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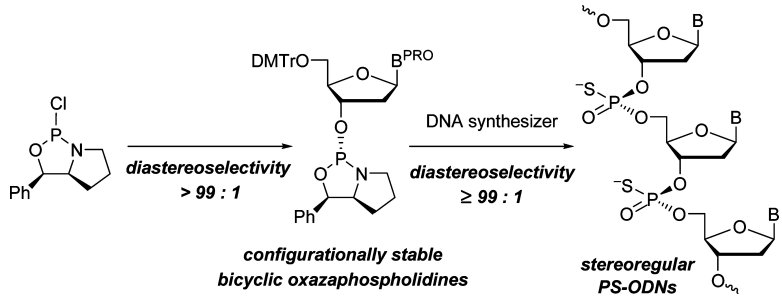

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Solid-Phase Synthesis of Stereoregular Oligodeoxyribonucleoside Phosphorothioates Using Bicyclic Oxazaphospholidine Derivatives as Monomer Units

Natsuhisa Oka, Mika Yamamoto, Terutoshi Sato, 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

Received July 24, 2008; E-mail: wada@k.u-tokyo.ac.jp

Abstract: Nucleoside 3′-*O*-bicylic oxazaphospholidine derivatives were designed as monomer units for a solid-phase synthesis of stereoregular oligodeoxyribonucleoside phosphorothioates (PS-ODNs). The *trans*-isomers of appropriately designed nucleoside 3′-*O*-bicyclic oxazaphospholidine derivatives were generated exclusively by the reaction between the 3′-OH of the corresponding protected nucleosides and 2-chloro-1,3,2-oxazaphospholidine derivatives. The resultant *trans*-oxazaphospholidine isomers were configurationally stable, and their diastereopurity was not impaired by epimerization in the presence of an acidic activator during the condensation on a solid support. As a result, the formation of both (*R*p)- and (*S*p)-phosphorothioate internucleotide linkages by using the oxazaphospholidine monomers and the acidic activator proceeded without any loss of diastereopurity (diastereoselectivity ≥99:1). In addition, *ab initio* molecular orbital calculations showed that the epimerization of oxazaphospholidine derivatives was most likely to proceed via an edge inversion process that was accelerated by *N*-protonation. The calculations rationalized not only the relations between the ring structure and the configurational stability of the oxazaphospholidines observed in this study but also the observations reported in the literature that the inversion of tricoordinated organophosphorus compounds were accelerated by acids or nucleophiles.

Introduction

Modification of oligodeoxyribonucleotides (ODNs) with phosphorothioate diester linkages (PS-linkages), in which one of the nonbridging oxygen atoms of natural phosphate diester linkages (PO-linkages) is replaced with a sulfur atom, has been widely used to create functional ODNs, such as probes for enzymatic reactions and nucleic acid drugs with improved nuclease stability and membrane permeability.[1,2] One of the most important features of the PS-linkage is its chirality.[2,3] Since the targets of the above-mentioned functional ODNs are chiral in most cases (e.g., enzymes, DNA, RNA, etc.), stereodefined PS-linkages are indispensable to create sophisticated functional ODNs.

In addition, the existence of PS-linkages in some bacterial genomic DNA has been revealed very recently.[4] It has been known that the genomic DNA from *Streptomyces lividans* DNA degradation phenotype undergoes Tris-dependent degradation during electrophoresis,[5] and a series of works confirmed that the phenomenon was attributed to an (*R*p)-PS-linkage at a specific site of the DNA.[4,6] It is expected that synthetic ODNs having stereodefined PS-linkages would be useful to elucidate the biosynthetic pathway and functions of the naturally occurring PS-linkages.

However, access to stereoregular PS-ODNs is still severely limited despite the considerable efforts by many research groups.[7−16] Currently, these ODNs are available via the oxathiaphospholane method,[3,8a−c] in which nucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholane) derivatives are used as monomers, though the method has some drawbacks, such as the time-consuming chromatographic isolation of the diastereopure monomers from ca. 1:1 mixtures of diastereomers, relatively

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low efficiency of the internucleotidic bond formation under strongly basic conditions, and requirement of another set of four nucleoside 3′-*O*-(2-oxo-1,3,2-oxathiaphospholane) monomers for the synthesis of stereoregular phosphate/phosphorothioate chimeric ODNs (PO/PS-chimeric ODNs) due to the incompatibility of the method with the conventional phosphoramidite method.[8c]

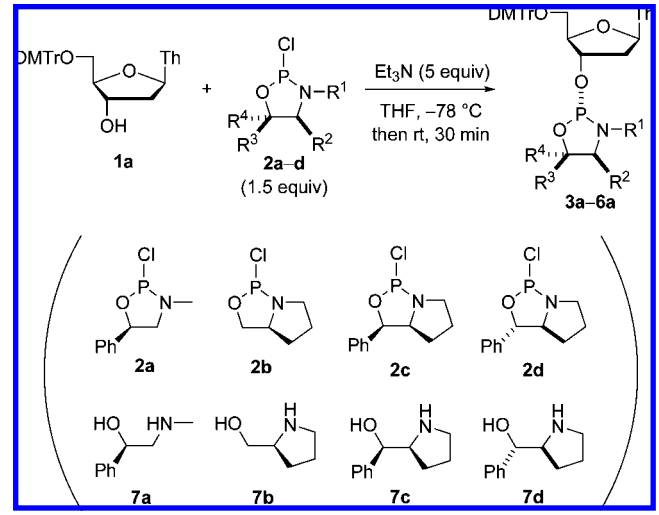
Under these circumstances, we have developed a method to synthesize stereoregular PS-ODNs using nucleoside 3′-*O*-(1,3,2-oxazaphospholidine) derivatives[11] as monomers, which were stereoselectively synthesized from enantiopure 1,2-amino alcohols and a less-nucleophilic acidic activator and applied to a manual solid-phase synthesis.[9] The method has some advantages, such as the diastereoselective formation of the monomers due to the chirality of the 1,2-amino alcohols and rapid condensation under mild acidic conditions. In addition, since the method consists of steps analogous to those of the conventional phosphoramidite method [(i) condensation promoted with a mild acidic activator; (ii) capping by acylating reagents; (iii) oxidation/sulfurization; (iv) 5′-*O*-detritylation], stereoregular PO/PS-chimeric ODNs may also be synthesized by switching the commonly used β-cyanoethyl phosphoramidites/oxazaphospholidines and/or oxidation/sulfurization. However, a non-negligible loss of diastereopurity during the condensation reactions was observed (up to 5−6% per cycle).[9b]

