DOI: 10.1002/ejoc.200500403

# Utility of Azolium Triflates as Promoters for the Condensation of a Nucleoside Phosphoramidite and a Nucleoside in the Agrawal's Stereoselective Synthesis of Nucleoside Phosphorothioates

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*Dedicated to Professor Wojciech J. Stec on the occasion of his 65th birthday*

**Keywords:** Oligonucleotides / Nitrogen heterocycles / Phosphoramidite / Solid-phase synthesis / Chirality

This paper demonstrates that some azolium triflates, such as *N*-phenylimidazolium triflate, benzimidazolium triflate and *N*-methylbenzimidazolium triflate, are more useful than 1*H*-tetrazole as promoters for the stereoselective condensation of a 5′-*O*-free nucleoside and a stereochemically pure 5′-*O*-(*p*,*p*′-dimethoxytrityl)-3′-*O*-{(4*R*)-1*H*,3*H*-pyrrolo[1,2-*c*]-1,3,2-oxazaphospholidin}-2-yl 2′-deoxyribonucleoside (*R*c-**1**) or 5′-*O*-(*p*,*p*′-dimethoxytrityl)-3′-*O*-{(4*S*)-1*H*,3*H*-pyrrolo[1,2-*c*]-1,3,2-oxazaphospholidin}-2-yl 2′-deoxyribonucleoside (*S*c-**2**) (Agrawal strategy). The azolium triflates allowed the stereoselective formation of an internucleotide phosphorothioate bond via the above-described condensation using a stereochemically pure phosphoramidite, followed by sulfurization using bis[3-triethoxysilylpropyl]tetrasulfide. The highest diastereoexcess values of the products in the synthesis of dideoxyribonucleoside phosphorothioates using a suitable azolium triflate such as benzimidazolium triflate, *N*-methylbenzimidazolium triflate or *N*-phenylimidazolium triflate were 90–96 % in solution phase or 80–88 % in solid phase; these values were higher than those obtained in the synthesis using 1*H*-tetrazole as a promoter for the condensation of a nucleoside phosphoramidite and a nucleoside. This paper also describes that studies on the absolute configurations of stereogenic phosphorus atoms in the phosphoramidites *R*c-**1** and *S*c-**2** by meansof two different existing methods, i.e., the Beaucage method, gave contrary conclusions, and thus the configurations should be determined by an absolutely reliable method, such as X-ray analysis.


## Introduction

Oligonucleotide phosphorothioates (PS-oligos) are important molecules in antisense therapy.[1] In the nucleoside phosphorothioate, the phosphorus atom becomes an asymmetric center to generate two diastereomers with every phosphorothioate function. Thus, there exist $2^n$ kinds of diastereomers in a PS-oligo with *n* phosphorothioate moieties. These $2^n$ isomers potentially have their own biological properties, some of which might be clinically undesirable. Thus, in order to avoid undesirable side effects, there is an increasing tendency to design a therapy that uses stereochemically pure PS-oligos rather than a mixture of diastereomers. Accordingly, a variety of methods for the stereoselective preparation of nucleoside phosphorothioate diesters have been developed. The most useful of these methods may be that achieved through stereoselective condensation of a 5′-*O*-free nucleoside and a stereochemically pure nucleoside 3′-cyclicphosphoramidite, i.e., 3′-oxazaphospholidine, in which the stereogenic carbon atom has an *R* or *S* configuration (an *R*c- or an *S*c-phosphoramidite, respectively), to form a dinucleoside phosphite, and the subsequent stereospecific sulfurization, followed by the stereospecific conversion of the resulting phosphorothioate triester to the diester. Representative methods in this category include Agrawal's method,[2] Just's two methods[3,4] and Wada's method.[5] In the synthesis of stereodefined PS-oligos via the above strategy, it is an important requirement that stereochemically pure *R*c- and *S*c-nucleoside 3′-oxazaphospholidines, which are used as monomer units, can be easily prepared. From this point of view, the Agrawal method is the most favorable among the existing methods. For example, in the synthesis of deoxyribonucleotides according to the Agrawal method, the requisite monomer units, *R*c-**1** and *S*c-**2**, are prepared in only three steps start-

