Rp*)-**3b**	(*Sp*)-C_SU	(*Sp*)-**5b**	96	>1:99
7	(*Rp*)-**3c**	(*Sp*)-A_SU	(*Sp*)-**5c**	95	>1:99
8	(*Rp*)-**3d**	(*Sp*)-G_SU	(*Sp*)-**5d**	97	>1:99

[a]Reagents and conditions: (i) (*Sp*)- or (*Rp*)-**3a−d** (0.13 M, 40 equiv), CMPT (1 M, 300 equiv), CH_3CN, rt, 5 min; (ii) DTD (0.3 M, 120 equiv), CH_3CN, rt, 10 min; (iii) 3% DCA, CH_2Cl_2, rt, 4 × 15 s; (iv) conc NH_3 aq/ EtOH (3:1, v/v), rt, 3 h. [b]Subscript S denotes PS-linkage. [c]Determined by RP-HPLC.

monomers in the presence of *N*-(cyanomethyl)pyrrolidinium triflate (CMPT) that we developed for the stereospecific condensation of oxazaphospholidine monomers.[19] The resultant dinucleoside phosphite intermediates were then sulfurized with *N*,*N*′-dimethylthiuram disulfide (DTD)[20] to give dinucleoside phosphorothioate triester intermediates. Finally, the 5′-*O*-(4,4′-dimethoxytrityl) (DMTr) group was removed by treat-

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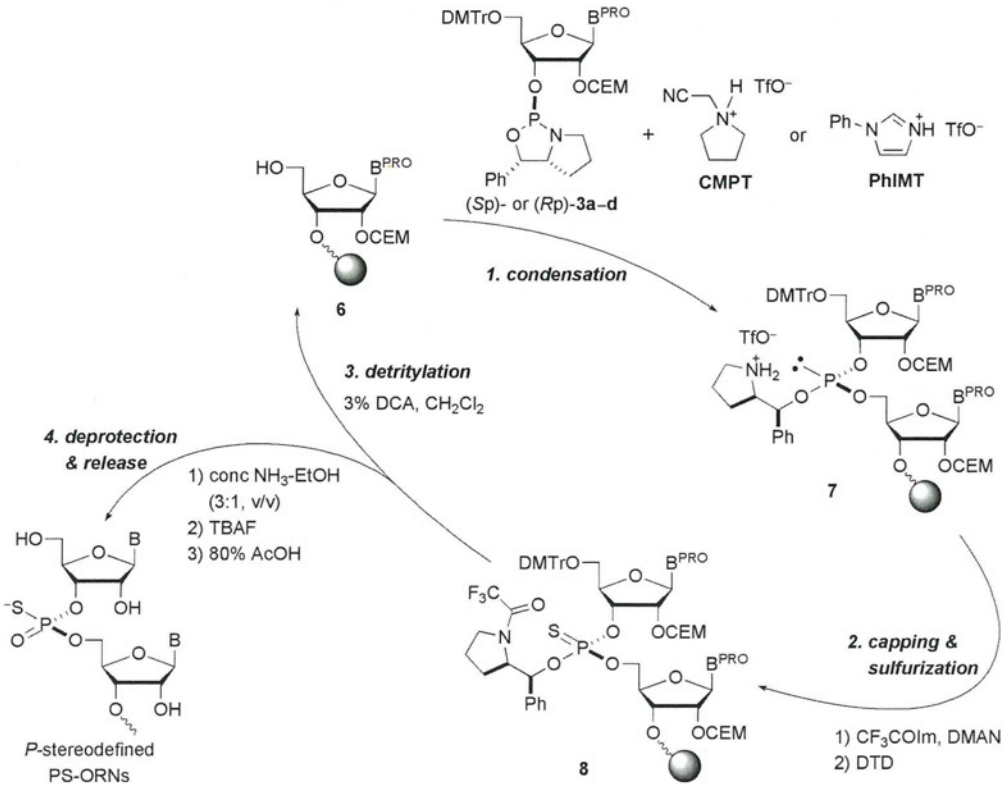
Scheme 1. Synthetic Cycle for *P*-Stereodefined PS-ORNs



ment with 3% dichloroacetic acid (DCA) in CH_2Cl_2, and the oligomer on the solid support was then treated with concentrated aqueous ammonia to deprotect the base and the PS-linkage and cleave the linker. HPLC analysis of the resultant crude 2′-*O*-CEM-protected dinucleoside phosphorothioates (*Rp*)- and (*Sp*)-**5a–d**[21] showed that the efficiency of the cycle (95–98%) and diastereopurity of the products (>99:1) were good enough for the synthesis of oligomers (Table 2).

Encouraged by these results, we extended this method to the synthesis of oligomers. The synthetic cycle is shown in Scheme 1. The cycle consists of the following steps: (1) chain elongation by a condensation reaction between the 5′-OH of a nucleoside or a PS-ORN on a solid support and one of the oxazaphospholidine monomers (**3a–d**) in the presence of an activator, (2) capping of any unreacted 5′-OH as well as the liberated secondary amino group of intermediate **7** using trifluoroacetylimidazole (CF_3COIm) and 1,8-bis-(dimethylamino)naphthalene (DMAN)[13,17] and the subsequent *P*-sulfurization with DTD, and (3) 5′-*O*-detritylation with 3% DCA. The capping step was added to the method shown in Table 2 in order to facilitate the purification of the products as well as to protect the secondary amino group derived from the chiral auxiliary. Trifluoroacetylation is used for capping in place of the conventional acetylation because the *N*-acetylated chiral auxiliaries would be too stable to be removed from the product.[13,17] After repeating the cycle to assemble the desired oligomer, the solid support is treated with a mixture of concentrated aqueous ammonia and ethanol in order to remove the protecting groups and chiral auxiliaries from the nucleobases and PS-linkages, respectively, and to cleave the succinate linker. The resultant PS-ORN bearing the 5′-*O*-DMTr and 2′-*O*-CEM protecting groups is then treated with a solution of TBAF in DMSO containing 0.5 vol % CH_3NO_2, a scavenger of acrylonitrile to deprotect the 2′-*O*-CEM groups.[16] Subsequent purification by RP-HPLC (DMTr-on and -off) affords the desired *P*-stereodefined PS-ORN.

First, four kinds of PS-ORN 4mers, all-(*Rp*)-PS-U_4 (**9a**), all-(*Sp*)-PS-U_4 (**9b**), all-(*Rp*)-PS-CAGU (**9c**), and all-(*Sp*)-CAGU (**9d**) (Table 3, entries 1–5), were manually synthesized, and the efficiency of the synthesis was evaluated from the average coupling yields and the RP-HPLC profiles of the resulting PS-ORNs[21] (2′-*O*-CEM-on–5′-*O*-DMTr-off and fully deprotected). The results showed that the efficiency of the synthesis was greatly improved by employing the 2′-*O*-CEM protection. Thus, the desired PS-ORNs **9a–d** were obtained in the average coupling yields of 94–98% when the coupling time was extended to 10–15 min (entries 2–5) from that used for the synthesis of 2mers (5 min). In contrast, we have previously reported that the average coupling yields were 67–90% when the same oligomers were synthesized by using the 2′-*O*-TBS-protected oxazaphospholidine monomers under similar conditions with a longer coupling time (20 min).[13] This improvement can be attributed to the enhanced reactivity of the new monomers bearing a less bulky CEM group as was the case with the phosphoramidite monomers.[16] Furthermore, the HPLC analyses showed that the 2′-*O*-CEM-protecting groups were removed from the resultant 4mers by treatment with TBAF virtually quantitatively without observable side reactions.[21]

Next, we applied the optimized reaction conditions for the synthesis of 4mers to the synthesis of 12mers (entries 6–12). As shown in entries 6 and 7, all-(*Rp*)-PS-U_{12} (**10a**) and all-(*Sp*)-PS-U_{12} (**10b**) were manually synthesized in excellent coupling yields under these conditions, although the isolated yields were rather low owing to partial loss of the product during a double purification procedure by RP-HPLC (DMTr-on and -off) (Figures 1 and 2, A and B). However, attempts to synthesize all-(*Rp*)-PS-$(CAGU)_3$ (**10c**) and all-(*Sp*)-PS-

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Table 3. Stereocontrolled Solid-Phase Synthesis of PS-ORN 4−12mers

entry	product		activator	coupling time (min)	coupling yield (%)[a,b]	isolated yield (%)
1	All-(*Rp*)-PS-U_4	**9a**	CMPT	5	91	
2	All-(*Rp*)-PS-U_4	**9a**	CMPT	10	98	
3	All-(*Sp*)-PS-U_4	**9b**	CMPT	10	96	
4	All-(*Rp*)-PS-CAGU	**9c**	CMPT	15	98	
5	All-(*Sp*)-PS-CAGU	**9d**	CMPT	15	94	
6	All-(*Rp*)-PS-U_{12}	**10a**	CMPT	15	99	12
7	All-(*Sp*)-PS-U_{12}	**10b**	CMPT	15	99	14
8	All-(*Rp*)-PS-$(CAGU)_3$	**10c**	CMPT	15	90 (68)	
9	All-(*Rp*)-PS-$(CAGU)_3$	**10c**	CMPT	15	92 (75[c])	
10	All-(*Sp*)-PS-$(CAGU)_3$	**10d**	CMPT	15	93 (80)	
11[d]	All-(*Rp*)-PS-$(CAGU)_3$	**10c**	PhIMT	15	94 (86)	6
12	All-(*Sp*)-PS-$(CAGU)_3$	**10d**	PhIMT	15	97 (92)	10

[a]Average coupling yield. [b]Average coupling yields of C^{ac}-monomers are given in parentheses. [c]Double coupling. [d]Synthesized on automated DNA synthesizer.

$(CAGU)_3$ (**10d**) under the same conditions resulted in lower average coupling yields (entries 8−10). The $DMTr^+$ assay showed that the coupling yields of the C^{ac}-monomers were particularly lower than those of the other monomers. Double coupling of the C^{ac}-monomer improved the yield only slightly (entry 9). The low coupling yield might be due to the relatively high basicity of cytosine, which could be initially protonated and thus hamper the protonation of its oxazaphospholidine moiety. To solve this problem, we employed *N*-phenylimidazolium triflate (PhIMT)[22] as the activator for the synthesis of **10c** and **10d**. As expected, the coupling yield of the C^{ac}-monomer improved, and the desired oligomers of mixed sequence (**10c** and **10d**) were isolated (Table 3, entries 11 and 12) (Figures 1 and 2, C and D). The diastereoselectivity of the coupling reaction using PhIMT (98:2 for *Rp*; >99:1 for *Sp*) was determined to be comparable or slightly lower than that obtained using CMPT by independently synthesizing (*Rp*)-U_SU and (*Sp*)-U_SU.[21] We also applied the method to an automated synthesis. The synthesis of all-(*Rp*)-PS-$(CAGU)_3$ (**10c**) was performed on an automated DNA synthesizer to demonstrate the applicability of the method to automation. The desired all-(*Rp*)-PS-$(CAGU)_3$ (**10c**) was obtained with average coupling yield of 94% as shown in entry 11. All-(*Sp*)-PS-$(CAGU)_3$ (**10d**) was also synthesized on an automated synthesizer with similar efficiency (av. 95%) to that obtained by a manual solid-phase synthesis.[21]

***P*-Configurational Assignment of *P*-Stereodefined PS-ORNs by Enzymatic Digestion.** Isolated PS-ORN 12mers **10a−d** were treated with snake venom phosphodiesterase[23] (svPDE) (*Rp*-specific) and nuclease P1[24] (nP1) (*Sp*-specific) for configurational assignments. After being incubated with svPDE or nP1 for 16 h at 37 °C, the PS-ORNs were analyzed by RP-HPLC.[21] All-(*Sp*)-PS-U_{10} (**10b**) and all-(*Sp*)-PS-$(CAGU)_3$ (**10d**) were completely digested with nP1, whereas only ca. 34% of **10b** and ca. 31% of **10d** were digested with svPDE. On the other hand, complete digestion was observed when all-(*Rp*)-PS-U_{10} (**10a**) and all-(*Rp*)-PS-$(CAGU)_3$ (**10c**) were treated with svPDE, whereas ca. 32% of **10a** and ca. 78% of **10c** were digested with nP1. The partial digestion is probably owing to the lower specificity of the enzymes.[23b,25] These experiments confirmed that the stereochemistry of the PS-ORNs synthesized using the new 2′-*O*-CEM oxazaphospholidine monomers was as expected, that is, (*Rp*)- and (*Sp*)-PS-linkages were formed from the (*Sp*)- and (*Rp*)-monomers, respectively.

Hybridization Properties of the *P*-Stereodefined PS-ORNs. Finally, the hybridization properties of the *P*-stereodefined all-(*Rp*)-PS-$(CAGU)_3$ (**10c**) and all-(*Sp*)-PS-$(CAGU)_3$ (**10d**) were studied. A UV denaturing study was carried out with four kinds of duplexes consisting of **10c** and **10d**, stereo-random-PS-$(CAGU)_3$, or an unmodified $(CAGU)_3$ and the complementary ORNs. As shown in Figure 3, the T_m value of the duplex containing of **10c** was slightly higher than that with unmodified $(CAGU)_3$ (ΔT_m = +3.7 °C). The stabilization effect of an (*Rp*)-PS-linkage (ΔT_m = ca. +0.4 °C per modification) was virtually the same as that obtained previously with all-(*Rp*)-PS-U_{10}.[13] In sharp contrast, the T_m values obtained with all-(*Sp*)-PS-$(CAGU)_3$ (**10d**) and stereo-random-PS-$(CAGU)_3$ were much lower than that of the unmodified duplex (ΔT_m = −10.8 and −4.2 °C, respectively). We have also shown the destabilizing effect of (*Sp*)-PS-linkages previously by using all-(*Sp*)-PS-U_{10}; however, no distinct T_m value was obtained.[13] Thus, the destabilizing effect of (*Sp*)- and stereo-random PS-linkages was evaluated more precisely in the current study using *P*-stereodefined PS-ORNs of mixed sequence.