The partial loss of the diastereopurity can be attributed to the epimerization of the oxazaphospholidine monomers in the presence of the acidic activator. Though epimerization of tricoordinate phosphoramidite derivatives is usually negligible under neutral conditions at ambient temperatures, it is known to be accelerated under acidic conditions and/or at higher temperatures.[17] Since the contact of the oxazaphospholidine monomers with the acidic activator prior to the reaction with the 5′-OH of nucleosides on a solid support is inevitable for a solid-phase synthesis, an oxazaphospholidine monomer that would not epimerize even in the presence of the acidic activator is required. The oxazaphospholidine derivative also has to fulfill other requirements for the synthesis of stereoregular PS-ODNs, such as the high diastereoselectivity of their synthesis, repetitive condensations with high efficiency, and the removal of the 1,2-amino alcohol moiety from the resultant PS-linkages without any loss of diastereopurity. In this paper, we describe the development of nucleoside 3′-*O*-bicyclic oxazaphospholidine derivatives that fulfill all of these requirements and their applications to the synthesis of stereoregular PS-ODNs on an automated DNA synthesizer.

Results and Discussion

Design and Synthesis of Configurationally Stable Nucleoside 3′-*O*-Bicyclic Oxazaphospholidine Derivatives. Thymidine 3′-*O*-oxazaphospholidine derivatives (**3a**−**6a**) were synthesized by the reaction between the 3′-OH of 5′-*O*-(dimethoxytrityl)thymidine [5′-*O*-(DMTr)thymidine, **1a**] and 2-chloro-1,3,2-oxazaphospholidine derivatives (**2a**−**d**) (Scheme 1).[9] Crude 2-chloro-1,3,2-oxazaphospholidine derivatives **2a**−**d** were synthesized from the corresponding 1,2-amino alcohols (**7a**−**d**) as previously reported and used without further purification.[9,18] We have reported that the stereochemistry of the reaction is kinetically controlled under these conditions and one of the two *P*-diastereomers of the oxazaphospholidine derivatives is preferentially generated from the (*R*p)- or (*S*p)-2-chloro-1,3,2-oxazaphospholidine derivative, which are rapidly epimerized between each other due to repetitive nucleophilic attacks of Cl^-. To design a configurationally stable nucleoside 3′-*O*-oxazaphospholidine derivative, we focused on proline-derived bicyclic oxazaphospholidine rings. The simplest one [Figure 1, (***R*p)-4a**] has been reported to generate only the *trans*-isomer of the nucleoside 3′-*O*-oxazaphospholidine,[11b,c] and we also obtained the *trans*-isomer almost exclusively (*trans*:*cis* = 98:2) under the kinetic conditions.[19] However, the diastereomer ratio of (***R*p)-4a** was decreased to 93:7 by treatment with 2 equiv of an acidic activator, which was used to activate the oxazaphospholidine monomers [*N*-(cyanomethyl)pyrrolidinium triflate (CMPT) **8**],[9] in CH_3CN-CD_3CN (4:1, v/v) for 4 h at rt, though the epimerization was significantly suppressed compared to that of the monocyclic oxazaphospholidine derivative (***R*p)-3a**[9] (*trans*:*cis* = 58:42 under the same conditions). On the basis of the results, we designed new bicyclic oxazaphospholidine deriva-

Scheme 1. Synthesis of Thymidine 3′-*O*-Oxazaphospholidine Derivatives **3a**−**6a**



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(18) The corresponding 1,2-amino alcohols (**7a**−**d**) and PCl_3 (1 equiv) were reacted in toluene in the presence of *N*-methylmorpholine (2 equiv) for 30 min at 0 °C to room temperature. The mixture was filtered under argon to remove the resultant *N*-methylmorpholine hydrochloride and concentrated to dryness to afford crude **2a**−**d**, which was used without further purification.
(19) See Supporting Information.

