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Enhancement of affinity of 2′-*O*-Me-oligonucleotides for complementary RNA by incorporating a stereoregulated boranophosphate backbone

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2′-*O*-Me-oligoribonucleotides bearing a stereoregulated boranophosphate backbone (2′-*O*-Me-PB-ORNs) were synthesized by using 2′-*O*-Me-ribonucleoside 3′-*O*-oxazaphospholidine monomers. A thermal denaturation study with the resultant diastereopure 2′-*O*-Me-PB-ORNs revealed that all-(*Sp*)-boranophosphate backbone had a large stabilizing effect on the duplex with complementary RNA.

Chemically modified oligonucleotides have lately gained significant attention as novel therapeutics to silence target mRNAs sequence-specifically.[1] Among many types of modifications developed so far, the combination of 2′-*O*-alkylribose (e.g., Me, 2-methoxyethyl) and phosphorothioate (PS) backbone has been used most commonly for therapeutic oligonucleotides under clinical trials and those approved for marketing because it provides high affinity for complementary RNA, nuclease resistance, and favorable pharmacokinetic properties.[2] However, the PS backbone has some drawbacks, i.e., it lowers the affinity of the oligonucleotides to complementary RNA which is enhanced by the 2′-*O*-alkyl modification and also causes non-specific bindings to proteins and off-target effects.[2,3]

To develop a novel type of therapeutic oligonucleotides that would overcome these limitations, we focused on boranophosphate (PB), another type of backbone modification in which a non-bridging oxygen of each phosphodiester internucleotidic linkage is substituted by a BH_3 group.[4] PB-oligonucleotides have recently attracted much attention as promising therapeutic oligonucleotides owing to their high potency for gene suppression,[5] higher stability to nucleases than PS,[6] RNase H activity,[7] and high lipophilicity, which would be favorable for efficient cellular uptake.[5c] Lower toxicity than PS-modified oligonucleotides is also expected.[5a,c,8] Furthermore, they have been expected as target-specific ^{10}B carriers for boron neutron capture therapy (BNCT).[4b,c]

However, it is also known that the PB-modification has a much larger destabilizing effect on the duplex formation with complementary RNA than that of the PS counterpart, which may have been discouraging its use in therapeutic oligonucleotides.[6a,9] In this Communication, we describe that use of the PB-backbone can be advantageous even in terms of duplex formation when PB is properly stereoregulated. Thus, 2′-*O*-Me-PB-ORN bearing an all-(*Sp*)-PB-backbone (Figure 1, **1**) was found to form a much more stable duplex with the complementary RNA than that of 2′-*O*-Me-ORN bearing an unmodified phosphate backbone, while the all-(*Rp*)-PB-backbone (**2**) showed a large destabilizing effect on the duplex formation, indicating that the reported destabilization of duplexes by non-stereoregulated PB-backbones is attributed to (*Rp*)-PB-linkages. Stereocontrol of *P*-chiral therapeutic oligonucleotides has been a challenge over the past years,[10] and there is no efficient method for the stereocontrolled synthesis of PB-oligonucleotides except for enzymatic syntheses which can provide only all-(*Sp*)-oligo(ribo)nucleotides and cannot generally applied to the synthesis of sugar-modified oligonucleotides.[5a,7b,11] In this study, we synthesized 2′-*O*-Me-PB-ORNs in a stereocontrolled manner by the oxazaphospholidine method that we have developed for the synthesis of various *P*-modified oligonucleotides.[9f,12] We herein describe preliminary results on the stereocontrolled synthesis of 2′-*O*-Me-PB-ORNs and analysis of their duplex formation.

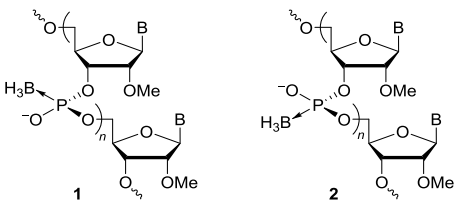
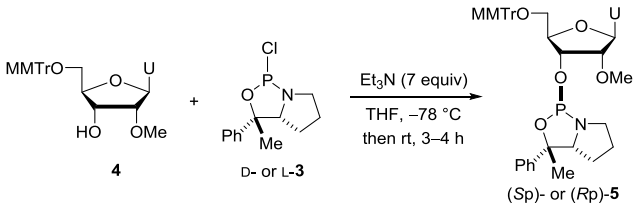


Figure 1 All-(*Sp*)- and all-(*Rp*)-2′-*O*-Me-PB-ORNs **1** and **2**.