■ CONCLUSION

In conclusion, we successfully developed a method for synthesizing *P*-stereodefined PS-ORNs of mixed sequence by employing 2′-*O*-CEM protection in the oxazaphospholidine method. The coupling efficiency of the 2′-*O*-CEM-protected oxazaphospholidine monomers was greater than that of the 2′-*O*-TBS-protected monomers. The resultant *P*-stereodefined PS-ORNs of mixed sequence enabled us to demonstrate that a PS-ORN−ORN duplex was stabilized by incorporating (*Rp*)-PS-linkages, whereas it was largely destabilized with (*Sp*)- or stereo-random-PS-linkages. The expanded availability of *P*-stereodefined PS-ORNs should promote their use in therapeutic studies. Furthermore, the stabilizing effect of (*Rp*)-PS-linkages can support the design of miRNA-based drug candidates, which require a much higher hybridization potential to target RNAs than that needed for siRNA.[2]

■ EXPERIMENTAL SECTION

***O^6*-Cyanoethyl-*N^2*-phenoxyacetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-cyanoethoxymethyl)-guanosine 11.** N^2-Phenoxyacetyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-cyanoethoxymethyl)-guanosine (11.18 g, 14.9 mmol) was dried by repeated coevaporations with dry toluene and dissolved in dry CH_2Cl_2 (100 mL) under argon. DMAP (0.092 g, 0.75 mmol), triethylamine (8.3 mL, 60 mmol), and 2-mesitylenesulfonyl chloride (3.92 g, 17.9 mmol) were successively added, and the mixture was stirred for 30 min at rt. A saturated $NaHCO_3$ aqueous solution (50 mL) was then added. The organic layer was separated and washed with saturated $NaHCO_3$ aqueous solutions (2 × 50 mL). The washings were combined and extracted with $CHCl_3$ (2 × 30 mL). The organic

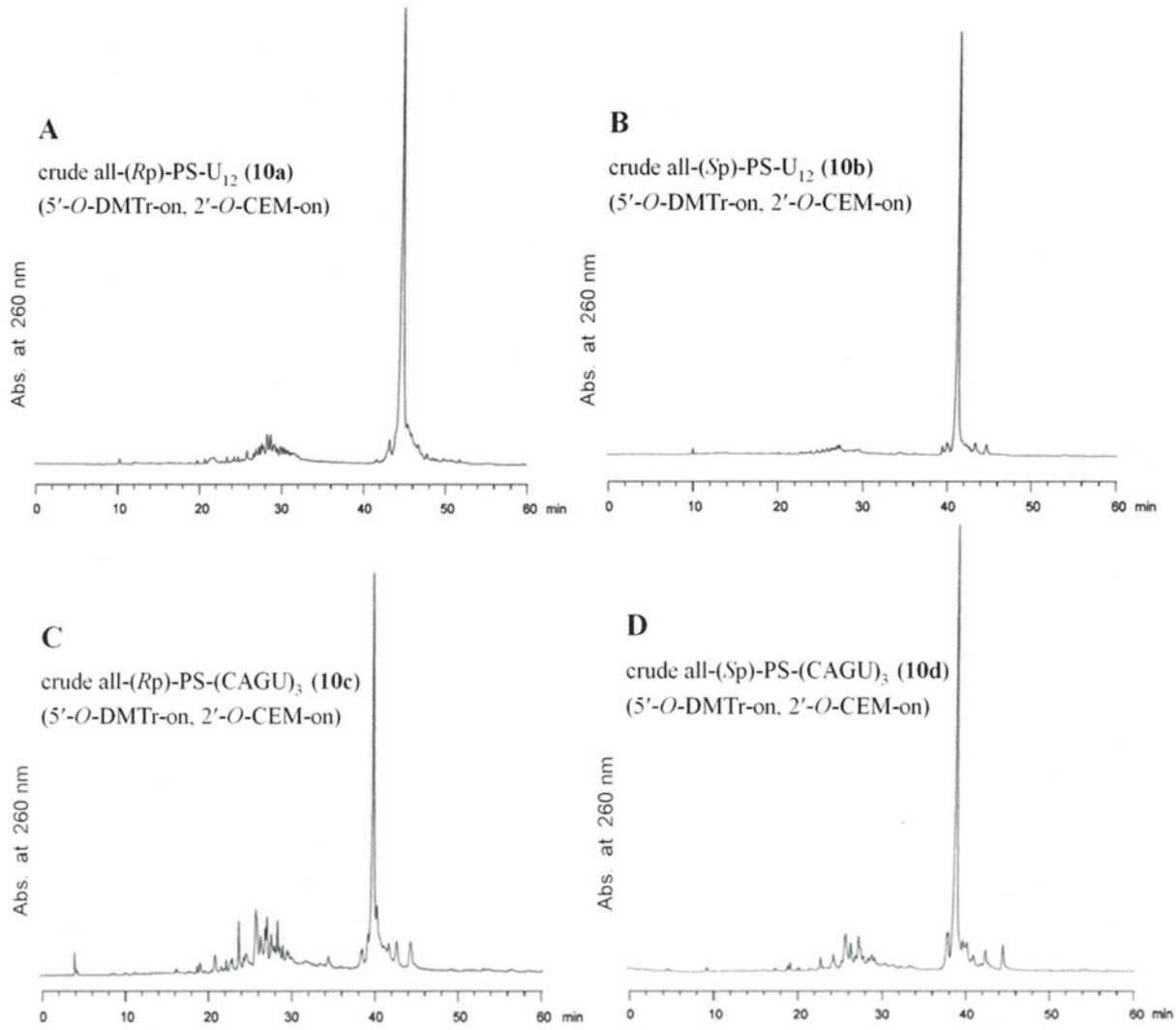

Figure 1. RP-HPLC profiles of PS-ORNs bearing 5′-*O*-DMTr and 2′-*O*-CEM groups: (A) crude all-(*R*p)-PS-U_{12} (**10a**); (B) crude all-(*S*p)-PS-U_{12} (**10b**); (C) crude all-(*R*p)-PS-$(CAGU)_3$ (**10c**); (D) crude all-(*S*p)-PS-$(CAGU)_3$ (**10d**). RP-HPLC was performed with a linear gradient of 0–60% CH_3CN in 0.1 M TEAA buffer (pH 7.0) over 60 min at 30 °C and a rate of 0.5 mL/min.

layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried by repeated coevaporations with dry toluene and dissolved in dry CH_2Cl_2 (100 mL) under argon. *N*-Methylpyrrolidine (15.9 mL, 149 mmol) was added at 0 °C, and the mixture was stirred for 40 min at the same temperature. 2-Cyanoethanol (10.1 mL, 149 mmol) and DBU (3.3 mL, 22 mmol) were then added, and the mixture was further stirred for 25 min at 0 °C. A 1.0 M KH_2PO_4 aqueous solution (50 mL) was then added, and the organic layer was separated and washed with 1.0 M KH_2PO_4 aqueous solutions (2 × 50 mL). The washings were combined and extracted with $CHCl_3$ (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [ethyl acetate–hexane (50:50, v/v to 100:0, v/v)]. The fractions containing **11** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford **11** (6.20 g, 7.8 mmol, 52%) as a pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (1H, brs, 2-NH), 8.31 (1H, s, 8-H), 7.40–7.38 (2H, dd, J = 8.0, 8.0 Hz, 3-H of Pac), 7.11–6.98 (3H, m, 2,4-H of Pac), 6.16 (1H, s, 1′-H), 5.20 (1H, d, J = 7.2 Hz, OCH_2O of CEM), 5.10 (1H, d, J = 7.2 Hz, OCH_2O of CEM), 4.85–4.78 (2H, m, O^6-OCH_2CH_2CN), 4.69 (2H, s, CH_2 of Pac), 4.53 (1H, dd, J = 4.2, 9.3 Hz, 2′-H), 4.35–4.17 (3H, m, 3′-H, 5′-H), 4.08–4.01 (2H, m, 4′-H, OCH_2CH_2CN, of CEM), 3.92–3.78 (1H, m, OCH_2CH_2CN, of CEM), 3.02 (2H, t, J = 6.8 Hz, O^6-OCH_2CH_2CN), 2.66–2.61 (2H, t, J = 6.6 Hz, OCH_2CH_2CN of CEM), 1.12–0.95 (28H, m, *i*-Pr). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 159.6, 156.9, 152.0, 150.8, 139.8, 129.9, 122.6, 119.0, 117.8, 116.8, 114.9, 94.7, 88.4, 81.5, 78.6, 68.4, 67.9, 63.1, 61.7, 59.4, 18.8, 18.1, 17.5, 17.3, 17.3, 17.3, 17.1, 17.0, 16.9, 16.8, 13.3, 13.0, 12.9, 12.6. ESI-HRMS: m/z calcd for $C_{37}H_{54}N_7O_9Si_2^+$ $[(M + H)^+]$ 796.3516, found 796.3527.

***O*^{6}-Cyanoethyl-*N*^{2}-phenoxyacetyl-2′-*O*-(2-cyanoethoxymethyl)-guanosine 12.** Compound **11** (1.92 g, 2.4 mmol) was dried by repeated coevaporations with dry toluene and dissolved in dry THF (5.0 mL) under argon. $Et_3N{\cdot}3HF$ (0.40 mL, 2.4 mmol) was added at 35 °C, and the mixture was stirred for 2 h at the same temperature. The mixture was then diluted with $CHCl_3$ (50 mL) and washed with saturated $NaHCO_3$ aqueous solutions (3 × 30 mL). The washings were combined and extracted with $CHCl_3$ (2 × 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [$CHCl_3$–MeOH (100:3, v/v)] to afford **12** (0.86 g, 1.6 mmol, 64%) as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.73 (1H, brs, 2-NH), 8.57 (1H, s, 8-H), 7.33–7.28 (2H, dd, 7.8, 7.8 Hz, 3-H of Pac), 6.96–6.93 (3H, m, 2,4-H of Pac), 6.09 (1H, d, J = 6.0 Hz, 1′-H), 5.38 (1H, d, J = 5.1 Hz, 3′-OH), 5.11–4.98 (3H, m, 5′-OH, OCH_2O of CEM), 4.78–4.65 (5H, m, 2′-H, O^6-OCH_2CH_2CN, CH_2 of Pac), 4.36 (1H, dd, J = 3.6, 4.7 Hz, 3′-H), 3.98 (1H, d, J = 3.6 Hz, 4′-H), 3.72–3.51 (3H, m, 5′-H,

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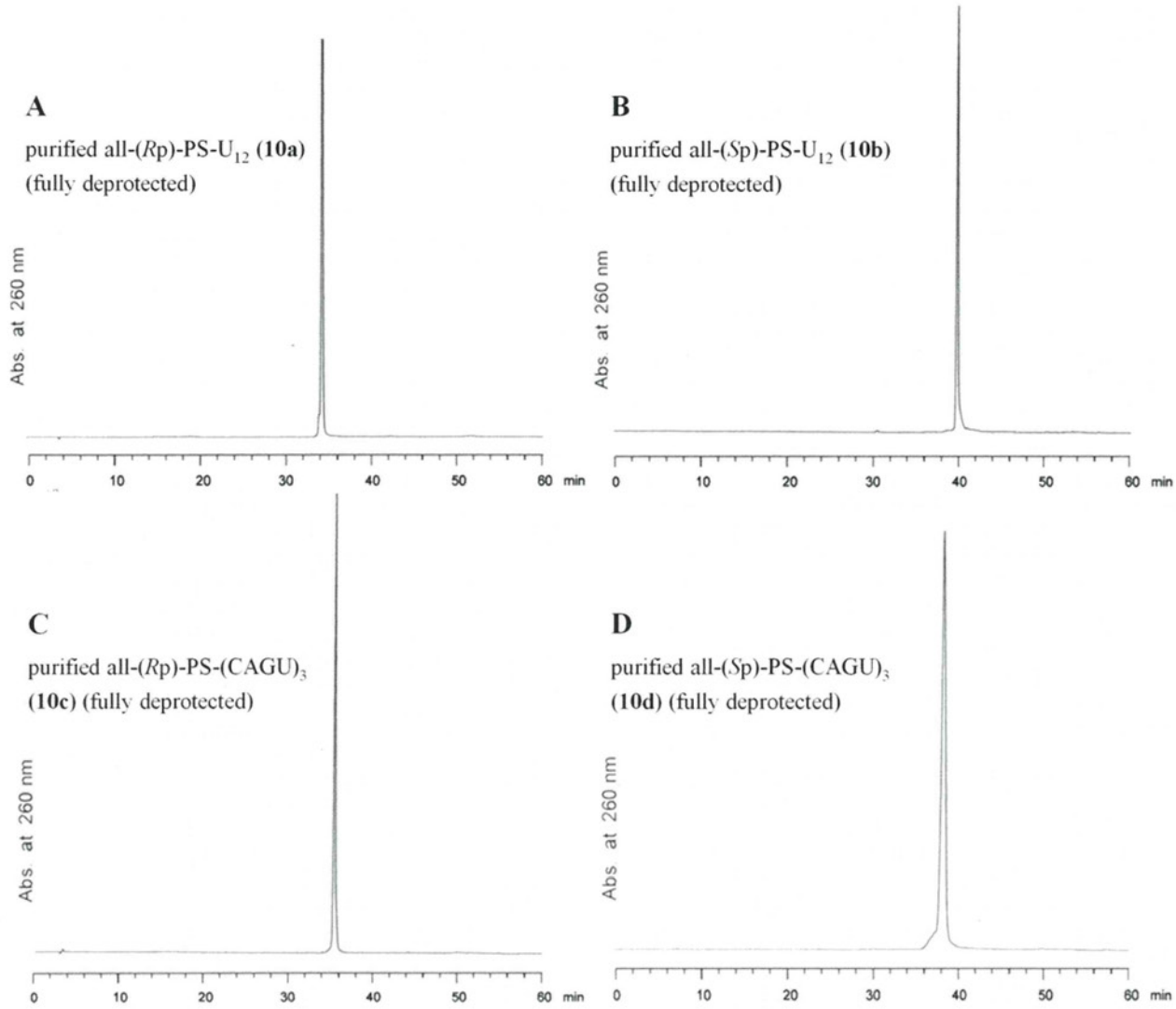

Figure 2. RP-HPLC profiles of fully deprotected and purified PS-ORNs: (A) all-(*Rp*)-PS-U_{12} (**10a**); (B) all-(*Sp*)-PS-U_{12} (**10b**); (C) all-(*Rp*)-PS-$(CAGU)_3$ (**10c**); (D) purified all-(*Sp*)-PS-$(CAGU)_3$ (**10d**). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) over 60 min at 30 °C and a rate of 0.5 mL/min.

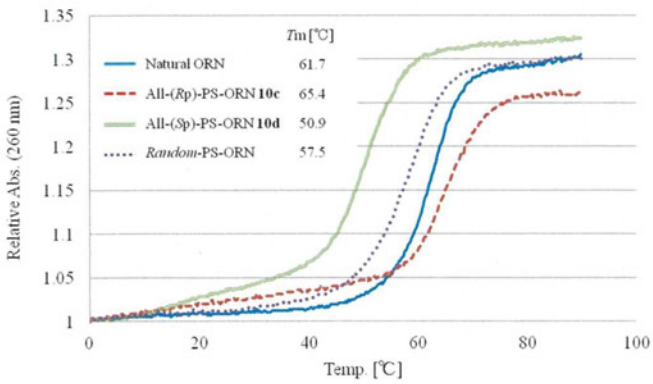

Figure 3. UV melting curves of PS-$(CAGU)_3$-PO-$(ACUG)_3$.

OCH_2CH_2CN, of CEM), 3.47–3.25 (1H, m, OCH_2CH_2CN of CEM), 3.22–3.16 (2H, t, J = 5.9 Hz, O^6-OCH_2CH_2CN), 2.66–2.50 (2H, m, OCH_2CH_2CN of CEM). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 160.0, 156.8, 152.1, 150.6, 142.6, 129.8, 122.5, 119.6, 117.6, 116.9, 114.8, 95.7, 88.4, 86.9, 79.9, 70.8, 67.5, 63.3, 62.4, 61.9, 18.7, 18.0. ESI-HRMS: m/z calcd for $C_{25}H_{28}N_7O_8^+$ $[(M + H)^+]$ 554.1994, found 554.1996.