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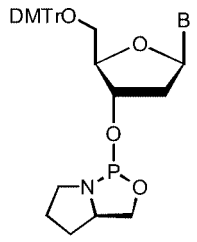
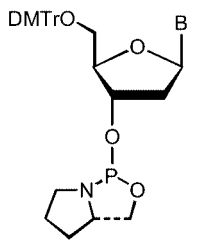



*Rc*-**1a**, B = Ade$^{Bz}$
*Rc*-**1b**, B = Cyt$^{Bz}$
*Rc*-**1c**, B = Gua$^{dmf}$
*Rc*-**1d**, B = Thy




*Sc*-**2a**, B = Ade$^{Bz}$
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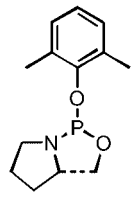
*Sc*-**2c**, B = Gua$^{dmf}$
*Sc*-**2d**, B = Thy

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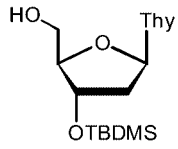



**4**

ing from commercially supplied D- and L-prolines [(*R*)- and (*S*)-prolines], respectively. Moreover, all the steps in the preparation give products of excellent purity, and thus there is no need for tedious purification. By contrast, deoxyribonucleotide syntheses by the Just and Wada methods require a longer pathway than the Agrawal method for the preparation of desired *R*c- and *S*c-nucleoside oxazaphospholidines and sometimes troublesome column chromatography. For example, in one of the Just approaches, desired *R*c- and *S*c-oxazaphospholidine building units are constructed in 6 steps starting from D- and L-xyloses, respectively.[3] The other Just method produces the requisite building units in 10 steps starting from L- and $^{d}$–$N_b$–benzyl tryptophan methyl esters.[4] The Wada method requires a 6-step transformation using L- and D-mandelic acids as starting materials to obtain monomer units. Further, in this method, tedious purification by column chromatography at the final stage of the preparation is necessary to obtain pure *R*c- and *S*c-oxazaphospholidines. However, the Agrawal method also has a drawback—i.e., it has inferior stereoselectivity for the formation of nucleoside phosphorothioates, which is another crucial requirement.[6] Thus, each of the existing methods has its merits and demerits. The above-mentioned ease of acquisition of monomer units, *R*c-**1** and *S*c-**2**, in the Agrawal method is among the greatest of the merits, and in fact this quality is hard to do without in oligonucleotide synthesis. Thus, if it were possible to raise the low selectivity in the condensation of *R*c-**1** or *S*c-**2** with a 5′-*O*-free nucleoside to a high selectivity, the Agrawal method would become a truly useful tool for the stereocontrolled synthesis of nucleoside phosphorothioates because the subsequent sulfurization is achieved in a stereospecific manner. The stereoselectivity of the objective condensation strongly depends on the reaction conditions. For example, the reaction promoter may affect the selectivity. Thus, we investigated the effect of various kinds of promoters on the stereoselective condensation of *R*c-**1** or *S*c-**2** with a 5′-*O*-free nucleoside. The results showed that some kinds of azolium triflate promoters were more effective than 1*H*-tetrazole, which is generally employed by the Agrawal method to gain high stereoselectivity. In addition, this paper describes our attempts to clarify the absolute configurations of stereogenic phosphorus atoms in *R*c-**1** and *S*c-**2**, which have not yet been precisely determined.

## Results and Discussion

### Preparation of Nucleoside 3′-Oxazaphospholidines (Agrawal Phosphoramidites) and Determination of the Absolute Configuration of the Stereogenic Phosphorus Atom in the Products

The nucleoside 3′-oxazaphospholidines (Agrawal phosphoramidites), *R*c-**1a**–*R*c-**1d** and *S*c-**2a**–*S*c-**2d**, were prepared according to previously-reported methods.[2] In these compounds, it is important to define the absolute configurations of the stereogenic phosphorus atoms (*p*-configurations) in the phosphoramidites. Agrawal and his co-workers inferred that *R*c-**1** and *S*c-**2** have *R*- and *S*-configurations, respectively, based on a report that substitution reactions of *P*-chloro-oxazaphospholidines involving carbon-, oxygen-, and nitrogen-based nucleophiles gave products with an overall retention of configuration.[7] Thus *R*c-**1** and *S*c-**2**, which are prepared by a method analogous to this substitution reaction, would have a structure with *R*p- and *S*p-configurations, respectively. This conclusion might be correct, but we think that it is necessary to carry out more straightforward investigations using *R*c-**1** and *S*c-**2** themselves to verify the structure. Thus, in analogy with the two different methods reported by Beaucage[8] and Arzoumanian,[9] we attempted to determine the *p*-configurations of the Agrawal phosphoramidites by representatively using *R*c-**1d** and *S*c-**2d**.