First, we synthesized the monomers (*Sp*)- and (*Rp*)-**5** for the synthesis of (*Sp*)- and (*Rp*)-PB-linkages, respectively (Table 1). 5′-*O*-MMTr-2′-*O*-Me-uridine **4** was allowed to react with proline-derived 2-chlorooxazaphospholidines D- and L-**3**[12d] to afford the *trans*-isomers with diastereoselectivity ranging from 97:3 to 99:1, which were isolated in 51–59% yields by column chromatography on 3-aminopropyl-functionalized silica gel. The *trans* to *cis* ratios were determined by NMR according to our previous reports.[12d]

Table 1 Synthesis of oxazaphospholidine monomers.



entry	product[a]	yield (%)	*trans*:*cis*[b]
1	(*Sp*)-**5**	59	99:1 (159.0, 151.4)
2	(*Rp*)-**5**	51	97:3 (159.4, 152.3)

[a] R = 5′-*O*-(4-monomethoxytrityl)-2′-*O*-methyl-uridin-3′-yl [b] The chemical shifts of ^{31}P NMR are given in parentheses.

Using these monomers, we investigated the solid-phase synthesis of 2′-*O*-Me-PB-ORNs. The synthetic cycle is shown in Scheme 1. The monomers (*Sp*)- and (*Rp*)-**5** were condensed with the 5′-OH of 2′-*O*-Me-uridine anchored to a highly cross-linked polystyrene (HCP) support via a succinate linker in the presence of *N*-(cyanomethyl)dimethylammonium triflate (CMMT) **6**[12b] as an activator. MMTr-protected monomers were employed because 4,4′-dimethoxytrityl group was not tolerated in the presence of CMMT.[12b] The resultant phosphite intermediates **7** were then treated with 1% trifluoroacetic acid in CH_2Cl_2–Et_3SiH[12d] for the removal of 5′-*O*-MMTr group and stereospecific conversion to the *H*-phosphonate oligoribonucleotides (2′-*O*-Me-PH-ORNs) **8**. In this process, the chiral auxiliary was removed in the form of a carbocation, which was then reduced by Et_3SiH.[9e,12d,13] Further elongation of **8** was carried out by repeating this cycle. Finally, 2′-*O*-Me-PH-ORNs **8** were converted into 2′-*O*-Me-PB-ORNs by silylation and boronation with *N*,*O*-bis-(trimethylsilyl)acetamide (BSA) and BH_3·SMe_2 in DMAc,[9f] and the linker was cleaved by NH_3 to afford the desired 2′-*O*-Me-PB-ORNs.

First, we synthesized (*Rp*)- and (*Sp*)-2′-*O*-Me-PB-ORN 2mers (**9**, **10**) with this method. Reversed-phase HPLC (RP-HPLC) analysis of the crude **9** and **10** showed that they were synthesized with good yields (89–93%) and diastereoselectivities (96:4 to >99:1), indicating that the whole synthesis and deprotection process proceeded without compromising the diastereopurity of the monomers (Table 2).[14]

Encouraged by these results, we synthesized longer oligomers. All-(*Rp*)- and all-(*Sp*)-2′-*O*-Me-PB-ORN 4mers (**11**, **12**) were synthesized without much trouble as observed by RP-HPLC profiles of the crude mixtures[14] (Table 3, entries 1, 2). It should be noted that 2′-*O*-Me-PH-ORNs **8** can be used as precursors of other *P*-modified 2′-*O*-Me-ORNs as we previously reported with the oligodeoxyribonucleotide (ODN) counterparts.[12d] Thus, 2′-*O*-Me-ORN 4mers bearing PS-backbones (**13**, **14**) and those bearing *N*-[(2-dimethylamino)ethyl]phosphoramidate backbones (**15**, **16**) were synthesized from the common precursors **8** (entries 3–6). Furthermore, we applied this method to the synthesis of 2′-*O*-Me-PB-ORN 10mers (**17**, **18**). Although the efficiency of the synthesis and isolation process remain to be optimized and the isolated yields were low, the desired 10mers **17** and **18** with fully stereoregulated PB-backbones were obtained (Table 3, entries 7–8).