O^6-Cyanoethyl-N^2-phenoxyacetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(2-cyanoethoxymethyl)-guanosine 1d. Compound **12** (0.86 g, 1.6 mmol) was dried by repeated coevaporations with dry pyridine and dissolved in dry pyridine (5.0 mL) under argon. DMTrCl (0.80 g, 2.4 mmol) was added at 0 °C, and the mixture was stirred for 9 h at rt. MeOH (5 mL) was then added, and the mixture was concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), and the solution was washed with saturated $NaHCO_3$ aqueous solutions (3 × 30 mL). The washings were combined and extracted with $CHCl_3$ (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [ethyl acetate–hexane–pyridine (50:50:0.5, v/v/v to 100:0:0.5, v/v/v)] to afford **1d** (1.21 g, 1.4 mmol, 91%) as a pale yellow foam. ^1H NMR (300 MHz, $CDCl_3$) δ 8.84 (1H, brs, 2-NH), 8.10 (1H, s, 8-H), 7.42–6.99 (14H, m, 2-H of *p*-An, Ph of Pac and DMTr), 6.79 (4H, d, J = 8.1 Hz, 3-H of *p*-An), 6.24 (1H, d, J = 3.9 Hz, 1′-H), 5.03, 4.96 (2H, 2d, J = 7.1 Hz, OCH_2O of CEM), 4.90–4.79 (3H, m, 2′-H, O^6-OCH_2CH_2CN), 4.68–4.61 (3H, m, 3′-H, CH_2 of Pac), 4.26 (1H, d, J = 3.3 Hz, 4′-H), 3.78–3.65 (8H, m, OMe, OCH_2CH_2CN of CEM), 3.49 (2H, d, J = 3.9 Hz, 5′-H), 3.02 (2H, t, J = 6.6 Hz, O^6-OCH_2CH_2CN), 2.62 (1H, d, J = 6.3 Hz, 3′-OH), 2.50 (2H, t, J = 6.2 Hz, OCH_2CH_2CN of CEM). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 159.7, 158.5, 156.9, 152.8, 150.8, 149.7, 144.4, 140.9,

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135.5, 135.4, 130.0, 129.8, 128.1, 127.9, 127.0, 122.5, 118.7, 117.6, 116.8, 114.9, 113.1, 95.6, 87.0, 86.6, 83.9, 80.2, 70.2, 67.7, 63.4, 63.1, 61.7, 55.2, 18.8, 18.0. ESI-HRMS: m/z calcd for $C_{46}H_{46}N_7O_{10}^+$ [(M + H)$^+$] 856.3301, found 836.3300.

(*Sp*)-U-Monomer [(*Sp*)-3a]. A Typical Procedure for the Synthesis of 3a–d. 5′-*O*-DMTr-2′-*O*-CEM-uridine (0.94 g, 1.5 mmol) was dried by repeated coevaporations with dry pyridine and dry toluene and dissolved in freshly distilled THF (5.0 mL) under argon. Triethylamine (1.5 mL, 10.5 mmol) and a 0.5 M solution of the 2-chloro-1,3,2-oxazaphospholidine derivative D-**2** in freshly distilled THF (9.3 mL, 4.7 mmol) were successively added at −78 °C, and the mixture was stirred for 1.5 h at rt. The mixture was then diluted with $CHCl_3$ (400 mL) and washed with a saturated $NaHCO_3$ aqueous solutions (3 × 100 mL). The washings were combined and extracted with $CHCl_3$ (2 × 30 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was then purified by silica gel column chromatography [NH silica gel, hexane–ethyl acetate–triethylamine (20:10:0.03 to 10:20:0.03, v/v/v)]. The fractions containing (*Sp*)-**3a** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Sp*)-**3a** (0.89 g, 1.1 mmol, 71%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (1H, brs, 3-NH), 8.11 (1H, d, J = 8.4 Hz, 6-H), 7.41–7.16 (14H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr), 6.85 (4H, dd, J = 2.1, 9.0 Hz, 3-H of *p*-An), 5.98 (1H, d, J = 1.5 Hz, 1′-H), 5.79 (1H, d, J = 6.3 Hz, 5″-H), 5.20 (1H, d, J = 8.4 Hz, 5-H), 4.89–4.81 (3H, m, 3′-H, OCH_2O of CEM), 4.32–4.25 (2H, m, 2′-H, 4′-H), 3.87–3.68 (10H, m, 5′-H, 4″-H, OCH_2CH_2CN, OMe), 3.54–3.41 (2H, m, 5′-H, 6″-H), 3.14–3.03 (1H, m, 6″-H), 2.47 (2H, ddd, J = 1.8, 6.0, 6.0 Hz, OCH_2CH_2CN), 1.68–1.59 (2H, m, 7″-H), 1.28–1.17 (1H, m, 8″-H), 1.01–0.88 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0, 158.7, 158.7, 150.1, 144.0, 139.8, 137.7 (d, $^3J_{PC}$ = 4.0 Hz), 135.0, 134.8, 130.3, 129.0, 128.4, 128.3, 128.2, 128.0, 127.7, 127.2, 125.3, 117.9, 113.2, 113.2, 102.2, 94.6, 88.4, 87.1, 82.3 (d, $^2J_{PC}$ = 9.5 Hz), 81.8 (d, $^3J_{PC}$ = 4.3 Hz), 78.5, 69.3 (d, $^2J_{PC}$ = 15.5 Hz), 67.3 (d, $^2J_{PC}$ = 3.2 Hz), 62.9, 60.2, 55.2, 47.0 (d, $^2J_{PC}$ = 34.4 Hz), 27.9, 25.9 (d, $^3J_{PC}$ = 3.4 Hz), 18.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ 159.4. ESI-HRMS: m/z calcd for $C_{47}H_{51}N_5O_{10}P^+$ [(M + H)$^+$] 835.3103, found 835.3104.

(*Sp*)-C^{ac}-Monomer [(*Sp*)-3b]. Crude (*Sp*)-**3b** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-*N*^{4}-acetylcytidine **1b** (0.67 g, 1.0 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, toluene–ethyl acetate–triethylamine (10:20:0.03, v/v/v)]. The fractions containing (*Sp*)-**3b** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Sp*)-**3b** (0.66 g, 0.75 mmol, 75%) as a pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.07 (1H, brs, 4-NH), 8.61 (1H, d, J = 7.5 Hz, 6-H), 7.44–7.16 (14H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr), 6.99 (1H, d, J = 7.5 Hz, 5-H), 6.86 (4H, dd, J = 1.8, 8.7 Hz, 3-H of *p*-An), 5.98 (1H, s, 1′-H), 5.78 (1H, d, J = 6.3 Hz, 5″-H), 5.04 (1H, d, J = 6.9 Hz, OCH_2O of CEM), 4.95 (1H, d, J = 6.9 Hz, OCH_2O of CEM), 4.78 (1H, ddd, J = 4.8, 9.3, 9.3 Hz, 3′-H), 4.34–4.27 (2H, m, 2′-H, 4′-H), 3.88–3.73 (10H, m, 5′-H, 4″-H, OCH_2CH_2CN, OMe), 3.56–3.39 (2H, m, 5′-H, 6″-H), 3.12–3.00 (1H, m, 6″-H), 2.49 (2H, t, J = 6.9 Hz, OCH_2CH_2CN), 2.24 (3H, s, Ac), 1.78–1.58 (2H, m, 7″-H), 1.28–1.11 (1H, m, 8″-H), 0.99–0.86 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 162.6, 158.7, 154.8, 144.7, 144.0, 137.8 (d, $^3J_{PC}$ = 4.3 Hz), 135.1, 135.0, 135.0, 130.3, 128.3, 128.3, 128.0, 127.6, 127.2, 125.3, 118.0, 113.2, 113.2, 113.2, 96.5, 94.6, 90.0, 87.1, 82.4 (d, $^2J_{PC}$ = 9.5 Hz), 81.3 (d, $^3J_{PC}$ = 4.0 Hz), 78.6, 68.6 (d, $^2J_{PC}$ = 14.9 Hz), 67.2 (d, $^2J_{PC}$ = 3.2 Hz), 63.0, 59.7, 55.2, 46.9 (d, $^2J_{PC}$ = 35.4 Hz), 29.7, 27.9, 25.9 (d, $^3J_{PC}$ = 3.8 Hz), 24.9, 18.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ 159.0. ESI-HRMS: m/z calcd for $C_{47}H_{51}N_5O_{10}P^+$ [(M + H)$^+$] 876.3368, found 876.3365.

(*Sp*)-A^{ac}-Monomer [(*Sp*)-3c]. Crude (*Sp*)-**3c** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-*N*^{6}-acetyladenosine **1c** (0.70 g, 1.0 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, toluene–ethyl acetate–triethylamine (50:50:0.1, v/v/v)]. The fractions containing (*Sp*)-**3c** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Sp*)-**3c** (0.46 g, 0.51 mmol, 51%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (1H, s, 2-H), 8.51 (1H, brs, 6-NH), 8.21 (1H, s, 8-H), 7.42–7.22 (14H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr), 6.81 (4H, d, J = 6.9 Hz, 3-H of *p*-An), 6.22 (1H, d, J = 5.1 Hz, 1′-H), 5.79 (1H, d, J = 6.6 Hz, 5″-H), 5.07–4.98 (2H, m, 2′-H, 3′-H), 4.84 (1H, d, J = 7.2 Hz, OCH_2O of CEM), 4.76 (1H, d, J = 7.2 Hz, OCH_2O of CEM), 4.36 (1H, dd, J = 3.9, 8.1 Hz, 4′-H), 3.87 (1H, ddd, J = 6.0, 10.5, 10.5 Hz, 4″-H), 3.78 (6H, s, OMe), 3.64–3.44 (4H, m, 5′-H, 6″-H, OCH_2CH_2CN), 3.40 (1H, dd, J = 3.9, 10.8 Hz, 5′-H), 3.17–3.06 (1H, m, 6″-H), 2.61 (3H, s, Ac), 2.37 (2H, t, J = 6.3 Hz, OCH_2CH_2CN), 1.72–1.56 (2H, m, 7″-H), 1.28–1.19 (1H, m, 8″-H), 1.02–0.92 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 158.6, 152.4, 151.0, 149.1, 144.3, 141.7, 138.0 (d, $^3J_{PC}$ = 4.0 Hz), 135.5, 135.4, 130.16, 128.3, 128.2, 127.9, 127.6, 127.0, 126.6, 126.0, 125.4, 122.2, 117.5, 113.1, 94.9, 87.1, 86.7, 83.6 (d, $^3J_{PC}$ = 3.7 Hz), 82.4 (d, $^2J_{PC}$ = 9.5 Hz), 78.0, 70.7 (d, $^2J_{PC}$ = 12.0 Hz), 67.3 (d, $^2J_{PC}$ = 3.2 Hz), 62.9, 62.2, 55.2, 53.1, 50.7, 47.1 (d, $^2J_{PC}$ = 34.7 Hz), 39.6, 27.9, 26.6, 26.0, 25.8 (d, $^3J_{PC}$ = 18.6 Hz), 20.6, 18.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ 156.1. ESI-HRMS: m/z calcd for $C_{48}H_{51}N_7O_9P^+$ [(M + H)$^+$] 900.3480, found 900.3479.

(*Sp*)-G^{ce,pac}-Monomer [(*Sp*)-3d]. Crude (*Sp*)-**3d** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-*O*^{6}-cyanoethyl-*N*^{2}-phenoxyacetylguanosine **1d** (1.28 g, 1.5 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, hexane–ethyl acetate–triethylamine (30:10:0.04 to 10:20:0.03, v/v/v)]. The fractions containing (*Sp*)-**3d** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Sp*)-**3d** (0.87 g, 0.82 mmol, 54%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (1H, brs, 2-NH), 8.13 (1H, s, 8-H), 7.45–6.98 (19H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr, Ph of Pac), 6.79 (4H, d, J = 6.6 Hz, 3-H of *p*-An), 6.23 (1H, d, J = 4.8 Hz, 1′-H), 5.76 (1H, d, J = 6.6 Hz, 5″-H), 4.99–4.75 (6H, m, 2′-H, 3′-H, OCH_2O of CEM, O^6-OCH_2CH_2CN), 4.62 (2H, s, CH_2 of Pac), 4.39 (1H, d, J = 3.3 Hz, 4′-H), 3.85 (1H, ddd, J = 6.0, 10.4, 10.4 Hz, 4″-H), 3.77 (6H, s, OMe), 3.62–3.42 (5H, m, 5′-H, 6″-H, OCH_2CH_2CN of CEM), 3.19–3.03 (3H, m, 6″-H, O^6-OCH_2CH_2CN), 2.34 (2H, t, J = 6.3 Hz, OCH_2CH_2CN of CEM), 1.75–1.59 (2H, m, 7″-H), 1.28–1.17 (1H, m, 8″-H), 1.01–0.89 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 159.7, 158.6, 157.0, 153.0, 151.0, 144.3, 140.8, 137.9 (d, $^3J_{PC}$ = 3.8 Hz), 135.4, 135.4, 130.1, 129.8, 128.5, 128.3, 128.2, 127.9, 127.6, 127.0, 125.4, 122.4, 118.6, 117.6, 116.8, 114.9, 113.2, 94.8, 86.8, 86.5, 83.8 (d, $^3J_{PC}$ = 3.7 Hz), 82.3 (d, $^2J_{PC}$ = 9.5 Hz), 78.0, 70.6 (d, $^2J_{PC}$ = 12.9 Hz), 67.7, 67.3 (d, $^2J_{PC}$ = 3.2 Hz), 63.0, 62.6, 61.8, 55.2, 47.1 (d, $^2J_{PC}$ = 34.9 Hz), 27.9, 25.9 (d, $^3J_{PC}$ = 3.5 Hz), 18.6, 18.0. ^{31}P NMR (121 MHz, $CDCl_3$) δ 157.5. ESI-HRMS: m/z calcd for $C_{57}H_{58}N_8O_{11}P^+$ [(M + H)$^+$] 1061.3957, found 1061.3977.