First, the determination of the *p*-configurations was carried out according to the Beaucage method. Computer-modeling analysis using Spartan 04 indicated that the energy-minimized conformers of *S*c-**2d** with *R*p- and *S*p-configurations are those shown as *S*c,*R*p-**2d** and *S*c,*S*p-**2d**, respectively, in Figure 1. This result reveals that proximal through-space interactions occur between *H*-8b of the pyrrolidine ring and *H*-2′ of the sugar moiety (2.27 Å) in the case of *S*c,*R*p-**2d** and between *H*-8a of the pyrrolidine ring and *H*-5′ of the sugar moiety (2.62 Å) in the case of *S*c,*S*p-**2d**. To determine which is the real *S*c-**2d**, ROESY and

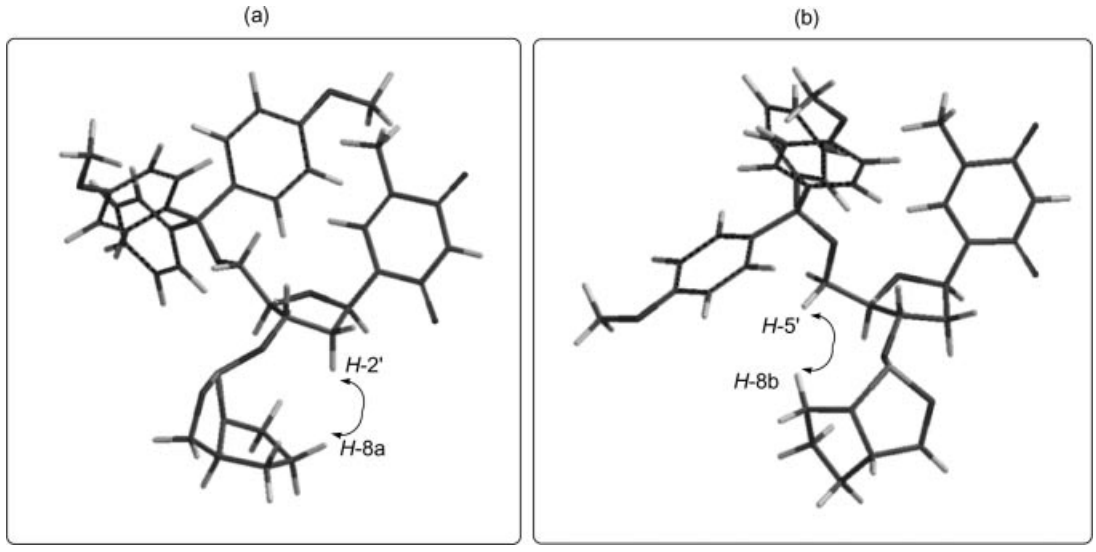



Figure 1. Energy-minimized comformers of (a) *S*c,*R*p-**2d** and (b) *S*c,*S*p-**2d**. Geometry optimization was carried out by the HF/6-31G$^+$.

GOESY NMR spectra were measured. The resulting spectra are shown in supporting information Figure S1 (ROESY) and Figure S2 (GOESY). These spectra showed the NOE signal resulting from the interaction between the pyrrolidine *H*-8a and sugar *H*-5′. However, no NOE was observed between pyrrolidine *H*-8b and sugar *H*-2′. This result revealed that *S*c-**2d** has the *S*p-configuration, which is consistent with the configuration assigned by Agrawal et al. In a similar manner, the *p*-configuration of *R*c-**1d** was determined to be *R*.

