Tetrahedron Letters 53 (2012) 4361–4364

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

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

ARTICLE INFO

Article history:
Received 25 April 2012
Revised 1 June 2012
Accepted 6 June 2012
Available online 13 June 2012

Keywords:
Stereocontrolled synthesis
Boranophosphate
Oxazaphospholidine approach
H-Phosphonate

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]

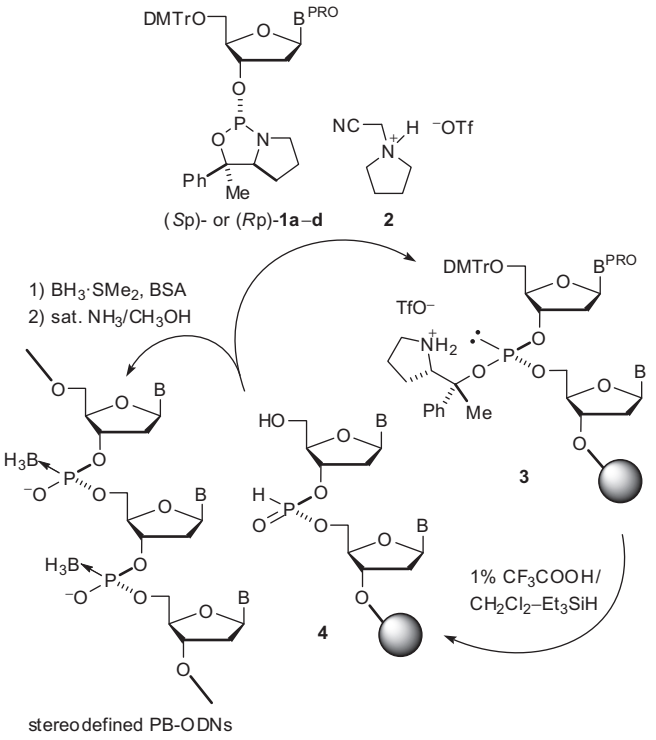
The first problem has been successfully overcome by several methods developed recently: an *H*-phosphonate method[11] using base-unprotected nucleoside 3′-*H*-phosphonate monomers,[12] a phosphoramidite method using base protecting groups unreactive to the boronating agents (e.g., 4,4′,4″-trimethoxytrityl (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

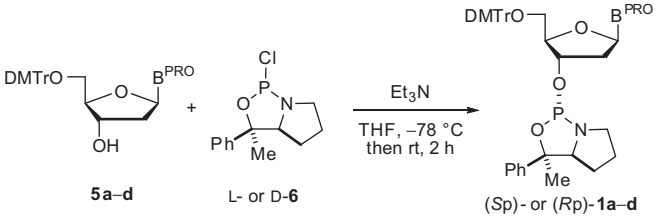
* Corresponding author. Tel./fax: +81 4 7136 3612.
E-mail address: wada@k.u-tokyo.ac.jp (T. Wada).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.06.015



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

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



Entry	1	B[PROb]	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.

Acknowledgments

We thank Professor Kazuhiko Saigo (Kochi University of Technology) for helpful suggestions. We also thank Dr. Mamoru Shimizu for helpful discussions and the synthesis of stereo-random PB-ODNs. This research was financially supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Japan.

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.