(*Rp*)-U-Monomer [(*Rp*)-3a]. Crude (*Rp*)-**3a** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-uridine **1a** (0.63 g, 1.0 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, toluene–ethyl acetate–triethylamine (60:40:0.1, v/v/v)]. The fractions containing (*Rp*)-**3a** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Rp*)-**3a** (0.52 g, 0.62 mmol, 62%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (1H, brs, 3-NH), 8.07 (1H, d, J = 8.1 Hz, 6-H), 7.41–7.19 (14H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr), 6.79 (4H, dd, J = 8.1, 8.1 Hz, 3-H of *p*-An), 5.95 (1H, d, J = 1.2 Hz, 1′-H), 5.75 (1H, d, J = 6.6 Hz, 5″-H), 5.16 (1H, d, J = 8.1 Hz, 5-H), 5.01, 4.94 (2H, 2d, J = 7.2 Hz, OCH_2O of CEM), 4.89 (1H, ddd, J = 6.9, 8.4, 8.4 Hz, 3′-H), 4.35 (1H, dd, J = 1.2, 4.8 Hz, 2′-H), 4.21 (1H, d, J = 8.1 Hz, 4′-H), 3.94–3.87 (3H, m, 4″-H, OCH_2CH_2CN), 3.77, 3.74 (6H, 2s, OMe), 3.63–3.52 (3H, m, 5′-H, 6″-H), 3.09–3.03 (1H, m, 6″-H), 2.67 (2H, ddd, J = 2.7, 6.5, 6.5 Hz, OCH_2CH_2CN), 1.65–1.56 (2H, m, 7″-H), 1.25–1.19 (1H, m, 8″-H), 1.03–0.91 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0, 158.7, 158.6, 150.1, 144.3, 140.0, 138.0 (d, $^3J_{PC}$ = 4.0 Hz), 135.0, 130.3, 130.1, 128.3, 128.2, 128.0, 127.6, 127.2, 125.5, 117.9, 113.2,

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113.2, 102.1, 94.6, 88.5, 87.1, 82.6 (d, $^2J_{PC}$ = 9.6 Hz), 81.8, 78.4 (d, $^3J_{PC}$ = 3.2 Hz), 69.4 (d, $^2J_{PC}$ = 14.4 Hz), 67.3 (d, $^2J_{PC}$ = 3.2 Hz), 63.1, 60.3, 55.2, 55.2, 47.1 (d, $^2J_{PC}$ = 34.7 Hz), 28.1, 26.0 (d, $^3J_{PC}$ = 3.7 Hz), 18.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ 158.2. ESI-HRMS: m/z calcd for $C_{45}H_{48}N_4O_{10}P^+$ [$(M + H)^+$] 835.3103, found 835.3104.

(*Rp*)-C^{ac}-Monomer [(*Rp*)-3b]. Crude (*Rp*)-**3b** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-N^4-acetylcytidine **1b** (1.01 g, 1.5 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, hexane−ethyl acetate−triethylamine (20:10:0.03 to 10:30:0.03, v/v/v)]. The fractions containing (*Rp*)-**3b** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Rp*)-**3b** (0.69 g, 0.79 mmol, 53%) as a pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.39 (1H, brs, 4-NH), 8.54 (1H, d, J = 7.5 Hz, 6-H), 7.42−7.16 (14H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr), 6.88 (1H, d, J = 7.5 Hz, 5-H), 6.84−6.77 (4H, dd, J = 6.0, 9.0 Hz, 3-H of *p*-An), 5.94 (1H, s, 1′-H), 5.72 (1H, d, J = 6.3 Hz, 5″-H), 5.15 (1H, d, J = 6.9 Hz, OCH_2O of CEM), 4.98, (1H, d, J = 6.9 Hz, OCH_2O of CEM), 4.84 (1H, ddd, J = 4.8, 9.3, 9.3 Hz, 3′-H), 4.33−4.26 (2H, m, 2′-H, 4′-H), 3.96−3.86 (3H, m, 4″-H, OCH_2CH_2CN), 3.77, 3.75 (6H, 2s, OMe), 3.69−3.50 (3H, m, 5′-H, 6″-H), 3.15−3.04 (1H, m, 6″-H), 2.78−2.59 (2H, m, OCH_2CH_2CN), 2.24 (3H, s, Ac), 1.67−1.51 (2H, m, 7″-H), 1.28−1.17 (1H, m, 8″-H), 1.01−0.93 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 162.6, 158.7, 158.6, 154.9, 144.8, 144.2, 138.0 (d, $^3J_{PC}$ = 4.0 Hz), 135.1, 130.3, 130.1, 128.3, 128.2, 128.0, 127.6, 127.2, 125.5, 118.1, 113.2, 98.4, 96.4, 94.4, 90.1, 87.1, 82.6 (d, $^2J_{PC}$ = 9.5 Hz), 81.4, 78.2, 68.8 (d, $^2J_{PC}$ = 14.6 Hz), 67.3 (d, $^2J_{PC}$ = 3.5 Hz), 63.0, 59.9, 55.2, 47.1 (d, $^2J_{PC}$ = 34.7 Hz), 28.0, 25.9 (d, $^3J_{PC}$ = 3.5 Hz), 24.9, 18.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ 158.0. ESI-HRMS: m/z calcd for $C_{47}H_{51}N_4O_{10}P^+$ [$(M + H)^+$] 876.3368, found 876.3367.

(*Rp*)-A^{ac}-Monomer [(*Rp*)-3c]. Crude (*Rp*)-**3c** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-N^6-acetyladenosine **1c** (0.70 g, 1.0 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, toluene−ethyl acetate−triethylamine (20:10:0.03, v/v/v)]. The fractions containing (*Rp*)-**3c** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Rp*)-**3c** (0.44 g, 0.49 mmol, 49%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (1H, brs, 6-NH), 8.61 (1H, s, 2-H), 8.26 (1H, s, 8-H), 7.45−7.16 (14H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr), 6.76 (4H, d, J = 9.0 Hz, 3-H of *p*-An), 6.24 (1H, d, J = 4.8 Hz, 1′-H), 5.77 (1H, d, J = 6.3 Hz, 5″-H), 5.06−4.95 (2H, m, 2′-H, 3′-H), 4.90, 4.84 (2H, 2d, J = 7.2 Hz, OCH_2O of CEM), 4.36 (1H, dd, J = 3.0, 6.0 Hz, 4′-H), 3.88 (1H, ddd, J = 6.3, 10.5, 10.5 Hz, 4″-H), 3.81−3.51 (10H, m, 5′-H, 6″-H, OMe, OCH_2CH_2CN), 3.41 (1H, dd, J = 3.6, 10.5 Hz, 5′-H), 3.18−3.06 (1H, m, 6″-H), 2.60 (3H, s, Ac), 2.49 (2H, t, J = 6.3 Hz, OCH_2CH_2CN), 1.69−1.59 (2H, m, 7″-H), 1.28−1.18 (1H, m, 8″-H), 0.98−0.91 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 158.5, 158.5, 152.3, 150.9, 149.2, 144.4, 141.9, 138.0 (d, $^3J_{PC}$ = 3.7 Hz), 135.5, 135.4, 130.1, 130.1, 128.3, 128.2, 127.8, 127.6, 126.9, 125.5, 122.2, 117.5, 113.1, 95.2, 87.3, 86.7, 83.6, 82.8 (d, $^2J_{PC}$ = 10.0 Hz), 78.0, 71.0 (d, $^2J_{PC}$ = 10.6 Hz), 67.3 (d, $^2J_{PC}$ = 3.2 Hz), 63.1, 62.4, 55.2, 47.0 (d, $^2J_{PC}$ = 34.9 Hz), 28.1, 25.8 (d, $^3J_{PC}$ = 18.1 Hz), 18.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ 157.0. ESI-HRMS: m/z calcd for $C_{48}H_{51}N_7O_9P^+$ [$(M + H)^+$] 900.3480, found 900.3480.

(*Rp*)-G^{ce,pac}-Monomer [(*Rp*)-3d]. Crude (*Rp*)-**3d** was synthesized from 5′-*O*-DMTr-2′-*O*-CEM-O^6-cyanoethyl-N^2-phenoxyacetylguanosine **1d** (0.86 g, 1.0 mmol) following the typical procedure described above and purified by silica gel column chromatography [NH silica gel, toluene−ethyl acetate−triethylamine (80:20:0.1, v/v/v)]. The fractions containing (*Rp*)-**3d** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to afford (*Rp*)-**3d** (0.45 g, 0.42 mmol, 42%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (1H, brs, 2-NH), 8.10 (1H, s, 8-H), 7.42−7.00 (19H, m, 5″-Ph, 2-H of *p*-An, Ph of DMTr, Ph of Pac), 6.76 (4H, d, J = 6.6 Hz, 3-H of *p*-An), 6.23 (1H, d, J = 5.1 Hz, 1′-H), 5.74 (1H, d, J = 6.3 Hz, 5″-H), 5.01−4.82 (6H, m, 2′-H, 3′-H, OCH_2O of CEM, O^6-OCH_2CH_2CN), 4.63 (2H, s, CH_2 of Pac), 4.35 (1H, d, J = 2.7 Hz, 4′-H), 3.90−3.42 (12H, m, 5′-H, 4″-H, 6″-H, OMe, OCH_2CH_2CN), 3.19−3.00 (3H, m, 6″-H, O^6-OCH_2CH_2CN), 2.48 (2H, t, J = 6.3 Hz, OCH_2CH_2CN of CEM), 1.73−1.59 (2H, m, 7″-H), 1.29−1.12 (1H, m, 8″-H), 1.01−0.87 (1H, m, 8″-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 159.7, 158.5, 158.5, 157.0, 153.0, 151.0, 144.4, 140.8, 138.0 (d, $^3J_{PC}$ = 4.1 Hz), 135.5, 135.4, 130.1, 130.0, 129.8, 128.2, 128.1, 127.9, 127.6, 127.0, 125.5, 122.4, 118.7, 117.6, 116.8, 114.9, 113.1, 95.1, 86.7, 86.6, 83.7, 82.7 (d, $^2J_{PC}$ = 9.5 Hz), 78.2 (d, $^3J_{PC}$ = 3.5 Hz), 71.1 (d, $^2J_{PC}$ = 12.0 Hz), 67.7, 67.3 (d, $^2J_{PC}$ = 3.2 Hz), 63.2, 62.6, 61.8, 55.2, 47.0 (d, $^2J_{PC}$ = 34.7 Hz), 28.0, 25.9 (d, $^3J_{PC}$ = 3.5 Hz), 18.6, 18.0. ^{31}P NMR (121 MHz, $CDCl_3$) δ 156.8. ESI-HRMS: m/z calcd for $C_{57}H_{58}N_8O_{11}P^+$ [$(M + H)^+$] 1061.3957, found 1061.3964.

A General Procedure for Solid-Phase Synthesis of PS-ORNs. Manual solid-phase synthesis of *P*-stereodefined PS-ORNs was performed according to the procedure given in Table 4 by using 5′-*O*-DMTr-uridine-loaded HCP or CPG (0.5 μmol). Automated solid-phase synthesis (**10c,d**) was performed according to the procedure given in Table 5 by using 5′-*O*-DMTr-uridine-loaded HCP (0.25 μmol). After the chain elongation by repeating steps 1−8 in Table 4 or 5, the 5′-*O*-DMTr group was removed by treatment with 3% DCA in CH_2Cl_2 except for those isolated ones, for which the DMTr group was left as a purification handle. The deprotection of the bases and the PS-linkages and the cleavage of the linker were performed by treatment with a 25% NH_3 aqueous solution−EtOH (3:1, v/v) (6 mL) for 3 h (for 2mers), 12 h (for 4mers), or 48 h (for 12mers) at rt. The resultant crude products were analyzed (2−4mers) or purified (12mers) by RP-HPLC. Fractions containing the desired PS-ORN 12mers (2′-*O*-CEM-on−5′-*O*-DMTr-on) were collected and lyophilized. The residue was then treated with a 0.5 M TBAF solution in dry DMSO containing 0.5% CH_3NO_2 (400 μL) for 5 h at rt and diluted with a 0.1 M TEAA buffer solution (pH 7.0) (40 mL). The mixture was purified with a Sep-pak C18 cartridge. The Sep-pak was washed twice with a 0.1 M TEAA buffer solution (pH 7.0) (5.0 mL) to remove DMSO, TBAF,

Table 4. Procedure for Manual Solid-Phase Synthesis of PS-ORNs

step	operation	reagents and solvents	time
1	detritylation	3% DCA in CH_2Cl_2	4 × 15 s
2	washing	(i) CH_2Cl_2, (ii) dry CH_3CN, (iii) drying *in vacuo*	
3	condensation	0.13 M monomer **3a−d**, 1 M activator, dry CH_3CN	5, 10, or 15 min
4	washing	(i) dry CH_3CN, (ii) drying *in vacuo*	
5	capping	0.5 M CF_3COIm and 1 M DMAN in dry THF	30 s
6	washing	(i) dry THF, (ii) dry CH_3CN, (iii) drying *in vacuo*	
7	sulfurization	0.3 M DTD in dry CH_3CN	10 min
8	washing	(i) dry CH_3CN, (ii) drying *in vacuo*	

Table 5. Procedure for Automated Solid-Phase Synthesis of PS-ORNs

step	operation	reagents and solvents	time
1	detritylation	3% DCA in CH_2Cl_2	49 s
2	washing	dry CH_3CN	
3	condensation	0.15 M monomer **3a−d**, 1 M activator, dry CH_3CN	15 min
4	washing	dry CH_3CN	
5	capping	0.5 M CF_3COIm and 1 M DMAN in dry THF	30 s
6	washing	dry CH_3CN	
7	sulfurization	0.3 M DTD in dry CH_3CN	6 min
8	washing	dry CH_3CN	

and CH_3NO_2, and then the desired PS-ORNs were eluted with 80% CH_3CN and lyophilized. The residue was then treated with an 80% AcOH aqueous solution (500 μL) for 1 h at rt and diluted with a 2 M TEAA buffer solution (pH 7.0) (20 mL). The mixture was desalted with a Sep-pak C18 cartridge. The Sep-pak was washed twice with a 0.1 M TEAA buffer solution (pH 7.0) (5.0 mL), and then the product was eluted with 40% CH_3CN. The eluate was concentrated under reduced pressure and purified by RP-HPLC to afford the desired PS-ORNs. Isolated yields were determined by UV quantitation at 260 nm. All-(*Rp*)-PS-U_{12} **10a**, 12% isolated yield, MALDI-TOF MS: *m*/*z* calcd for $C_{108}H_{132}N_{24}O_{83}P_{11}S_{11}^-$ [(M − H)$^-$] 3785.09, found 3788.62. All-(*Sp*)-PS-U_{12} **10b**, 14% isolated yield, MALDI-TOF MS: *m*/*z* calcd for $C_{108}H_{132}N_{24}O_{83}P_{11}S_{11}^-$ [(M − H)$^-$] 3785.09, found 3789.25. All-(*Rp*)-PS-$(CAGU)_3$ **10c**, 6% isolated yield, MALDI-TOF MS: *m*/*z* calcd for $C_{114}H_{141}N_{45}O_{71}P_{11}S_{11}^-$ [(M − H)$^-$] 3968.29, found 3971.19. All-(*Sp*)-PS-$(CAGU)_3$ **10d**, 10% isolated yield, MALDI-TOF MS: *m*/*z* calcd for $C_{114}H_{141}N_{45}O_{71}P_{11}S_{11}^-$ [(M − H)$^-$] 3968.29, found 3972.53.

Enzymatic Digestion of *P*-Stereodefined PS-ORNs. *Digestion with svPDE.* An aqueous solution (20 μL, pH 8.5) containing a purified PS-ORN (**10a–d**) (1.0 nmol), svPDE (0.1×10^{-3} unit), 100 mM Tris-HCl, and 15 mM $MgCl_2$ was incubated for 16 h at 37 °C. The mixture was then diluted with 0.1 M TEAA buffer (pH 7.0) (80 μL), heated for 1 min at 100 °C to inactivate the enzyme, filtrated, and analyzed by RP-HPLC.

Digestion with nP1. An aqueous solution (20 μL, pH 7.2) containing a purified PS-ORN (**10a–d**) (1.0 nmol), nuclease P1 (1 unit), 50 mM CH_3COONa, and 1 mM $ZnCl_2$ was incubated for 16 h at 37 °C. The mixture was then diluted with 0.1 M TEAA buffer (pH 7.0) (80 μL), heated at 100 °C for 1 min to inactivate the enzyme, filtrated, and analyzed by RP-HPLC.