Finally, hybridization affinity of the 2′-*O*-Me-PB-ORN 10mers **17** and **18** for complementary RNA was studied by UV-melting experiments with 2′-*O*-Me-U_{10} **19** and unmodified U_{10} **20** as standards.

Scheme 1 Solid-phase synthesis of stereoregulated 2′-*O*-Me-PB-ORNs.

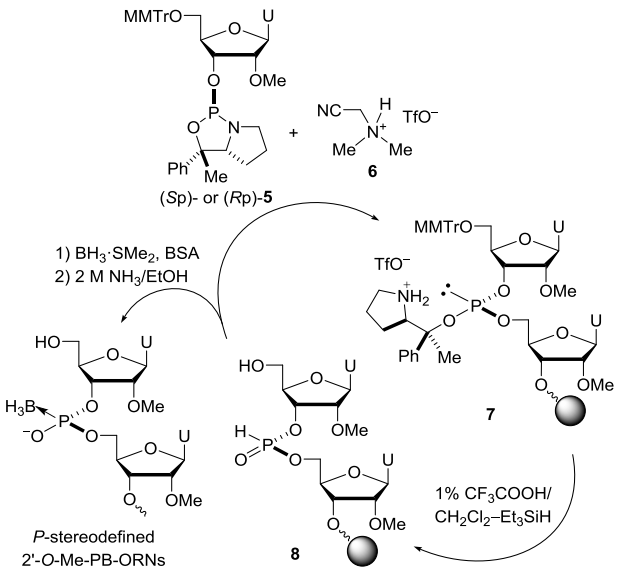


Table 2 Synthesis of (*Rp*)- and (*Sp*)-2′-*O*-Me-PB-ORN 2mers **9** and **10**.

entry	monomer	product[a]		yield (%)[b]	*Rp*:*Sp*[b]
1	(*Sp*)-**5**	(*Rp*)-U*$_B$U*	**9**	93	>99:1
2	(*Rp*)-**5**	(*Sp*)-U*$_B$U*	**10**	89	4:96

[a] Subscript B = boranophosphate diester. U* = 2′-*O*-Me-uridine. [b] Determined by RP-HPLC.

Table 3 Synthesis of stereoregulated 2′-*O*-Me-PB-, PS-, and PN-ORN 4–10mers **11**–**18**.

entry	product[a]		yield (%)
1	all-(*Rp*)-(U*$_B$)$_3$U*	**11**	75[b]
2	all-(*Sp*)-(U*$_B$)$_3$U*	**12**	62[b]
3	all-(*Rp*)-(U*$_S$)$_3$U*	**13**	82[b]
4	all-(*Sp*)-(U*$_S$)$_3$U*	**14**	85[b]
5	all-(*Rp*)-(U*$_N$)$_3$U*	**15**	62[b]
6	all-(*Sp*)-(U*$_N$)$_3$U*	**16**	67[b]
7	all-(*Rp*)-(U*$_B$)$_9$U*	**17**	5[c]
8	all-(*Sp*)-(U*$_B$)$_9$U*	**18**	2[c]

[a] Subscript S = phosphorothioate diester; N = *N*-[(2-dimethylamino)ethyl]-phosphoramidate. [b] Determined by RP-HPLC. [c] Isolated yield.

As shown in Table 4, the T_m values of the duplex of all-(*Sp*)-2′-*O*-Me-PB-U_{10} **18** with rA_{10} **21** were 33.7 °C and 40.0 °C at low and high ionic strength, respectively, which were higher than those of both unmodified

Figure 2 UV melting curves of the duplexes of stereoregulated 2′-*O*-Me-PB-U_{10}, 2′-*O*-Me-U_{10}, and unmodified U_{10} with complementary RNA.