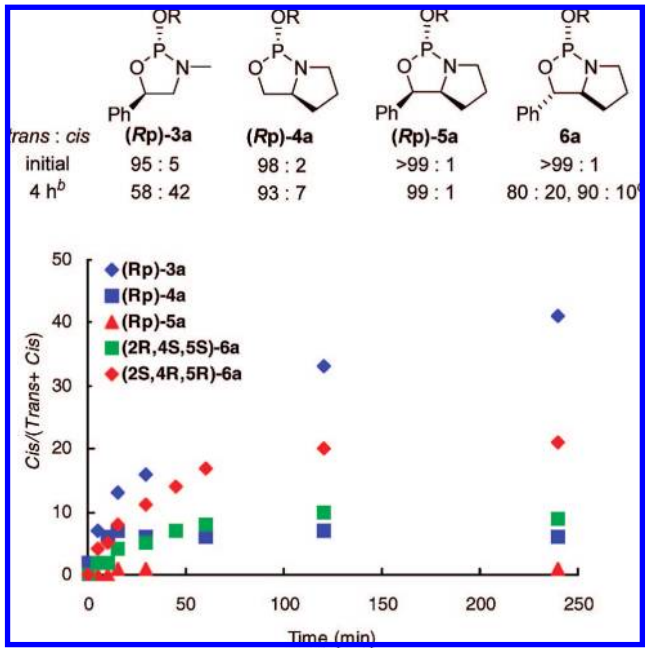
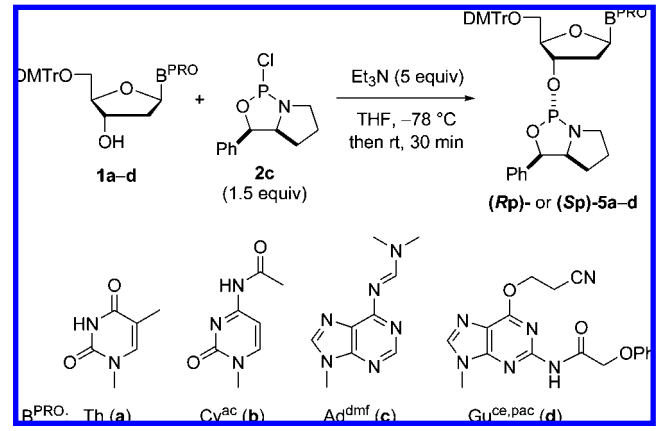


Figure 1. Ratios of *trans*:*cis* and time course of epimerization of thymidine 3′-*O*-oxazaphospholidine derivatives **3a**−**6a**. Notes: (*a*) RO = 5′-*O*-(DMTr)thymidin-3′-yl. (*b*) Diastereomer ratios of **3a**−**6a** after being treated by 2 equiv of CMPT in CH_3CN-CD_3CN (4:1, v/v) at room temperature for 4 h (^{31}P NMR). (*c*) Diastereomer ratios of (2*S*,4*R*,5*R*)-**6a** and (2*R*,4*S*,5*S*)-**6a** were 80:20 and 90:10, respectively.

tives having a phenyl group at the 5-position [(***R*p)-5a**, **6a**[20]] and carried out their synthesis under the same conditions to find that both of these oxazaphospholidine rings gave the *trans*-isomers of the thymidine 3′-*O*-oxazaphospholidines, exclusively. In addition, the epimerization in the presence of CMPT was almost completely suppressed for **(*R*p)-5a**, whereas 10−20% *cis*-isomers were generated for **6a**. These results showed that the newly designed bicyclic oxazaphospholidine ring structure of **(*R*p)-5a** generated the *trans*-isomer of the corresponding thymidine 3′-*O*-oxazaphospholidine derivative exclusively without being contaminated by the *cis*-isomer and also has virtually complete configurational stability, thus fulfilling two of the four requirements for the synthesis of stereoregular PS-ODNs mentioned above. By using the oxazaphospholidine ring, (*R*p)- and (*S*p)-nucleoside 3′-*O*-oxazaphospholidine monomers corresponding to the four kinds of nucleobases [**(*R*p)-** and **(*S*p)-5a**−**d**] were synthesized under the same conditions. The *trans*-isomers were generated exclusively in all of the cases and isolated in modest yields (Table 1).[21]

As described above, the proline-derived *trans*-nucleoside 3′-*O*-bicyclic oxazaphospholidine derivatives **(*R*p)-4a**,**(*R*p)-5a**, and **6a** had markedly increased configurational stability compared to the monocyclic **(*R*p)-3a**, and the rate of *trans* to *cis*

Table 1. Synthesis of Nucleoside 3′-*O*-Oxazaphospholidine Monomers



entry	oxazaphospholidine		isolated yield (%)	*trans*:*cis*
1	(*S*p)-Th	**(*S*p)-5a**	54	>99:1
2	(*S*p)-Cy^{ac}	**(*S*p)-5b**	50	>99:1
3	(*S*p)-Ad^{dmf}	**(*S*p)-5c**	58	>99:1
4	(*S*p)-Gu^{ce,pac}	**(*S*p)-5d**	45	>99:1
5	(*R*p)-Th	**(*R*p)-5a**	51	>99:1
6	(*R*p)-Cy^{ac}	**(*R*p)-5b**	52	>99:1
7	(*R*p)-Ad^{dmf}	**(*R*p)-5c**	43	>99:1
8	(*R*p)-Gu^{ce,pac}	**(*R*p)-5d**	44	>99:1

epimerization in the presence of CMPT **8** decreased in the following order: **(*R*p)-3a** > **6a** > **(*R*p)-4a** > **(*R*p)-5a** as shown in Figure 1. To rationalize these results, we carried out ab initio molecular orbital (MO) calculations.

The mechanism of inversion process at the phosphorus atoms of tricoordinate organophosphorus compounds has not been fully clarified.[22−24] In particular, little is known about the mechanism of the inversion process of phosphorus-containing chiral heterocyclic compounds, despite the fact that these compounds have been widely used to synthesize optically pure organophosphorus compounds such as phosphine-based ligands[25] and biologically active compounds.[2,3,7−13,24,26] It has been reported that nucleophilic species (e.g., amines, water, Cl^-) and acids (e.g., amine hydrochlorides) accelerate the inversion process, and their repetitive nucleophilic attacks to the phosphorus atom are proposed to be involved.[17,26] However, it cannot rationalize the fact that the inversion is also accelerated in the case that the nucleophilic species and the leaving group at the phosphorus

(20) The oxazaphospholidine **6a** was synthesized as a mixture of two isomers having either a (2*S*,4*R*,5*R*)- or a (2*R*,4*S*,5*S*)-oxazaphospholidine ring by using racemic 1,2-amino alcohol **7d**. The 1,2-amino alcohols **7c**,**d** were synthesized according to the procedures reported in the literature. The details are given in Supporting Information. (a) Bejjani, J.; Chemla, F.; Audouin, M. *J. Org. Chem.* **2003**, *68*, 9747–9752. (b) Meyers, A. I.; Ten Hoeve, W. *J. Am. Chem. Soc.* **1980**, *102*, 7125–7126.