Thermal Denaturating Experiment. An aqueous solution (200 μL, pH 7.0) containing a 1:1 ratio of complementary ORNs (0.45 nmol each), 10 mM phosphate, 100 mM NaCl, and 0.1 mM EDTA was deaerated for 10 min under reduced pressure. An aliquot (165 μL) was placed in a 1 cm path length quartz cell. The solution was then heated at a rate of 5 °C/min from rt to 90 °C, kept for 10 min at 90 °C, and cooled at a rate of −2 °C/min from 90 to 0 °C to hybridize the ORNs. After being kept for 90 min at 0 °C, the solution was gradually heated for denaturating experiments. UV absorbance values (260 nm) were recorded at intervals of 0.5 °C while the temperature was ramped at a rate of 0.5 °C/min from 0 to 90 °C under Ar.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H, ^{13}C, and ^{31}P NMR spectra, HPLC profiles and UV melting curves. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: wada@k.u-tokyo.ac.jp.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Bumcrot, D.; Manoharan, M.; Koteliansky, V.; Sah, D. W. Y. *Nat. Chem. Biol.* **2006**, *2*, 711–719. (b) Shukla, S.; Sumaria, C. S.; Pradeepkumar, P. I. *ChemMedChem* **2010**, *5*, 328–349.

(2) Seto, A. G. *Int. J. Biochem. Cell Biol.* **2010**, *42*, 1298–1305.

(3) Echeverri, C. J.; Perrimon, N. *Nat. Rev. Genet.* **2006**, *7*, 373–384.

(4) (a) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99–134. (b) Guga, P.; Koziołkiewicz, M. *Chem. Biodiversity* **2011**, *8*, 1642–1681.

(5) Oka, N.; Wada, T. *Chem. Soc. Rev.* **2011**, *40*, 5829–5843.

(6) Frederiksen, J. K.; Piccirilli, J. A. *Methods Enzymol.* **2009**, *468*, 289–309.

(7) (a) Yano, J.; Ohgi, T.; Ueda, T. Patent WO2006/043521-A1, CAN: 144:450874. (b) Nawrot, B.; Rębowska, B.; Michalak, O.; Bulkowski, M.; Błaziak, D.; Guga, P.; Stec, W. J. *Pure Appl. Chem.* **2008**, *80*, 1859–1871. (c) Wrzesinski, J.; Wichłacz, A.; Nijakowska, D.; Rebowska, B.; Nawrot, B.; Ciesiołka, J. *New J. Chem.* **2010**, *34*, 1018–1026.

(8) Matzura, H.; Eckstein, F. *Eur. J. Biochem.* **1968**, *3*, 448–452.

(9) Sierzchała, A.; Okruszek, A.; Stec, W. J. *J. Org. Chem.* **1996**, *61*, 6713–6716.

(10) Lesnikowski, Z. J. *Nucleosides Nucleotides* **1992**, *11*, 1621–1638.

(11) (a) Almer, H.; Stawinski, J.; Strömberg, R. *J. Chem. Soc., Chem. Commun.* **1994**, 1459–1460. (b) Almer, H.; Stawinski, J.; Strömberg, R. *Nucleic Acids Res.* **1996**, *24*, 3811–3820.

(12) Efimov, V. A.; Molchanova, N. S.; Chakhmakhcheva, O. G. *Nucleosides Nucleotides Nucleic Acids* **2007**, *26*, 1087–1093.

(13) Oka, N.; Kondo, T.; Fujiwara, S.; Maizuru, Y.; Wada, T. *Org. Lett.* **2009**, *11*, 967–970.

(14) Somoza, Á. *Chem. Soc. Rev.* **2008**, *37*, 2668–2675.

(15) (a) Scaringe, S. A.; Wincott, F. E.; Caruthers, M. H. *J. Am. Chem. Soc.* **1998**, *120*, 11820–11821. (b) Dellinger, D. J.; Timár, Z.; Myerson, J.; Sierzchala, A. B.; Turner, J.; Ferreira, F.; Kupihár, Z.; Dellinger, G.; Hill, K. W.; Powell, J. A.; Sampson, J. R.; Caruthers, M. H. *J. Am. Chem. Soc.* **2011**, *133*, 11540–11556. (c) Umemoto, T.; Wada, T. *Tetrahedron Lett.* **2004**, *45*, 9529–9531. (d) Semenyuk, A.; Földesi, A.; Johansson, T.; Estmer-Nilsson, C.; Blomgren, P.; Brännvall, M.; Kirsebom, L. A.; Kwiatkowski, M. *J. Am. Chem. Soc.* **2006**, *128*, 12356–12357. (e) Martin, A. R.; Lavergne, T.; Vasseur, J.-J.; Debart, F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4046–4049. (f) Lavergne, T.; Bertrand, J.-R.; Vasseur, J.-J.; Debart, F. *Chem.—Eur. J.* **2008**, *14*, 9135–9138. (g) Saneyoshi, H.; Ando, K.; Seio, K.; Sekine, M. *Tetrahedron* **2007**, *63*, 11195–11203. (h) Zhou, C.; Pathmasiri, W.; Honcharenko, D.; Chatterjee, S.; Barman, J.; Chattopadhyaya, J. *Can. J. Chem.* **2007**, *85*, 293–301. (i) Lackey, J. G.; Mitra, D.; Somoza, M. M.; Cerrina, F.; Damha, M. J. *J. Am. Chem. Soc.* **2009**, *131*, 8496–8502. (j) Cieślak, J.; Grajkowski, A.; Kauffman, J. S.; Duff, R. J.; Beaucage, S. L. *J. Org. Chem.* **2008**, *73*, 2774–2783.

(16) (a) Ohgi, T.; Masutomi, Y.; Ishiyama, K.; Kitagawa, H.; Shiba, Y.; Yano, J. *Org. Lett.* **2005**, *7*, 3477–3480. (b) Shiba, Y.; Masuda, H.; Watanabe, N.; Ego, T.; Takagaki, K.; Ishiyama, K.; Ohgi, T.; Yano, J. *Nucleic Acids Res.* **2007**, *35*, 3287–3296. (c) Nagata, S.; Hamasaki, T.; Uetake, K.; Masuda, H.; Takagaki, K.; Oka, N.; Wada, T.; Ohgi, T.; Yano, J. *Nucleic Acids Res.* **2010**, *38*, 7845–7857.

(17) Oka, N.; Yamamoto, M.; Sato, T.; Wada, T. *J. Am. Chem. Soc.* **2008**, *130*, 16031–16037.

(18) Ueda, T.; Nagata, S. Patent JP2011-121881A, CAN: 155:68060.

(19) (a) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2002**, *124*, 4962–4963. (b) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2003**, *125*, 8307–8317.

(20) Song, Q.; Wang, Z.; Sanghvi, Y. S. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 629–633.

(21) See the Supporting Information.

(22) Hayakawa, Y.; Kawai, R.; Hirata, A.; Sugimoto, J.; Kataoka, M.; Sakakura, A.; Hirose, M.; Noyori, R. *J. Am. Chem. Soc.* **2001**, *123*, 8165–8176.

(23) (a) Burgers, P. M. J.; Eckstein, F. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4798–4801. (b) Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1979**, *18*, 2825–2829.

(24) Potter, B. V. L; Connolly, B. A.; Eckstein, F. *Biochemistry* **1983**, *22*, 1369−1377.
(25) Griffiths, A. D.; Potter, B. V. L; Eperon, I. C. *Nucleic Acids Res.* **1987**, *15*, 4145−4162.

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There were errors in two of the chemical formulas in the version published ASAP August 29, 2012. The correct version reposted September 5, 2012.

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Stereocontrolled synthesis of oligodeoxyribonucleoside boranophosphates by an oxazaphospholidine approach using acid-labile *N*-protecting groups

Naoki Iwamoto, Natsuhisa Oka, Takeshi Wada *

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

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ABSTRACT

Oligodeoxyribonucleoside boranophosphates (PB-ODNs) were synthesized in a stereocontrolled manner via the corresponding *H*-phosphonates with fully deprotected nucleobases by using diastereopure 2′-deoxyribonucleoside 3′-*O*-oxazaphospholidine monomers bearing acid-labile protecting groups on the nucleobases. Using the resultant stereodefined PB-ODNs, we demonstrated that the thermal stability of the duplexes of PB-ODNs with complementary oligonucleotides was dependent on the configuration of their phosphorus atoms.

Oligonucleoside boranophosphates and their analogs have attracted much attention lately as potential therapeutic oligonucleotides.[1] They are resistant to nucleases, adequately lipophilic, which may facilitate transport across cell membranes, and have modest to high affinity for complementary RNA sequences.[1–3] Recent studies have shown that they serve as potent short interfering RNAs,[3a,4] being comparable with or more effective than natural siRNAs.[4] It has also been demonstrated that effective cell transfection was achieved with such P-boronated oligonucleotide analogs without the aid of transfection agents.[3] Furthermore, these types of oligonucleotide analogs have been expected to work as target-specific ^{10}B carriers for boron neutron capture therapy (BNCT).[1]

For these reasons, chemical synthesis of P-boronated oligonucleotide analogs has become a significant subject of research. However, there are still two major problems to be solved: first, nucleobases other than thymine bearing conventional acyl protecting groups suffer from severe side reactions caused by boronating agents, such as BH_3·THF and BH_3·SMe_2.[5] Thymine also becomes sensitive to such boronating agents after being silylated during the silylation step for the conversion of oligonucleoside *H*-phosphonate intermediates into boranophosphates.[6] Second, the phosphorus atoms of these P-boronated oligonucleotide analogs are chiral and proper stereocontrol should have a positive impact on their properties, such as the stability to nucleases and the affinity for target RNA strands.[7–10]

The first problem has been successfully overcome by several methods developed recently: an *H*-phosphonate method[11] using base-unprotected nucleoside 3′-*H*-phosphonate monomers,[12] a phosphoramidite method using base protecting groups unreactive to the boronating agents (e.g., 4,4′,4″-trimethoxytrityl (TMTr) groups),[3,13] and a boranophosphotriester method using nucleoside 3′-boranophosphate diester monomers.[2,14] However, all of these methods cannot control the stereochemistry at the phosphorus atoms and the products are obtained as mixtures of *P*-diastereomers. To date, there have been no reports for the stereoselective chemical synthesis of P-boronated oligonucleotide analogs.[15] The enzymatic synthesis[4,16] can incorporate only (*Sp*)-boranophosphate diester linkages into oligonucleotides due to the substrate recognition specificity of the enzymes. Thus, a new synthetic strategy to overcome both of these two problems is required.

Recently, we have developed a method for the stereocontrolled synthesis of oligodeoxyribonucleoside *H*-phosphonates (PH-ODNs) using diastereopure nucleoside 3′-*O*-oxazaphospholidine monomers,[17] and all-(*Rp*)- and (*Sp*)-tetrathymidylate boranophosphates have been synthesized by silylation and boronation of the resultant stereodefined *H*-phosphonate intermediates.[5,6,17,18] The side reactions on silylated thymine bases have been suppressed by using DMF as a solvent,[17] but attempts to synthesize PB-ODNs containing four kinds of nucleobases using conventional acyl protections resulted in failure due to significant side reactions on the acylated nucleobases by boronation even in DMF (data not shown).

Given this result, we turned to the synthesis of stereodefined PH-ODNs with unprotected nucleobases. As reported in the literature, unprotected nucleobases and the boronating agents can form adducts, but regenerate the unmodified nucleobases during the final deprotection of PB-ODNs.[11] Since base protection is necessary for the synthesis of the nucleoside 3′-*O*-oxazaphospholidine

* Corresponding author. Tel./fax: +81 4 7136 3612.
E-mail address: wada@k.u-tokyo.ac.jp (T. Wada).

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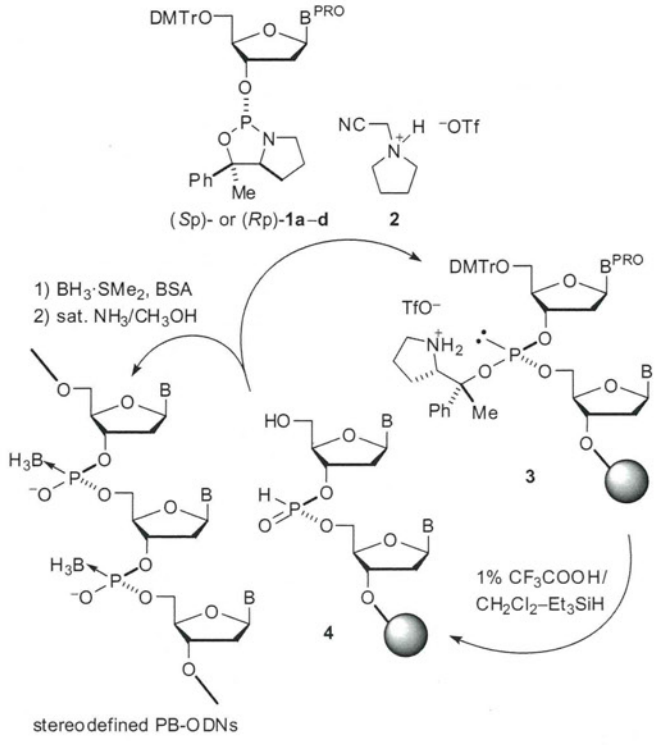
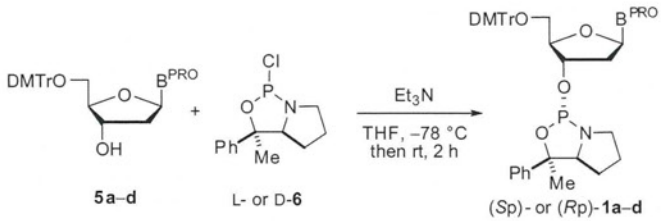


Scheme 1. Solid-phase synthesis of stereodefined PB-ODNs.