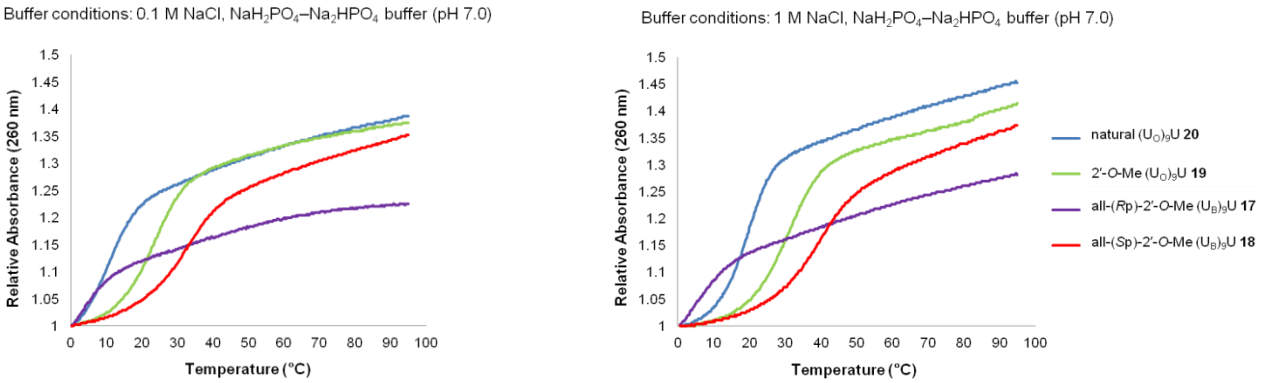


Table 4 T_m values of the duplexes of stereoregulated 2′-*O*-Me-PB-U_{10}, 2′-*O*-Me-PO-U_{10}, and unmodified U_{10} with complementary RNA.

entry	ORN	0.1 M NaCl, NaH_2PO_4–Na_2HPO_4 buffer (pH 7.0)			1 M NaCl, NaH_2PO_4–Na_2HPO_4 buffer (pH 7.0)		
		T_m (°C)	ΔT_m (°C)[a]	ΔT_m/PB (°C)[a]	T_m (°C)	ΔT_m (°C)[a]	ΔT_m/PB (°C)[a]
1	$(U_O)_9U$ **20**	10.4			21.2		
2	$(U^*_O)_9U^*$ **19**	24.9	+14.5		31.6	+10.4	
3	all-(*R*p)-$(U^*_B)_9U^*$ **17**	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]
4	all-(*S*p)-$(U^*_B)_9U^*$ **18**	33.7	+23.3 (+8.8)	+2.6 (+1.0)	40.0	+18.8 (+8.4)	+2.1 (+0.9)

[a] Differences in T_m and those per PB-modification relative to those of unmodified U_{10} **20** (or 2′-*O*-Me-U_{10} **19** in parentheses). [b] T_m was not observed.

U_{10} **20** (10.4 °C and 21.2 °C) and 2′-*O*-Me-U_{10} **19** (24.9 °C and 31.6 °C). Thus, the increase in the T_m per (*S*p)-PB-modification was estimated to be +0.9–1.1 °C. We have previously studied the effects of stereoregulated PS-backbones using PS-ORNs and evaluated the increase in T_m per (*R*p)-PS-modification to be +0.3–0.4 °C.[12e,f] Therefore, the stabilization effect of a stereoregulated PB-linkage was estimated to be more than two-fold larger than that of a PS-linkage, though a study using stereoregulated 2′-*O*-Me-PS-ORNs is necessary for more precise comparison. In contrast to the stabilizing effect of (*S*p)-PB-modification, the all-(*R*p)-PB-backbone largely destabilized the duplex. Thus, for the duplex of all-(*R*p)-2′-*O*-Me-PB-U_{10} **17** and rA_{10} **21**, T_m was not clearly observed but at much lower temperature than those of the standards **19** and **20** (entry 3).

In conclusion, the stereocontrolled synthesis of 2′-*O*-Me-PB-ORNs by the oxazaphospholidine method and a thermal denaturation study using the resultant 2′-*O*-Me-PB-ORNs revealed that the all-(*S*p)-PB-backbone had a larger stabilizing effect on duplex formation with complementary RNA than that of the PS counterpart. For further study on hybridization of 2′-*O*-Me-PB-ORNs, the synthesis of stereoregulated 2′-*O*-Me-PB-ORNs with mixed base sequences is in progress.