(21) The nucleoside 3′-*O*-oxazaphospholidine derivatives **5a**−**d** were partially decomposed on silica gel during chromatography, resulting in modest isolated yields. The isolated **5a**−**d** were stable for at least 1 year under proper storage conditions (at−30 °C in a glass vial flushed with an inert gas).

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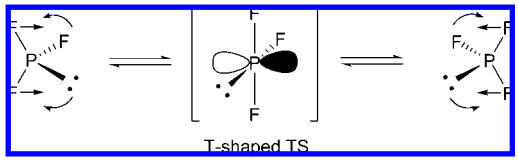


Figure 2. Edge inversion process.

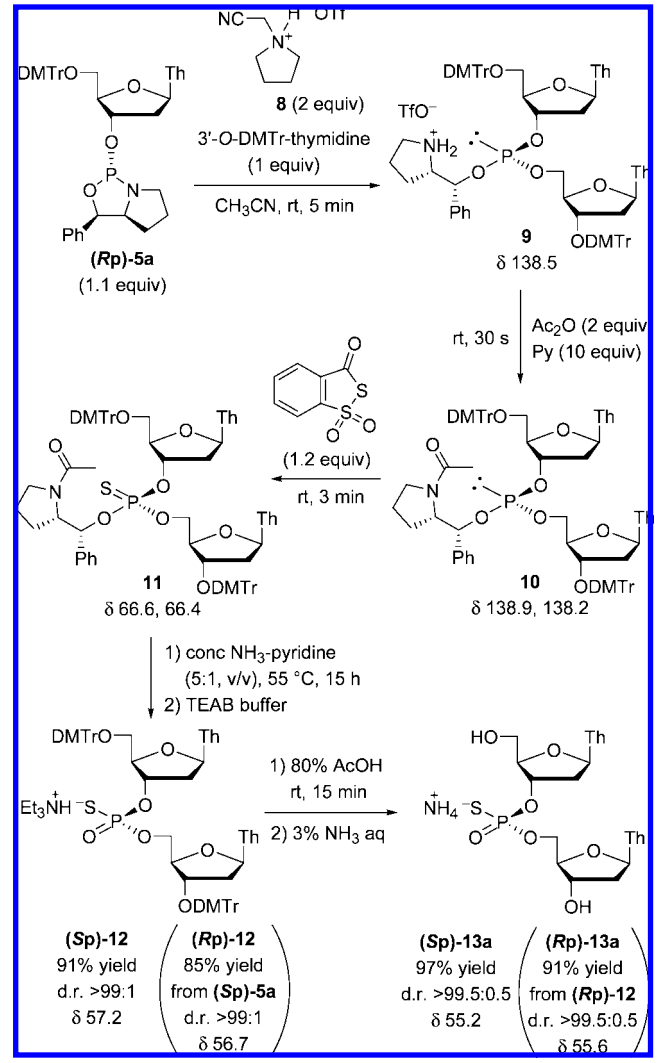
atom are different from each other. In addition, CMPT consisting of less nucleophilic components also accelerated the epimerization of the oxazaphospholidines as above. Therefore, elucidation of the epimerization mechanism of the oxazaphospholidine derivatives observed in this study would shed light on the unsolved inversion mechanism of tricoordinated organophosphorus compounds.

It has been calculated that the inversion of phosphine derivatives, such as PH_3, proceeds through a classical trigonal-planar transition state (vertex inversion) and those having electronegative substituents, such as PF_3, invert through a T-shaped transition state (edge inversion, Figure 2).[22a] As the first step of our MO calculations, the transition states of a model structure of (***Rp*)-3a** having a methyl group in the place of the 5′-*O*-(DMTr)thymidin-3′-yl group and its N^3-protonated form were calculated at the HF/6-31G* level. Two transition states were obtained for both the nonprotonated and the protonated forms, and all of these had a T-shaped structure,[19] indicating that the epimerization of the oxazaphospholidine derivatives proceeds through an edge inversion process. It is reasonable because a planar trigonal transition state has a theoretical O−P−N bond angle of ca. 120°, which would be significantly less stable for the oxazaphospholidines than the T-shaped one due to the existence of the five-membered oxazaphospholidine ring. The two of these transition states have the O^1 and the O^2 atoms at the apical positions, and the other two have the N^3 and the O^2 atoms at the apical positions. Since electron-withdrawing substituents at the apical positions stabilize the T-shaped transition states,[22c] the smallest activation energy is obtained for the one in which the extended P−N bond (2.90 Å) by N^3-protonation occupies the apical position.[19] The MO calculations thus showed that N^3-protonation of the oxazaphospholidine ring extended the P−N bond to accelerate an edge inversion process through a T-shaped transition state in which the P−NH^+ bond occupies the apical position.

The important thing here is that it has been shown both theoretically and experimentally that the edge inversion barrier at pnictogens is significantly reduced by coordination of nucleophilic species to the unoccupied orbital in the T-shaped transition state and the stabilization effect does not exist in a vertex inversion process.[27] Therefore, an edge inversion process is also supported by the reported acceleration of inversion by nucleophiles, especially those different from the leaving group on the phosphorus atom. In addition, another reported acceleration effect by acids is probably due to not only the coordination of the nucleophilic counteranion (e.g., Cl^-) but also the extension of the P−N bond by *N*-protonation.