Table 1
Synthesis of oxazaphospholidine monomers **1a–d**



Entry	1	B^PRO[b]	Yield (%)	*trans*:*cis*
1[a]	(*Sp*)-**1a**	Th	74	>99:1
2	(*Sp*)-**1b**	Cy^{tmt}	80	>99:1
3	(*Sp*)-**1c**	Ad^{dmt}	80	>99:1
4	(*Sp*)-**1d**	$Gu^{tse,dmt}$	76	>99:1
5[a]	(*Rp*)-**1a**	Th	83	>99:1
6	(*Rp*)-**1b**	Cy^{tmt}	88	>99:1
7	(*Rp*)-**1c**	Ad^{dmt}	80	>99:1
8	(*Rp*)-**1d**	$Gu^{tse,dmt}$	71	>99:1

[a] Data taken from Ref. 17.
[b] B^{PRO} = protected nucleobase; Th = thymin-1-yl; Cy^{tmt} = N^4-4,4′,4″-trimethoxytritylcytosin-1-yl; Ad^{dmt} = N^6-4,4′-dimethoxytrityladenin-9-yl; $Gu^{tse,dmt}$ = O^6-trimethylsilylethyl-N^2-4,4′-dimethoxytritylguanin-9-yl.

monomers, we employed acid-labile base protecting groups so that the nucleobases would be deprotected during the 5′-O-detritylation. The method is outlined in Scheme 1. Diastereopure nucleoside 3′-*O*-oxazaphospholidine monomers (*Sp*)- or (*Rp*)-**1a–d** were condensed with the 5′-OH of a nucleoside on solid support in the presence of *N*-(cyanomethyl)pyrrolidinium triflate (CMPT, **2**).[19] The resultant phosphite intermediates **3** were treated with 1% trifluoroacetic acid (TFA) in CH_2Cl_2–Et_3SiH[5a,14b] for the 5′-O-detritylation and the conversion of the phosphite triester linkage into an *H*-phosphonate diester stereospecifically.[17] Acid-labile protecting groups on the nucleobases were removed simultaneously. As a result, stereodefined PH-ODNs were synthesized on solid support with the nucleobases being fully deprotected (**4**). The desired PB-ODNs were given by silylation and boronation of **4**. We have already confirmed that oxazaphospholidine monomers do not form adducts with unprotected nucleobases.[20]

The synthesis of the nucleoside 3′-*O*-oxazaphospholidine monomers (*Sp*)- and (*Rp*)-**1a–d** is summarized in Table 1. 5′-*O*-DMTr-nucleosides **5a–d** bearing acid-labile TMTr (N^4-position of cytosine), DMTr (N^6-position of adenine and N^2-position of guanine), and trimethylsilylethyl (TSE)[21] (O^6-position of guanine) groups were allowed to react with the 2-chloro-1,3,2-oxazaphospholidine

Table 2
Synthesis of dinucleoside boranophosphates **7a–d**

Entry	7	dN_BT[b]	Yield[c] (%)	*Rp*:*Sp*[c]
1[a]	(*Rp*)-**7a**	(*Rp*)-T_BT	91	>99:1
2	(*Rp*)-**7b**	(*Rp*)-dC_BT	95	>99:1
3	(*Rp*)-**7c**	(*Rp*)-dA_BT	88	>99:1
4	(*Rp*)-**7d**	(*Rp*)-dG_BT	85	>99:1
5[a]	(*Sp*)-**7a**	(*Sp*)-T_BT	92	>1:99
6	(*Sp*)-**7b**	(*Sp*)-dC_BT	94	>1:99
7	(*Sp*)-**7c**	(*Sp*)-dA_BT	88	>1:99
8	(*Sp*)-**7d**	(*Sp*)-dG_BT	84	>1:99

[a] Data taken from Ref. 17.
[b] Subscript 'B' = boranophosphate diester linkage.
[c] Determined by RP-HPLC.

Table 3
Synthesis of *P*-stereodefined PB-ODNs

Entry	PB-ODN	Yield (%)
1	all-(*Rp*)-d($C_BA_BG_BT$) **8**	73[a]
2	all-(*Sp*)-d($C_BA_BG_BT$) **9**	54[a]
3	all-(*Rp*)-$(T_B)_{11}T$ **10**	13[b]
4	all-(*Sp*)-$(T_B)_{11}T$ **11**	19[b]

[a] Determined by RP-HPLC.
[b] Isolated yield.

derivatives L- or D-**6**, which were synthesized from L- or D-proline, respectively.[17] Only the *trans*-isomers were generated stereoselectively and isolated in good yields (Table 1).

Using these diastereopure monomers, we attempted to synthesize dinucleoside boranophosphates (*Rp*)- and (*Sp*)-**7a–d** on solid support by the method shown in Scheme 1. The monomers (*Sp*)- or (*Rp*)-**1a–d** were allowed to condense with the 5′-OH of thymidine attached to the support in the presence of CMPT **2** to afford the corresponding dinucleoside phosphite intermediates. The phosphite intermediates were then treated with 1% TFA in CH_2Cl_2–Et_3SiH (1:1, v/v) for the removal of the chiral auxiliary, base protecting groups and 5′-*O*-DMTr group to give the base-unprotected dinucleoside *H*-phosphonates. The resultant *H*-phosphonates were converted into the corresponding boranophosphates by using $BH_3{\cdot}SMe_2$ in DMF[17] or *N*,*N*-dimethylacetamide (DMAc) in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA). After being released from the solid support by treatment with saturated NH_3 in CH_3OH, the crude products were analyzed by reversed-phase HPLC (RP-HPLC). The analysis showed that diastereopure dinucleoside boranophosphates were obtained in modest to good yields without significant side reactions on the nucleobases (Table 2). It should be noted that the coupling reactions of the adenosine monomers (**1c**) were conducted using a lower concentration of CMPT (0.5 M) and a Lewis basic cosolvent 1-methyl-2-pyrrolidone to suppress the detritylation of the N^6-DMTr-adenine caused by the acidity of CMPT, whereas the other monomers (**1a**, **b**, **d**) were coupled using a higher concentration of CMPT (1.0 M) in CH_3CN. This should be the reason for the relatively low coupling yields for **1c** (entries 3, 7). The equally moderate yields for **1d** (entries 4, 8) may be due to the steric hindrance of the bulky N^2-DMTr group. Although the coupling yields still remain to be improved, it is noteworthy that the side reactions on silylated thymine as well as the other protected nucleobases during the boronation step were completely suppressed in either DMF or DMAc.

Next, we synthesized stereodefined PB-ODN 4mers containing a full set of nucleobases (Table 3, entries 1, 2) and dodecathymidylates [$(T_B)_{11}T$] (entries 3, 4) by this method. All-(*Rp*)- and all-(*Sp*)-$(T_B)_{11}T$ (**10**, **11**) were isolated by RP-HPLC for a thermal denaturation study. Configurational assignment was conducted by enzymatic digestion study using snake venom phosphodiesterase (svPDE) since it has been reported svPDE digests (*Sp*)-PB linkages stereospecifically over (*Rp*)-PB-linkages, though at a slower rate compared to that for natural phosphodiester linkages at dimer level.[9a,d] All-(*Sp*)-$(T_B)_{11}T$ (**11**) was digested completely as expected, whereas all-(*Rp*)-$(T_B)_{11}T$ (**10**) was also partially hydrolyzed.[22] Although it is still not clear whether the partial digestion was due to incomplete stereocontrol of the synthesis or imperfect stereospecificity of the enzyme,[23] it should be mentioned that the diastereoselectivity of the synthesis was >99% at least at the dimer level as shown in Table 2.

To evaluate the impact of the stereocontrol of PB-ODNs on their duplex formation, a thermal denaturating study was carried out with the duplexes of all-(*Rp*)-$(T_B)_{11}T$ (**10**), all-(*Sp*)-$(T_B)_{11}T$ (**11**), stereo-random $(T_B)_{11}T$ (**12**),[24] and natural dodecathymidylate [$(T_O)_{11}T$] (**13**) with the complementary dodecadeoxyadenylate d[$(A_O)_{11}A$] (**14**) and dodecadenylate r[$(A_O)_{11}A$] (**15**) at low and high ionic strength (Table 4). The data presented in Table 4 clearly show that the configuration of phosphorus atoms of PB-ODNs significantly affect the thermal stability of the duplexes. All-(*Sp*)-$(T_B)_{11}T$ (**11**) formed a duplex with d$(A_O)_{11}A$ (**14**) with T_m values of 11.9 and 19.6 °C at low and high ionic strength, respectively (entries 3, 7, left), though the duplex was much less stable than that of natural $(T_O)_{11}T$ (**13**) (entries 1, 5). In sharp contrast, no detectable T_m values were observed in the case of all-(*Rp*)-$(T_B)_{11}T$ (**10**) (entries 2, 6, left). Similarly, all-(*Sp*)-$(T_B)_{11}T$ (**11**) formed a duplex with r$(A_O)_{11}A$ (**15**) (entries 3, 7, right), whereas all-(*Rp*)-$(T_B)_{11}T$ (**10**) and stereo-random $(T_B)_{11}T$ (**12**) did not (entries 2, 4, 6, 8, right). The differences in T_m values between the duplexes all-(*Sp*)-$(T_B)_{11}T$–r$(A_O)_{11}A$

Table 4
Melting temperatures for duplexes formed with P-stereodefined or stereo-random PB-ODNs and natural DNA and RNA

Entry	ODN	d$(A_O)_{11}A$ **14**			r$(A_O)_{11}A$ **15**		
		T_m (°C)	ΔT_m[a] (°C)	ΔT_m/mod.[b] (°C)	T_m (°C)	ΔT_m[a] (°C)	ΔT_m/mod.[b] (°C)
0.1 M NaCl, NaH_2PO_4–Na_2HPO_4 buffer (pH 7.0)							
1	$(T_O)_{11}T$ **13**	34.8			30.4		
2	all-(*Rp*)-$(T_B)_{11}T$ **10**	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
3	all-(*Sp*)-$(T_B)_{11}T$ **11**	11.9	−22.9	−2.1	14.9	−15.5	−1.4
4	stereo-random $(T_B)_{11}T$ **12**	–[d]	–[d]	–[d]	–[c]	–[c]	–[c]
1 M NaCl, NaH_2PO_4–Na_2HPO_4 buffer (pH 7.0)							
5	$(T_O)_{11}T$ **13**	46.1			38.2		
6	all-(*Rp*)-$(T_B)_{11}T$ **10**	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]
7	all-(*Sp*)-$(T_B)_{11}T$ **11**	19.6	−26.5	−2.4	17.5	−20.7	−1.9
8	stereo-random $(T_B)_{11}T$ **12**	–[d]	–[d]	–[d]	–[c]	–[c]	–[c]

[a] Difference in T_m values between duplexes of PB-ODNs and natural counterparts.
[b] Difference in T_m values per boranophosphate modification.
[c] T_m value was not observed.
[d] Not determined.

(**11–15**) and $(T_O)_{11}T$–r$(A_O)_{11}A$ (**13–15**) were smaller (ΔT_m/mod. = −1.4 °C and −1.9 °C at low and high ionic strength, respectively) than those in the cases with $d(A_O)_{11}A$ (**14**) (ΔT_m/mod. = −2.1 °C and −2.4 °C at low and high ionic strength, respectively), being in consistent with the data we obtained with stereo-random PB-ODNs.[2]

Thus, the study showed that all-(*Sp*)-$(T_B)_{11}T$ (**11**) formed duplexes with complementary oligo(deoxy)adenylates (**14**, **15**), though with much lower T_m values than those obtained with the natural counterpart $(T_O)_{11}T$ (**13**). In contrast, all-(*Rp*)-$(T_B)_{11}T$ (**10**) did not form duplexes under the same conditions. The data indicate that the duplexes in which the BH_3 groups are oriented 'inward' are thermally more stable than those in which the BH_3 groups are oriented 'outward'. This stereo-dependence is generally similar to that of oligodeoxyribonucleoside phosphorothioates (PS-ODNs).[25] One of the reasons for the lower thermal stability obtained with the PB-ODNs is the sterically more demanding BH_3 groups compared to the oxygen atoms of natural ODNs.[1] However, a more intensive physicochemical study using stereodefined PB-ODNs containing four kinds of nucleobases would be necessary for full clarification of the stereo-dependence in the duplex formation of PB-ODNs because it has been reported that duplexes consisting of homothymidylates structurally differ in detail from those consisting of two oligomers of mixed base-sequence.[26]

In conclusion, we developed a method to synthesize stereodefined PB-ODNs at the oligomer level for the first time and clarified that the modifications of ODNs with (*Sp*)-boranophosphate linkages were better than those with (*Rp*)- and stereo-random counterparts in terms of the duplex formation. Although the duplexes of PB-ODNs used in this study were much less stable than those of natural ODNs, it should be improved by using PB-ODNs containing four kinds of nucleobases as we demonstrated previously with stereo-random PB-ODNs.[2] Further studies on the synthesis of duplexes formed with stereodefined PB-ODNs of mixed sequence and clarification of their properties are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.06.015.

References and notes

1. For a comprehensive review on nucleoside boranophosphates and their analogs, see: Li, P.; Sergueeva, Z. A.; Dobrikov, M.; Shaw, B. R. *Chem. Rev.* **2007**, *107*, 4746–4796.
2. Shimizu, M.; Saigo, K.; Wada, T. *J. Org. Chem.* **2006**, *71*, 4262–4269.
3. (a) Olesiak, M.; Krivenko, A.; Krishna, H.; Caruthers, M. H. *Phosphorus Sulfur Silicon* **2011**, *186*, 921–932; (b) Krishna, H.; Caruthers, M. H. *J. Am. Chem. Soc.* **2011**, *133*, 9844–9854.
4. (a) Hall, A. H. S.; Wan, J.; Shaughnessy, E. E.; Shaw, B. R.; Alexander, K. A. *Nucleic Acids Res.* **2004**, *32*, 5991–6000; (b) Hall, A. H. S.; Wan, J.; Spesock, A.; Sergueeva, Z.; Shaw, B. R.; Alexander, K. A. *Nucleic Acids Res.* **2006**, *34*, 2773–2781.
5. (a) Sergueeva, Z. A.; Sergueev, D. S.; Ribeiro, A. A.; Summers, J. S.; Shaw, B. R. *Helv. Chim. Acta* **2000**, *83*, 1377–1391; (b) Sergueeva, Z. A.; Sergueev, D. S.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 941–945.
6. (a) Zhang, J.; Terhorst, T.; Matteucci, M. D. *Tetrahedron Lett.* **1997**, *38*, 4957–4960; (b) Higson, A. P.; Sierzchala, A.; Brummel, H.; Zhao, Z.; Caruthers, M. H. *Tetrahedron Lett.* **1998**, *39*, 3899–3902.
7. For a review on *P*-chiral oligonucleotide analogs, see: Oka, N.; Wada, T. *Chem. Soc. Rev.* **2011**, *40*, 5829–5843.
8. Chen, Y.-Q.; Qu, F.-C.; Zhang, Y.-B. *Tetrahedron Lett.* **1995**, *36*, 745–748.
9. (a) Huang, F.; Sood, A.; Spielvogel, B. F.; Shaw, B. R. *J. Biol. Struct. Dyn.* **1993**, *1*, a078; (b) Li, H.; Huang, F.; Shaw, B. R. *Bioorg. Med. Chem.* **1997**, *5*, 787–795; (c) Wang, J. X.; Sergueev, D. S.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 951–955; (d) Johnson, C. N.; Spring, A. M.; Sergueev, D.; Shaw, B. R.; Germann, M. W. *Biochemistry* **2011**, *50*, 3903–3912.
10. Enya, Y.; Nagata, S.; Kitagawa, H.; Takagaki, K.; Oka, N.; Wada, T.; Ohgi, T.; Yano, J. *Bioorg. Med. Chem.* **2008**, *16*, 9154–9160.
11. Sergueev, D. S.; Sergueeva, Z. A.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 789–795.
12. Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710–12721.
13. McCuen, H. B.; Noé, M. S.; Sierzchala, A. B.; Higson, A. P.; Caruthers, M. H. *J. Am. Chem. Soc.* **2006**, *128*, 8138–8139.
14. (a) Wada, T.; Shimizu, M.; Oka, N.; Saigo, K. *Tetrahedron Lett.* **2002**, *43*, 4137–4140; (b) Shimizu, M.; Wada, T.; Oka, N.; Saigo, K. *J. Org. Chem.* **2004**, *69*, 5261–5268.
15. For the stereocontrolled synthesis of PB-ODN *2mers*, see: (a) Jin, Y.; Just, G. *Tetrahedron Lett.* **1998**, *39*, 6433–6436; (b) Wada, T.; Maizuru, Y.; Shimizu, M.; Oka, N.; Saigo, K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3111–3114.
16. Wang, X.; Dobrikov, M.; Sergueev, D.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1151–1153.
17. Iwamoto, N.; Oka, N.; Sato, T.; Wada, T. *Angew. Chem., Int. Ed.* **2009**, *48*, 496–499.
18. Sergueev, D. S.; Shaw, B. R. *J. Am. Chem. Soc.* **1998**, *120*, 9417–9427.
19. Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2003**, *125*, 8307–8317.
20. Oka, N.; Maizuru, Y.; Shimizu, M.; Wada, T. *Nucleosides Nucleotides Nucleic Acids* **2010**, *29*, 144–154.
21. Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2257–2260.
22. See Supplementary data.
23. Burgers, P. M. J.; Eckstein, F. *J. Biol. Chem.* **1979**, *254*, 6889–6893.
24. Stereo-random $(T_B)_{11}T$ **12** was synthesized by the boranophosphotriester method described in Ref. 2.
25. Guga, P.; Koziołkiewicz, M. *Chem. Biodivers.* **2011**, *8*, 1642–1681.
26. (a) Latimer, L. J. P.; Hampel, K.; Lee, J. S. *Nucleic Acids Res.* **1989**, *17*, 1549–1561; (b) Kibler-Herzog, L.; Kell, B.; Zon, G.; Shinozuka, K.; Mizan, S.; Wilson, W. D. *Nucleic Acids Res.* **1990**, *18*, 3545–3555; (c) Boczkowska, M.; Guga, P.; Stec, W. J. *Biochemistry* **2002**, *41*, 12483–12487.