Notes and references

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† Electronic Supplementary Information (ESI) available: experimental details, HPLC profiles, and NMR spectra. See DOI: 10.1039/b000000x/

1 R. Kole, A. R. Kraine and S. Altman, *Nat. Rev. Drug Discov.*, 2012, **11**, 125.
2 M. Dirin and J. Winkler, *Expert Opin. Biol. Ther.*, 2013, **13**, 875.
3 S. M. Freier, and K.-H. Altmann, *Nucleic Acids Res.*, 1997, **25**, 4429.
4 (a) A. Sood, B. R. Shaw and B. F. Spielvogel, *J. Am. Chem. Soc.*, 1990, **112**, 9000. (b) P. Li, Z. A. Sergueeva, M. Dobrikov and B. R. Shaw, *Chem. Rev.* 2007, **107**, 4746. (c) A. R. Martin, J.-J. Vasseur and M. Smietana, *Chem. Soc. Rev.*, 2013, **42**, 5684.
5 (a) A. H. S. Hall, J. Wan, E. E. Shaughnessy, B. R. Shaw and K. A. Alexander, *Nucleic Acids Res.*, 2004, **32**, 5991. (b) A. H. S. Hall, J. Wan, A. Spesock, Z. Sergueeva, B. R. Shaw and K. A. Alexander, *Nucleic Acids Res.*, 2006, **34**, 2773. (c) M. Olesiak, A. Krivenko, H. Krishna and M. H. Caruthers, *Phosphorus Sulfur Silicon*, 2011, **186**, 921.
6 (a) D. S. Sergueev and B. R. Shaw, *J. Am. Chem. Soc.*, 1998, **120**, 9417. (b) Y. Enya, S. Nagata, H. Kitagawa, K. Takagaki, N. Oka, T. Wada, T. Ohgi and J. Yano, *Bioorg. Med. Chem.*, 2008, **16**, 9154.
7 (*a*) V. K. Rait and B. R. Shaw, *Antisense Nucleic Acid Drug Dev.* 1999, **9**, 53; (*b*) X. Wang, M. Dobrikov, D. Sergueev and B. R. Shaw, *Nucleosides Nucleotides Nucleic Acids*, 2003, **22**, 1151.
8 I. H. Hall, B. S. Burnham, K. G. Rajendran, S. Y. Chen, A. Sood, B. F. Spielvogel and B. R. Shaw, *Biomed Phar.*, 1993, **47**, 79.
9 (a) J. Zhang, T. Terhorst and M. D. Matteucci, *Tetrahedron Lett.*, 1997, **38**, 4957. (b) A. P. Higson, A. Sierzchala, H. Brummel, Z. Zhao and M. H. Caruthers, *Tetrahedron Lett.*, 1998, **39**, 3899. (c) H. A. Brummel and M. H. Caruthers, *Tetrahedron Lett.*, 2002, **43**, 749. (d) J. X. Wang, D. S. Sergueev and B. R. Shaw, *Nucleosides Nucleotides Nucleic Acids*, 2005, **24**, 951. (e) M. Shimizu, K. Saigo and T. Wada, *J. Org. Chem.*, 2006, **71**, 4262. (f) N. Iwamoto, N. Oka and T. Wada, *Tetrahedron Lett.*, 2012, **53**, 4361. (g) S. Uehara, S. Hiura, R. Higashida, N. Oka and T. Wada, *J. Org. Chem.*, 2014, **79**, 3465.
10 N. Oka and T. Wada, *Chem. Soc. Rev.*, 2011, **40**, 5829.
11 D. Sergueev, A. Hasan, M. Ramaswamy and B. R. Shaw, *Nucleosides Nucleotides*, 1997, **16**, 1533.

12 (a) N. Oka, T. Wada and K. Saigo, *J. Am. Chem. Soc.*, 2002, **124**, 4963. (b) N. Oka, T. Wada and K. Saigo, *J. Am. Chem. Soc.*, 2003, **125**, 8307. (c) N. Oka, M. Yamamoto, T. Sato and T. Wada, *J. Am. Chem. Soc.*, 2008, **130**, 16031. (d) N. Iwamoto, N. Oka, T. Sato and T. Wada, *Angew. Chem. Int. Ed.*, 2009, **48**, 496. (e) N. Oka, T. Kondo, S. Fujiwara, Y. Maizuru and T. Wada, *Org. Lett.*, 2009, **11**, 967. (f) Y. Nukaga, K. Yamada, T. Ogata, N. Oka and T. Wada, *J. Org. Chem.*, 2012, **77**, 7913.
13 Z. A. Sergueeva, D. S. Sergueev and B. R. Shaw, *Nucleosides Nucleotides Nucleic Acids*, 2001, **20**, 941.
14 See the Supplementary Information.