Our MO calculations thus rationalized the reported observation on the inversion of tricoordinated phosphorus compounds. Therefore the model was applied to the other oxazaphospholidine structures (**4a**, **5a**, **6a**) to obtain the activation energy of the edge inversion process to find that the calculated energy values increased in the following order: (***Rp*)-3a** < **6a** < (***Rp*)-4a** < (***Rp*)-5a**, which was consistent with the order of configurational stability obtained in this study.[19] Though the possibility of stabilization by nucleophiles cannot be excluded, the calculations showed that the epimerization of the oxazaphospholidine derivatives in the presence of less-nucleophilic CMPT is most likely to proceed via an edge process, which is accelerated by the extension of the P−N bond by N^3-protonation, and the edge inversion energy barrier for those with a bicyclic oxazaphospholidine ring, especially **(*Rp*)-5a**, is significantly larger than that for the monocyclic one, resulting in the configurational stability.

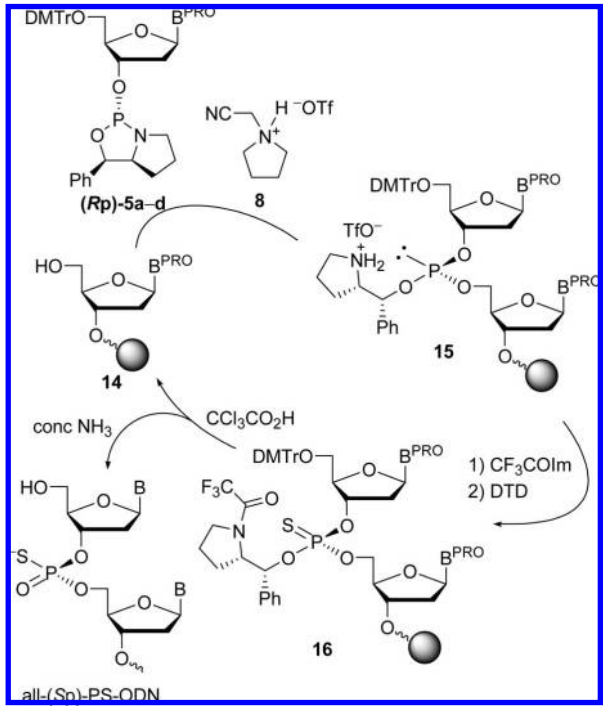
Scheme 2. Solution-Phase Synthesis of Dithymidine Phosphorothioates **(*Rp*)-** and **(*Sp*)-13a**



Solution-Phase Synthesis of (*Sp*)- and (*Rp*)-Dithymidine Phosphorothioates. The oxazaphospholidine derivative **(*Rp*)-5a** with the best configurational stability was then applied to the stereocontrolled synthesis of internucleotidic PS-linkages. First, dithymidine phosphorothioate was synthesized in solution-phase by using **(*Rp*)-5a** as the monomer unit (Scheme 2). The oxazaphospholidine **(*Rp*)-5a** was allowed to condense with a 3′-*O*-protected thymidine in the presence of CMPT **8** to afford a dithymidine phosphite intermediate (**9**) stereospecifically. Acetylation of the protonated secondary amino group of **9** and

(27) (a) Dixon, D. A.; Arduengo, A. J. *Int. J. Quantum Chem., Symp.* **1988**, *22*, 85–98. (b) Yamamoto, Y.; Chen, X.; Akiba, K.-y. *J. Am. Chem. Soc.* **1992**, *114*, 7906–7907. (c) Yamamoto, Y.; Chen, X.; Kojima, S.; Ohdoi, K.; Kitano, M.; Doi, Y.; Akiba, K.-y. *J. Am. Chem. Soc.* **1995**, *117*, 3922–3932.

Scheme 3. Synthesis Cycle for Stereoregular PS-ODNs



the following sulfurization of the phosphorus atom afforded a fully protected dithymidine phosphorothioate triester (**11**). Two ^{31}P NMR signals were observed for **10** and **11** due to the existence of the acetamide rotamers.[9,10] Deprotection of the PS-linkage of **11** required a long treatment with concentrated ammonia at an elevated temperature but proceeded without loss of diastereopurity to afford a 3′,5′-*O*-protected dithymidine phosphorothioate [**(Sp)-12**] in an excellent yield. The desired fully deprotected dithymidine phosphorothioate (***Sp*)-13a** was obtained in an excellent yield by 3′,5′-*O*-detritylation under usual acidic conditions. The configuration and diastereopurity of **13a** was confirmed by RP-HPLC.[19] Diastereopure **(*Rp*)-13a** was also obtained in an excellent yield from (***Sp*)-5a**. Thus, the newly designed bicyclic oxazaphospholidine monomers gave both (*R*p)- and (*S*p)-diastereopure PS-linkages in solution phase efficiently.