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Synthesis of nucleoside 5′-boranophosphorothioate derivatives using an *H*-boranophosphonate monoester as a precursor

Natsuhisa Oka [a,*], Yuji Takayama [a], Kaori Ando [a], Takeshi Wada [b]

[a] Department of Chemistry, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[b] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

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ABSTRACT

We developed a method to convert a nucleoside 5′-*H*-boranophosphonate monoester into the corresponding nucleoside 5′-boranophosphorothioate monoester through temporary protection of the *H*-boranophosphonate monoester moiety as a diester with 9-fluorenylmethanol, subsequent sulfurization of the P–H group and removal of the 9-fluorenylmethyl group. Although the isolation of the resultant boranophosphorothioate monoester was found to be difficult due to instability of the compound, this new method proved to be useful to synthesize some conjugates of the nucleoside 5′-boranophosphorothioate with other biomolecules, such as cholesterol and an amino acid.

Chemically modified 5′-nucleotide analogs have been studied for years for their potential as antiviral and anticancer agents inhibiting the biosynthesis of disease-related nucleic acids in cells.[1] *P*-Boronated 5′-nucleotide analogs have been developed relatively recently, but have attracted much attention lately.[2] These analogs are highly lipophilic and stable to nucleases, which would favor their transport to the targets without degradation. Some of these analogs have been reported to show antineoplastic,[3] anti-inflammatory,[4] and hypolipidemic[5] activities in vitro and in vivo. Studies toward the development of antiviral *P*-boronated nucleotide analogs have also been reported.[6–8] Furthermore, such boronated nucleotide analogs are potentially useful as ^{10}B carriers for boron neutron capture therapy.[9]

However, only limited kinds of *P*-boronated 5′-nucleotide analogs are currently available due to the lack of synthetic methods. To solve this problem, we have recently developed nucleoside *H*-boranophosphonate derivatives.[10] These compounds are also categorized as *P*-boronated nucleotide analogs but distinctively different from the others in that they have a hydrogen atom on their phosphorus atom. Thus, the phosphorus atom can be further functionalized by deprotonation and subsequent reaction with electrophiles (Fig. 1). We expect that this property makes the *H*-boranophosphonate derivatives versatile precursors of various *P*-boronated nucleotide analogs. In addition, nucleoside 5′-*H*-boranophosphonate monoesters can be modified by condensation with other molecules, such as alcohols and amines (Fig. 1).

In this study, we focused on the synthesis of nucleoside 5′-boranophosphorothioate derivatives using a nucleoside 5′-*H*-boranophosphonate monoester as a precursor. The target molecules are one of the *P*-boronated 5′-nucleotide analogs in which two of the three non-bridging oxygen atoms of the phosphomonoester group are replaced with a borane group and a sulfur atom. These molecules are considered as potential therapeutic nucleotide analogs because such double modifications have been reported to enhance the lipophilicity and stability to enzymatic hydrolysis significantly.[11] However, there are only a few reports on their synthesis in the literature and their properties have not been studied in detail.[6a,11,12] We expected that the application of *H*-boranophosphonate derivatives as precursors would facilitate access to the boranophosphorothioate derivatives. Furthermore, sulfurization of the *H*-boranophosphonate monoester would be a good model of other P–H modifications with electrophiles, such as chalcogenization, alkylation, and acylation.

We have already demonstrated that a dinucleoside *H*-boranophosphonate could be converted into the corresponding dinucleoside boranophosphorothioate by treatment with S_8 and Et_3N.[10] However, in the case of an *H*-boranophosphonate monoester, the free P–OH group should be protected before sulfurization. In fact, when a thymidine *H*-boranophosphonate monoester derivative (Scheme 1, **1**), which was synthesized by condensation of 3′-*O*-benzoylthymidine with pyridinium *H*-boranophosphonate according to our previous procedure,[10] was subjected to the same

* Corresponding author. Tel./fax: +81 58 293 2564.
E-mail address: oka@gifu-u.ac.jp (N. Oka).

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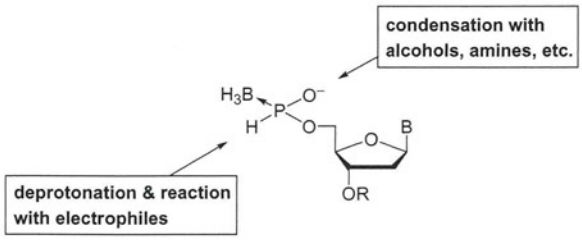
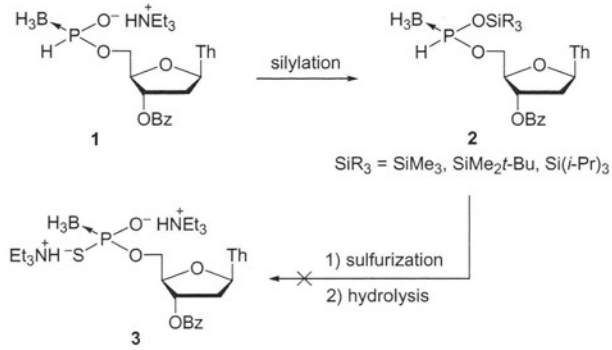
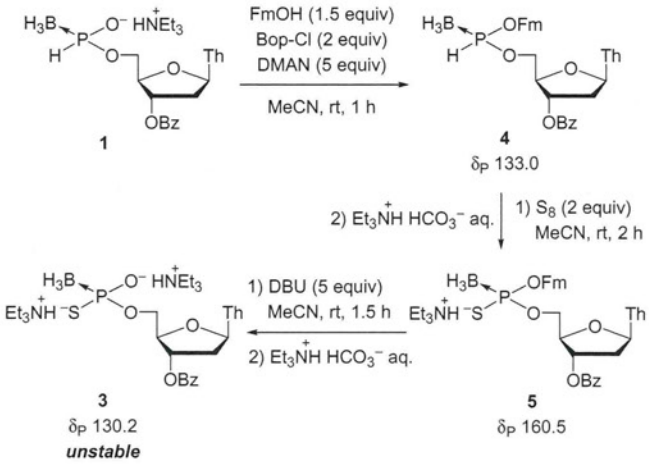


Figure 1. Approaches to derivatization of nucleoside 5′-*H*-boranophosphonate monoesters.



Scheme 1. Attempted synthesis of thymidine 5′-boranophosphorothioate monoester **3** via silyl esters **2**.

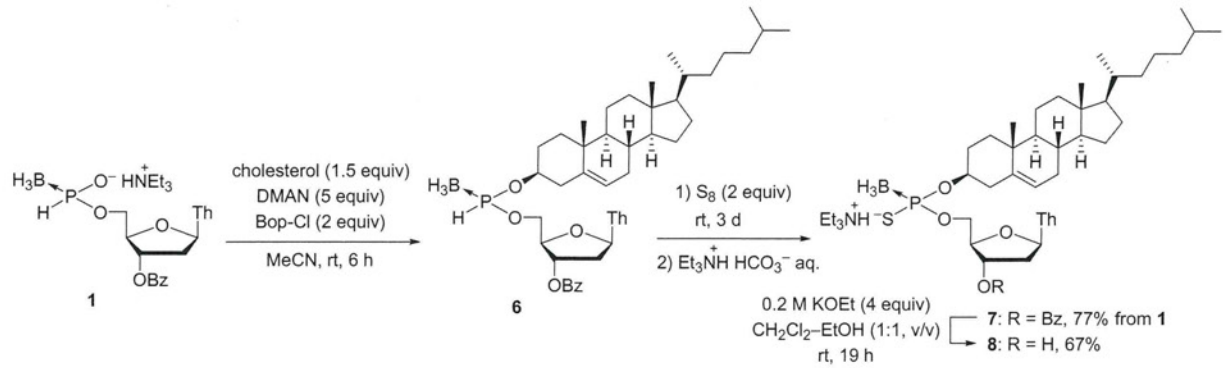


Scheme 2. Synthesis of thymidine 5′-boranophosphorothioate monoester **3** via 9-fluorenylmethyl esters **4** and **5**.

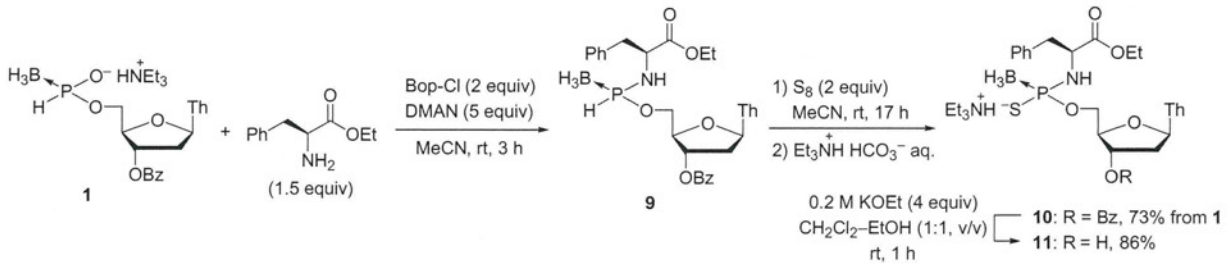
sulfurization conditions, multiple unidentified side products were generated. So, we decided to protect the *H*-boranophosphonate monoester group temporarily and employed the protection as silyl esters first (Scheme 1). Compound **1** was converted into silyl esters **2** by treatment with *N*,*O*-bis(trimethylsilyl)acetamide, TBDMSCl or *i*-Pr_3SiCl, and the resultant silyl esters **2** were treated with S_8 or Beaucage reagent[13] without isolation. The reactions were conducted in MeCN and THF in the presence of Et_3N or 1,8-bis(dimethylamino)naphthalene (DMAN). However, none of these reactions gave the desired boranophosphorothioate derivative **3** and most of the starting material **1** was recovered after aqueous work-up. The use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to accelerate the deprotonation of the P–H group resulted in the formation of a complex mixture.

These results can be attributed to the relatively low acidity of the P–H group of the silyl esters **2** which have a highly polarized $O^{\delta-}$–$Si^{\delta+}$ bond adjacent to the same phosphorus atom. The deprotonation of the P–H group should be easier if the compound **1** is protected as a diester with an alcohol because a C–O bond is generally much less-polarized than a Si–O bond.[14] In fact, it has been reported that the reaction of $(EtO)_2P(\rightarrow BH_3)H$ with aldehydes at the phosphorus atom was readily promoted by deprotonation with *i*-Pr_2NEt, whereas that of (*i*-Pr_3SiO)(EtO)$P(\rightarrow BH_3)H$ was not.[15] On the basis of this hypothesis, we employed the protection as a 9-fluorenylmethyl (Fm) ester because the protection could be easily carried out by condensation of the compound **1** with 9-fluorenylmethanol (FmOH) and it would also be easily deprotected by treatment with an amine under anhydrous conditions (Scheme 2). First, the compound **1** was allowed to condense with FmOH in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl) and Et_3N. However, the product **4** once formed was slowly converted back into the starting material **1** in the reaction mixture. It was because of the β-elimination of the compound **4** promoted by Et_3N. It was confirmed by a ^1H NMR analysis, which showed the formation of dibenzofulvene in the crude mixture. To prevent this side reaction, we replaced Et_3N with DMAN because the latter has been reported not to decompose 2-cyanoethyl-protected boranophosphate triesters by β-elimination.[16] The triethylammonium cation of **1** was also exchanged prior to the condensation by the addition of DMAN and subsequent repeated coevaporation with toluene. In this case, the desired product **4** was obtained without decomposition. The product **4** was then treated with S_8 without purification to synthesize the boranophosphorothioate diester derivative **5**. A ^{31}P NMR analysis showed that the sulfurization proceeded smoothly without observable side reactions. Finally, the compound **5** was treated with DBU to remove the Fm group. The deprotection itself proceeded without any side reactions. However, we found that the compound **3** was unstable and always partially decomposed during the purification by silica gel column chromatography. There is a report on the synthesis and isolation of a nucleoside 5′-boranophosphorothioate monoester in modest yield,[11a] but every attempt to isolate the product **3** (e.g., reversed-phase and ion-exchange chromatography, countercation exchange and precipitation) failed in our hands. It has also been reported that nucleoside 5′-boranophosphate monoesters are more sensitive to hydrolysis than the natural nucleoside 5′-phosphates, especially under acidic conditions.[17] Because 3′-*O*-Bz-thymidine was observed as a decomposition product, the partial decomposition of the compound **3** can also be attributed to the cleavage of the P–O bond by hydrolysis.

Thus, the study showed that the desired 5′-boranophosphorothioate monoester was difficult to isolate. However, it was also demonstrated that the nucleoside 5′-*H*-boranophosphonate monoester derivative could be smoothly converted into the boranophosphorothioate counterpart as planned. Given this result, we next explored the possibility of developing conjugates of the nucleoside 5′-boranophosphorothioate monoester with biomolecules in which the unstable monoester moiety would be trapped into a more stable form. Conjugates of therapeutic 5′-nucleotides with biomolecules, such as lipophilic sterols and amino acids, have been widely studied to improve the therapeutic effects of the parent 5′-nucleotides.[1] Such conjugates are much more lipophilic than the parent phosphate monoesters, which is advantageous for their delivery into cells. It has been reported that the conjugates are gradually hydrolyzed in cells to regenerate the 5′-nucleotides.[1] We expect that the strategy may also be useful for the delivery of relatively unstable 5′-nucleotide analogs to targets, such as the compound **3** shown above. New conjugates of *P*-boronated 5′-nucleotide analogs may also exert therapeutic activity by themselves. There have been a few reports on the synthesis of *P*-boronated 5′-nucleotide–amino acid conjugates in the literature



Scheme 3. Synthesis of thymidine 5′-boranophosphorothioate–cholesterol conjugate **8** via *H*-boranophosphonate intermediate **6**.