Automated Solid-Phase Synthesis of Stereoregular PS-ODNs. Next, the method was applied to the solid-phase synthesis of stereoregular PS-ODNs on a DNA synthesizer. The synthesis cycle is shown in Scheme 3. The monomers **5a**−**d** were allowed to condense with the 5′-OH of a nucleoside anchored to a highly cross-linked polystyrene (HCP)[28] (**14**) in the presence of CMPT **8** to afford a phosphite intermediate (**15**). Since we found that the removal of *N*-acetylated chiral auxiliaries from PS-linkages by treatment with conc NH_3 caused partial strand cleavage at an oligomer level, *N*-trifluoroacetylimidazole (CF_3COIm) was used to cap the secondary amino group of **15** and any unreacted 5′-OH on the support. No desulfurization was observed under the capping conditions.[29] It was also confirmed that any of the 5′-OH remaining on the solid-support were quantitatively capped under the conditions. The synthesis cycle consists of the condensation, capping, and the following sulfurization by a cost-effective sulfurizing reagent *N*,*N*′-dimethylthiuram disulfide (DTD)[30] and 5′-*O*-detritylation. After the chain elongation by repeating the cycle, the protected PS-ODNs on the support were deprotected and cleaved from the support by treatment with concentrated NH_3. The *N*-trifluoroacetylated chiral auxiliary was removed without observable strand cleavage, though the complete removal required a long treatment as the oligomers lengthened (15−48 h). First, (*R*p)- and (*S*p)-dinucleoside phosphorothioates were synthesized by this method to find that the formation of PS-linkages was successfully conducted with excellent yields and diastereoselectivity (Table 2, 96−99% yields, dr ≥99:1). Stereoregular PS-ODNs 8−12mers (**17**−**21**, Table 3) were prepared efficiently by using the cycle and isolated by RP-HPLC (Table 3, Figures 3 and 4). The PS-ODNs were characterized by MALDI-TOF-MS, and their diastereopurity was confirmed by enzymatic digestion.[19] The HPLC profiles shown in Figures 3 and 4 showed that all the PS-ODNs were produced with excellent diastereoselectivity. Thus, the configurationally stable bicyclic oxazaphospholidine monomers proved to be useful for the synthesis of stereodefined (*R*p)- and (*S*p)-PS-linkages at the oligomer level.

Table 2. Synthesis of dinucleoside phosphorothioates **(*Rp*)-** and **(*Sp*)-13a−d**

entry	dN_ST[a]		yield (%)[b]	*Rp*:*Sp*[b]
1	(*R*p)-T_ST	**(*Rp*)-13a**	98	>99:1
2	(*R*p)-dC_ST	**(*Rp*)-13b**	98	>99:1
3	(*R*p)-dA_ST	**(*Rp*)-13c**	98	>99:1
4	(*R*p)-dG_ST	**(*Rp*)-13d**	96	>99:1
5	(*S*p)-T_ST	**(*Sp*)-13a**	97	>1:99
6	(*S*p)-dC_ST	**(*Sp*)-13b**	99	1:99
7	(*S*p)-dA_ST	**(*Sp*)-13c**	98	>1:99
8	(*S*p)-dG_ST	**(*Sp*)-13d**	96	1:99

[a] Subscript "S" = PS-linkage. [b] Yield and diastereomer ratio of products were determined by RP-HPLC.

Table 3. Synthesis of Stereoregular PS-ODNs **17**−**21**.

no.	oligonucleotide[a]	coupling yield (%)[b]	isolated yield (%)[c]
17	all-(*R*p)-$T_ST_ST_ST_ST_ST_ST_ST_ST_ST_ST$	99	34
18	all-(*S*p)-$T_ST_ST_ST_ST_ST_ST_ST_ST_ST_ST$	98	32
19	(*Rp*,*Rp*,*Rp*,*Rp*,*Rp*,*Sp*,*Sp*)-$T_ST_ST_ST_ST_ST_ST_ST_ST_ST$	98	30
20	all-(*R*p)-d[$C_SA_SG_ST_SC_SA_SG_ST_SC_SA_SG_ST$]	96	16
21	all-(*S*p)-d[$C_SA_SG_ST_SC_SA_SG_ST_SC_SA_SG_ST$]	95	12

[a] Subscript "S" = PS-linkage. [b] Average coupling yield was estimated by $DMTr^+$ assay. [c] Isolated by RP-HPLC.

Conclusion

In summary, the nucleoside 3′-*O*-bicyclic oxazaphospholidines designed as monomer units to synthesize stereodefined PS-internucleotide linkages were generated with complete diastereoselectivity and were also configurationally stable even in the presence of an acidic activator. Automated synthesis of PS-linkages by using the monomers proceeded without any loss of diastereopurity, and stereoregular PS-ODNs were also synthesized efficiently. The method should facilitate the synthesis of stereoregular PS-ODNs and may also become useful to provide stereoregular PO/PS-chimeric ODNs since the method uses a series of reactions similar to those for the conventional phosphoramidite method. In addition, ab initio MO calculations

(28) McCollum, C.; Andrus, A. *Tetrahedron Lett.* **1991**, *32*, 4069–4072.
(29) In contrast, serious desulfurization was observed when $(CF_3CO)_2O$ was used in the place of CF_3COIm as reported in the literature. (a) Heliński, J.; Skrzypczyński, Z.; Wasiak, J.; Michalski, J. *Tetrahedron Lett.* **1990**, *31*, 4081–4084. (b) Bruzik, K. S.; Stec, W. J. *J. Org. Chem.* **1990**, *55*, 6131–6135.
(30) Song, Q.; Wang, Z.; Sanghvi, Y. S. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 629–633.

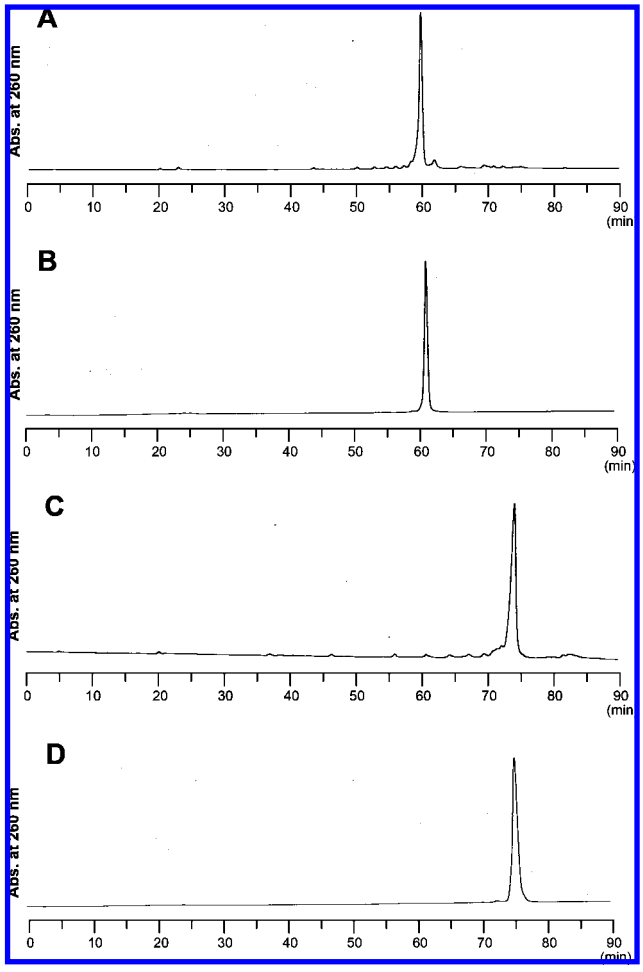


Figure 3. Reversed-phase HPLC profiles of PS-T_{10}: (A) crude all-(*R*p)-PS-T_{10} **17**; (B) purified **17**; (C) crude all-(*S*p)-PS-T_{10} **18**; (D) purified **18**. RP-HPLC was performed with a linear gradient of 0−20% acetonitrile in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C for 90 min at a rate of 0.5 mL/min.

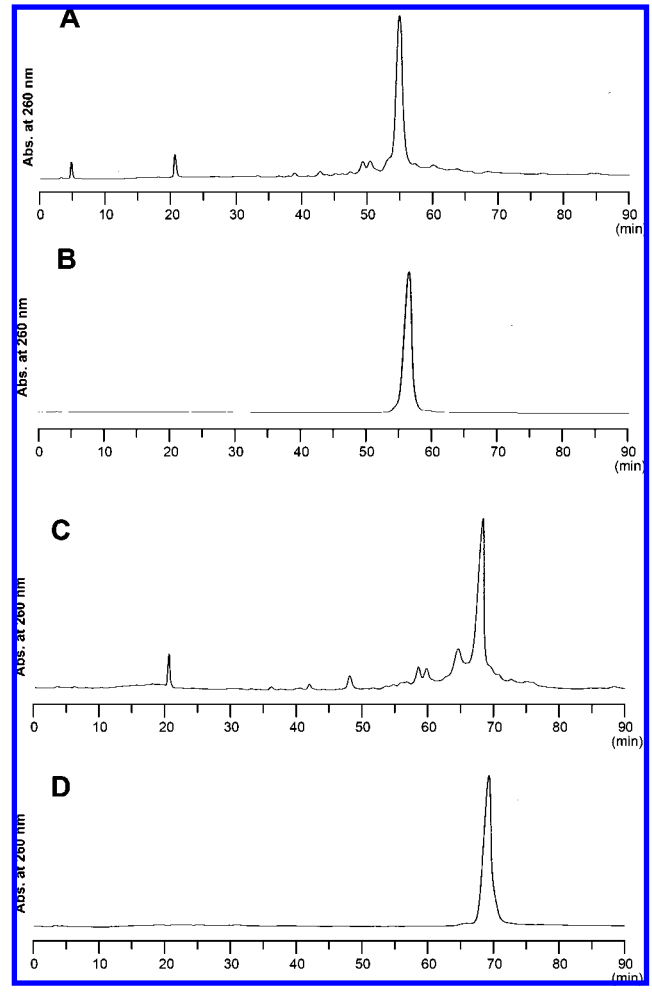


Figure 4. Reversed-phase HPLC profiles of PS-d[CAGT]$_3$: (A) crude all-(*R*p)-PS-d[CAGT]$_3$ **20**; (B) purified **20**; (C) crude all-(*S*p)-PS-d[CAGT]$_3$ **21**; (D) purified **21**. RP-HPLC was performed with a linear gradient of 0−20% acetonitrile in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C for 90 min at a rate of 0.5 mL/min.

showed that the epimerization of the oxazaphospholidines was most likely to proceed via an edge inversion process. The calculations rationalized the observations reported in the literature that the epimerization of tricoordinated organophosphorus compounds was accelerated by acids or nucleophiles. Though the effect of nucleophiles or coordinating solvents was not included in the calculations in this study because we consider the effect of *N*-protonation dominates, such calculations can also be carried out easily. Such calculations may be useful for the design of tricoordinated organophosphorus compounds, such as chiral ligands, because practical configurational stability of these compounds in organic solvents or in the presence of a small amount of impurities can be predicted.

Experimental Section

Ab Initio MO Calculations. The calculations were carried out using the Spartan'04[31] on a Dell Inc. PRECISION 650 workstation. Geometry optimizations and single-point energy calculations were carried out at the HF/6-31G* level.