Scheme 4. Synthesis of thymidine 5′-boranophosphorothioate–phenylalanine ethyl ester conjugate **11** via *H*-boranophosphonate intermediate **9**.

aiming to develop antiviral agents.[6] Development of a novel method using an *H*-boranophosphonate derivative as precursor would expand the availability of such conjugates.

We chose cholesterol as the first model of lipophilic biomolecules to synthesize a new *P*-boronated 5′-nucleotide conjugate **8** as shown in Scheme 3. Compound **1** was condensed with cholesterol by using Bop-Cl and DMAN to give the thymidine 5′-*H*-boranophosphonate–cholesterol conjugate **6**. We used DMAN even though the compound **6** would not undergo a β-elimination as in the case of the Fm ester **4** because the use of Et_3N caused a partial deboronation of the product **6** probably due to the coordination of Et_3N to the BH_3. The deboronation was completely suppressed by replacing Et_3N with DMAN. Because the compound **6** was found to be relatively unstable on silica gel, we sulfurized it without purification. The sulfurization reaction was slow but proceeded without observable side reactions to afford the 3′-*O*-Bz-thymidine 5′-boranophosphorothioate–cholesterol conjugate **7**, and the subsequent deprotection gave the final product **8**. Contrary to the boranophosphorothioate monoester derivative **3**, the compounds **7** and **8** were stable and isolated in good yield.

Next, we applied this method to the synthesis of a conjugate with an amino acid. As shown in Scheme 4, the compound **1** was allowed to condense with L-phenylalanine ethyl ester to give the intermediate **9** having a P–H group. Compound **9** was then sulfurized and treated with potassium ethoxide to give the desired thymidine 5′-boranophosphorothioate–phenylalanine ethyl ester conjugate **11** in good yield. Compounds **10** and **11** were also found to be stable in contrast to the monoester **3**.

In conclusion, we developed a method to synthesize nucleoside 5′-boranophosphorothioate derivatives using a nucleoside 5′-*H*-boranophosphonate monoester as a precursor. Although the nucleoside 5′-boranophosphorothioate monoester could not be isolated due to the instability of the compound, we consider that the new strategy using temporary protection of the *H*-boranophosphonate monoester moiety as a Fm diester will be useful to synthesize diverse *P*-doubly modified *P*-boronated 5′-nucleotide analogs. Many studies reported in the literature on the P–H functionalization of secondary phosphine–boranes by various reactions, such as alkylation and transition metal-catalyzed arylation,[18] as well as the recent study on the alkylation of *H*-boranophosphonate esters, such as $(EtO)_2P(\rightarrow BH_3)H$ and $(i\text{-}Pr_3SiO)(EtO)P(\rightarrow BH_3)H$,[15] would be informative to explore this possibility. Evaluation of biological activity of the nucleoside 5′-boranophosphorothioate conjugates which were successfully synthesized in this study is also intriguing and will be presented elsewhere.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.093.

References and notes

1. (a) Mehellou, Y.; Balzarini, J.; McGuigan, C. *ChemMedChem* **2009**, *4*, 1779; (b) Bobeck, D. R.; Schinazi, R. F.; Coats, S. J. *Antivir. Ther.* **2010**, *15*, 935.
2. Li, P.; Sergueeva, Z. A.; Dobrikov, M.; Shaw, B. R. *Chem. Rev.* **2007**, *107*, 4746.
3. Hall, I. H.; Hall, E. S.; Chi, L. K.; Shaw, B. R.; Sood, A.; Spielvogel, B. F. *Anticancer Res.* **1992**, *12*, 1091.
4. Rajendran, K. G.; Burnham, B. S.; Chen, S. Y.; Sood, A.; Spielvogel, B. F.; Shaw, B. R.; Hall, I. H. *J. Pharm. Sci.* **1994**, *83*, 1391.
5. Hall, I. H.; Burnham, B. S.; Rajendran, K. G.; Chen, S. Y.; Sood, A.; Spielvogel, B. F.; Shaw, B. R. *Biomed. Pharmacother.* **1993**, *47*, 79.
6. (a) Li, P.; Shaw, B. R. *Org. Lett.* **2002**, *4*, 2009; (b) Baraniak, J.; Kaczmarek, R.; Wasilewska, E. *Tetrahedron Lett.* **2004**, *45*, 671; (c) Li, P.; Shaw, B. R. *J. Org. Chem.* **2005**, *70*, 2171; (d) Khan, S. I.; Shaw, B. R. *Nucleosides Nucleotides* **2007**, *26*, 621.

7. (a) Dobrikov, M. I.; Grady, K. M.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 275; (b) Dobrikov, M. I.; Sergueeva, Z. A.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1651; (c) Li, P.; Dobrikov, M.; Liu, H.; Shaw, B. R. *Org. Lett.* **2003**, *5*, 2401; (d) Wang, G.; Boyle, N.; Chen, F.; Rajappan, V.; Fagan, P.; Brooks, J. L.; Hurd, T.; Leeds, J. M.; Rajwanshi, V. K.; Jin, Y.; Prhavc, M.; Bruice, T. W.; Cook, P. D. *J. Med. Chem.* **2004**, *47*, 6902.
8. (a) Meyer, P.; Schneider, B.; Sarfati, S.; Deville-Bonne, D.; Guerreiro, C.; Boretto, J.; Janin, J.; Véron, M.; Canard, B. *EMBO J.* **2000**, *19*, 3520; (b) Selmi, B.; Boretto, J.; Sarfati, S. R.; Guerreiro, C.; Canard, B. *J. Biol. Chem.* **2001**, *276*, 48466; (c) Deval, J.; Selmi, B.; Boretto, J.; Egloff, M. P.; Guerreiro, C.; Sarfati, S.; Canard, B. *J. Biol. Chem.* **2002**, *277*, 42097; (d) Matamoros, T.; Deval, J.; Guerreiro, C.; Mulard, L.; Canard, B.; Menéndez-Arias, L. *J. Mol. Biol.* **2005**, *349*, 451; (e) Deval, J.; Alvarez, K.; Selmi, B.; Bermond, M.; Boretto, J.; Guerreiro, C.; Mulard, L.; Canard, B. *J. Biol. Chem.* **2005**, *280*, 3838; (f) Barral, K.; Priet, S.; Sire, J.; Neyts, J.; Balzarini, J.; Canard, B.; Alvarez, K. *J. Med. Chem.* **2006**, *49*, 7799.
9. Barth, R. F.; Coderre, J. A.; Vicente, M. G. H.; Blue, T. E. *Clin. Cancer Res.* **2005**, *11*, 3987.
10. Higashida, R.; Oka, N.; Kawanaka, T.; Wada, T. *Chem. Commun.* **2009**, 2466.
11. (a) Lin, J.; Shaw, B. R. *Nucleosides Nucleotides* **2001**, *20*, 587; (b) Li, P.; Shaw, B. R. *Chem. Commun.* **2002**, 2890.
12. Syntheses of dinucleoside boranophosphorothioates (ref. 11a; Lin, J.; Shaw, B. R. *Chem. Commun.* **1999**, 1517), nucleoside 3′,5′-cyclic boranophosphorothioates (ref. 11b) and nucleoside α-*P*-borano, α-*P*-thiotriphosphates (Lin, J.; Shaw, B. R. *Chem. Commun.* **2000**, 2115; Lin, J.; Porter, K. W.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1019) have also been reported.
13. Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253.
14. Brook, M. A. In *Silicon in Organic, Organometallic, and Polymer Chemistry*; John Wiley & Sons, Ltd: New York, 2000; Chapter 2.
15. Belabassi, Y.; Antczak, M. I.; Tellez, J.; Montchamp, J.-L. *Tetrahedron* **2008**, *64*, 9181.
16. Shimizu, M.; Saigo, K.; Wada, T. *J. Org. Chem.* **2006**, *71*, 4262.
17. (a) Tomasz, J.; Shaw, B. R.; Porter, K.; Spielvogel, B. F.; Sood, A. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1373; (b) Li, H.; Hardin, C.; Shaw, B. R. *J. Am. Chem. Soc.* **1996**, *118*, 6606.
18. (a) Carboni, B.; Monnier, L. *Tetrahedron* **1999**, *55*, 1197; (b) Staubitz, A.; Robertson, A. P. M.; Sloan, M. E.; Manners, I. *Chem. Rev.* **2010**, *110*, 4023.

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Synthesis of Novel Oligocationic Peptides Which Bind to A-Type Nucleic Acid Duplexes

Yusuke Maeda, Rintaro Iwata, and Takeshi Wada

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan
e-mail: kk107354@mgs.k.u-tokyo.ac.jp

Several oligocationic peptides have been synthesized and the effects of peptides on the thermal stability of nucleic acid duplexes were investigated. Certain stabilization effects on A-type RNA-RNA duplexes were observed, whereas B-type DNA-DNA duplexes were not stabilized appreciably. These results suggested that the cationic peptides could selectively recognize the A-type RNA-RNA duplexes.

Keywords: nucleic acid duplex, oligocationic peptide, thermal stability

Introduction

Recently, nucleic acid drugs, especially RNA interference drugs (RNAi drugs), have been focused as new therapeutic agents. However, the current RNAi drugs are not sufficiently efficient due to their low membrane permeability and instability of siRNA in cells. Therefore, construction of a new RNAi drug delivery system (DDS) is highly demanded for practical applications. To increase the stability of siRNAs, a number of chemical modifications have been proposed. Another strategy to stabilize siRNAs is the use of molecules that can noncovalently bind to RNA molecules and protect them from nucleases. RNAi drugs are composed of double stranded RNAs existing A-type duplexes. In the previous study directing the development of DDS, we designed α-(1→4)-linked-2,6-diamino-2,6-dideoxy-D-glucopyranose oligomers targeting the phosphate groups in the major groove of A-type duplexes, which were different from B-type DNA-DNA duplexes [1]. The oligodiaminosaccharide could stabilize the RNA duplexes and showed the binding selectivity to RNA-RNA duplexes. However, the synthesis of various types of oligodiaminosaccharides requires multi steps and generally difficult. Thus we focus on cationic peptides as RNA binding molecules. Synthesis of peptides is easier than that of oligosaccharide and conjugation with other molecules such as signal peptides can be done in the same way of synthesizing the oligocationic peptides. We herein designed and synthesized various types of peptides, and compared the thermal stability of peptide-nucleic acids complexes. For example, peptides with various side chain lengths were designed to change the distance of amino groups, and peptides including aminoproline were designed to change the conformation of the peptide backbones. In this study, the correlation of the peptide structure and the thermal stability of peptide-RNA complexes were investigated.

Table 1. Melting temperatures of r(CGCGAAUUCGCG)$_2$ and d(CGCGAATTCGCG)$_2$

	RNA		DNA	
Peptide	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)
None	60.9		48.2	
Dap_8	62.0	1.1	50.8	2.6
Dab_8	68.3	7.4	49.5	1.3
Orn_8	67.0	6.1	51.3	3.1
Lys_8	65.4	4.5	49.7	1.5
Dab_7Amp_1	63.1	2.2	51.2	3.0
Dab_6Amp_2	65.4	4.5	50.6	2.4

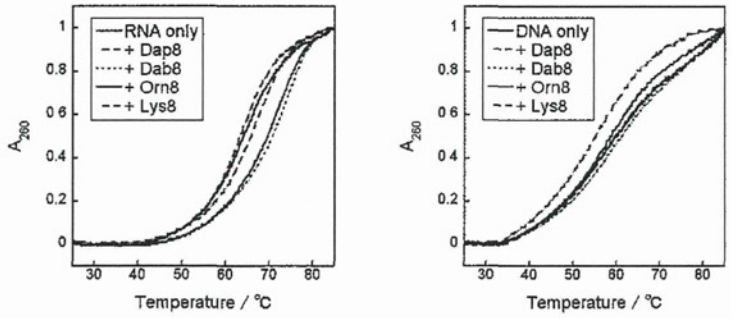


Fig.1. UV melting curve of r(CGCGAAUUCGCG)$_2$ and d(CGCGAATTCGCG)$_2$.

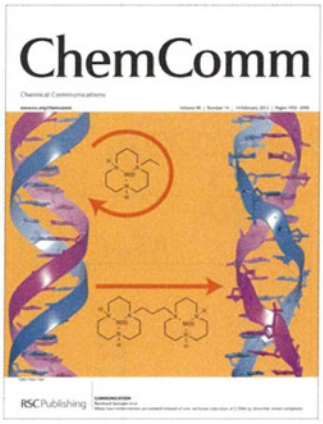
Results and Discussion

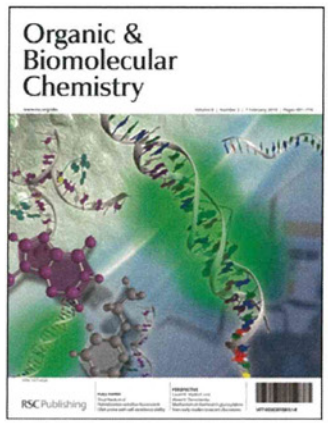
First, we compared the binding ability of oligocationic peptides, which have different side chain length, to the nucleic acid duplexes. These peptides are variously composed of lysine (Lys), ornithin (Orn), diaminobutyric acid (Dab), and diaminopropionic acid (Dap). To analyze the interaction between peptides and nucleic acid duplexes, CD spectrometry and UV melting experiments were performed. As the results of UV melting experiments, the melting temperatures (T_m) of peptide-RNA complexes were different according to the side chain length (Table 1, Fig. 1). Furthermore, tendency of thermal stability of peptide-RNA complexes were different from those of the peptide-DNA complexes. These differences suggested that the distance between the amino groups could generate the A-type RNA-RNA duplex binding selectivity.

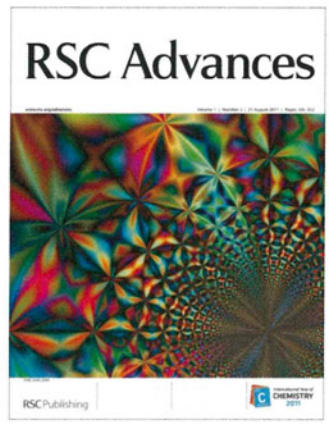
Next, oligocationic peptides with other backbone were designed to improve their RNA binding ability. Comparing with saccharide derivatives, peptides were more flexible and an introduction of aminoproline into the peptide chain would be effective to give a conformational rigidity. However, the T_m values were decreased by introducing of aminoproline (Table 1). The results suggested that the interaction of peptides and nucleic acids were disturbed by the aminoproline residue. These results also suggested that the amino group of peptides could recognize the phosphate groups in the major groove of RNA duplexes.

Reference

1. Iwata, R., Sudo, M., Nagafuji, K., and Wada, T. (2011) *J. Org. Chem.*, **76**, 5895-5906.







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