General Procedure for the Synthesis of the Nucleoside 3′-*O*-Oxazaphospholidine Monomers (*R*p)-5a−d. The appropriately protected 2′-deoxyribonucleoside (**1a−d**, 2.0 mmol) was dried by repeated co-evaporations with dry pyridine and dry toluene and dissolved in freshly distilled THF (6.0 mL) under argon. Et_3N (1.39 mL, 10 mmol) and a 0.5 M solution of the corresponding crude (4*S*,5*R*)-2-chloro-1,3,2-oxazaphospholidine [(4*S*,5*R*)-**2c**], which was prepared from an L-proline-derived 1,2-amino alcohol (α*R*,2*S*)-**7c**, in freshly distilled THF (6.0 mL, 3.0 mmol) were successively added dropwise to the solution at −78 °C with stirring. The mixture was stirred for 30 min at room temperature, poured into an ice-cold saturated $NaHCO_3$ aqueous solution (100 mL), and extracted with $CHCl_3$ (100 mL). The organic layer was washed with ice-cold saturated $NaHCO_3$ aqueous solutions (2 × 100 mL), and the combined aqueous layers were back-extracted with $CHCl_3$ (100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The resultant crude **(*R*p)-5a−d** was purified by silica gel column chromatography. The purification conditions, isolated yields, and characterizing data are given in Supporting Information. The monomers **(*S*p)-5a−d** were also synthesized according to the same method by using the protected nucleosides (**1a−d**) and (4*R*,5*S*)-2-chloro-1,3,2-oxazaphospholidine derivative [(4*R*,5*S*)-**2c**], which was prepared from the D-proline-derived amino alcohol (α*S*,2*R*)-**7c**.

(*R*p)-5a. Crude **(*R*p)-5a** obtained as above was purified by silica gel column chromatography [3.5 × 6 cm, 30 g of silica gel, hexane−ethyl acetate−triethylamine (20:80:2, v/v/v)]. The fractions containing **(*R*p)-5a** were combined, washed with a saturated $NaHCO_3$ aqueous solution (500 mL), dried over Na_2SO_4, filtered,

(31) Kong, J. et al. *Spartan'04*; Wavefunction, Inc.: Irvine, CA; *J. Comput. Chem.* **2000**, *21*, 1532−1548.

and concentrated to dryness under reduced pressure to afford **(*R*p)-5a** (0.772 g, 1.0 mmol, 51%) as a white foam. IR (KBr) 3412, 3182, 2953, 1690, 1607, 1582, 1509, 1465, 1366, 1252, 1177, 1154, 1076, 1034, 962, 915, 829, 793, 756, 706, 584, 559 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 9.46 (brs, 1H), 7.59 (s, 1H), 7.40−7.19 (m, 14H), 6.79 (d, J = 8.1 Hz, 4H), 6.44 (dd, J = 7.1, 6.0 Hz, 1H), 5.71 (d, J = 6.3 Hz, 1H), 4.93 (m, 1H), 4.12 (m, 1H), 3.81 (m, 1H), 3.75 (s, 6H), 3.56 (m, 1H), 3.47 (dd, J = 10.6, 2.4 Hz, 1H), 3.37 (dd, J = 10.6, 2.3 Hz, 1H), 3.16 (m, 1H), 2.58 (ddd, J = 13.6, 6.0, 2.7 Hz, 1H), 2.37 (ddd, J = 13.6, 7.1, 6.8 Hz, 1H), 1.63 (m, 2H), 1.41 (s, 3H), 1.19−1.12 (m, 1H), 0.99−0.85 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.9, 158.3, 158.3, 150.3, 144.0, 137.8 (d, $^3J_{PC}$ = 4.1 Hz), 135.3, 135.1, 135.0, 129.8, 129.8, 127.9, 127.9, 127.6, 127.2, 126.8, 125.1, 112.9, 110.9, 86.6, 85.2 (d, $^3J_{PC}$ = 2.0 Hz), 84.4, 82.1 (d, $^2J_{PC}$ = 9.5 Hz), 73.0 (d, $^2J_{PC}$ = 12.8 Hz), 67.1 (d, $^2J_{PC}$ = 3.2 Hz), 62.7, 55.0, 47.0 (d, $^2J_{PC}$ = 34.7 Hz), 39.9 (d, $^3J_{PC}$ = 4.6 Hz), 28.0, 25.8 (d, $^3J_{PC}$ = 3.4 Hz), 11.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ 155.2. FAB-HRMS: m/z calcd for $C_{42}H_{45}N_3O_8P^+$ $[(M + H)^+]$ 750.2939, found 750.2976.

Automated Solid-Phase Synthesis of Stereoregular PS-ODNs [(*R*p)- and (*S*p)-13a−d, 17−21]. The automated solid-phase synthesis of stereoregular PS-ODNs was performed on an Expedite 8909 Nucleic Acid Synthesis System (Applied Biosystems) according to the cycle shown in Supporting Information. The detailed protocol for the Expedite is also given in Supporting Information. All the PS-ODNs were synthesized using a thymidine-loaded highly cross-linked polystyrene (HCP) (0.5 μmol, 30 μmol/g, succinate linker) The fractions of the detritylation and the following washings were combined and diluted with a 0.1 M *p*-toluenesulfonic acid monohydrate solution in CH_3CN to make a 50.0 mL solution for every cycle to perform the $DMTr^+$ assay. Average coupling yields were 95−99%. After the synthesis, the HCP was dried in vacuo and treated with a 25% NH_3 aqueous solution−EtOH (5:1, v/v) (1 mL) for 15−48 h at 55 °C. The mixture was cooled to room temperature, and the HCP was removed by membrane filtration. The filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in H_2O (3 mL), washed with Et_2O (3 × 3 mL), and concentrated to dryness under reduced pressure. The purification and/or analysis of the resultant crude PS-ODNs were performed by using RP-HPLC with a linear gradient of acetonitrile (0−15%/45 min for the dimers **13a−d** or 0−20%/90 min for the oligomers **17−21**) in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C at a rate of 0.5 mL/min.

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Supporting Information Available: Experimental procedures, spectral data, and HPLC profiles and the full ref 30. This material is available free of charge via the Internet at http://pubs.acs.org.

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