Subsequently, we attempted to determine the *p*-configurations by the Arzoumanian method. According to a previous study,[9] in the phosphoramidite *S*c-**3** with a (4*S*)-1*H*,3*H*-pyrrolo[1,2-*c*]-1,3,2-oxazaphospholidin-2-yl skeleton, which is the same skeleton as in the phosphoramidite part in *S*c-**2d**, the magnitude of the coupling constant, $^2J_{PC}$, between the signals due to the phosphorus atom and the carbon atom β to the phosphorus atom is strongly controlled by the dihedral angle associating the lone-pair orbital on the phosphorus atom and the β-carbon. For example, in the *R*p- and *S*p-isomers, the $^2J_{PC}$ values between P and C-8 are estimated to be approximately 40 Hz and 0 Hz, respectively. In the $^{13}$C NMR spectrum of *S*c-**2d** (see Figure 2), the signal due to the C-8 actually appeared at $\delta$ = 47.01 ppm as a doublet with $^2J_{PC}$ = 35 Hz. Therefore, the *p*-configuration of *S*c-**2d** was suggested to be *R*. In a similar manner, it was suggested that the *p*-configuration of *R*c-**1d** is *S*.

It is surprising that these two methods provided different results. Since Arzoumanian et al. employed a sample with a skeleton identical to that of the phosphoramidite with **2d**, the result obtained by the Arzoumanian method would seem to be more reliable than the result attained by the Beaucage method, but, at this stage, we cannot conclusively state that *S*c-**2d** and *R*c-**1d** have *R*p and *S*p configurations, respectively. In conclusion, alternative, absolutely reliable methods, such as X-ray analysis, are necessary to conclusively determine the *p*-configuration of the Agrawal phosphoramidites.

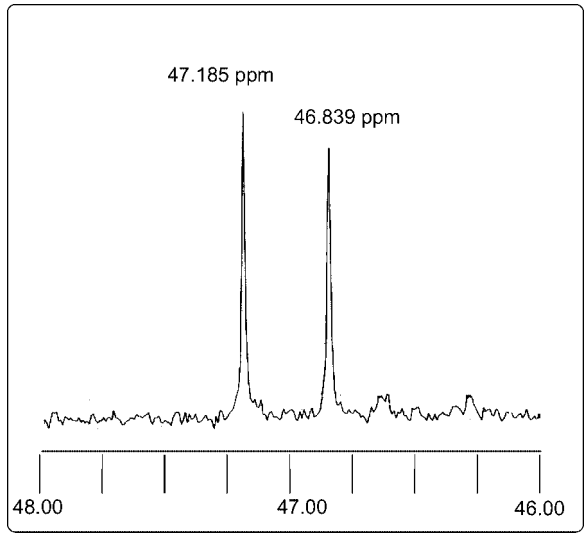
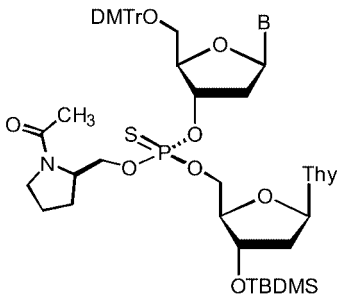



Figure 2. The $^{13}$C NMR spectrum of *S*c-**2d**.

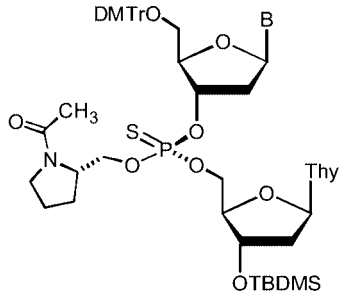
### Stereoselectivity in the Solution-Phase Synthesis of Dinucleoside Phosphorothioates

First, we investigated stereoselectivity in the solution-phase synthesis of *R*c,*R*p-**5** and *S*c,*S*p-**6** using various reagents for the activation of *R*c-**1** and *S*c-**2**. The activators include 1*H*-tetrazole, trifluoroacetic acid,[10] trichloroacetic acid,[10] 2,4-dinitrobenzoic acid,[10] *N*-phenylimidazolium triflate (*N*-PhIMT),[11] benzimidazolium triflate (BIT),[11] *N*-methylbenzimidazolium triflate (*N*-MeBIT),[11] *N*-(cyanomethyl)pyrrolidinium tetrafluoroborate (*N*-CMPyrTBF),[5] and 1-hydroxybenzotriazole (HOBT).[12] The synthesis was carried out according to the following procedure. The nucleoside phosphoramidite *R*c-**1** or *S*c-**2**




*Rc*,*Rp*-**5a**, B = Ade$^{Bz}$
*Rc*,*Rp*-**5b**, B = Cyt$^{Bz}$
*Rc*,*Rp*-**5c**, B = Gua$^{dmf}$
*Rc*,*Rp*-**5d**, B = Thy




*Sc*,*Sp*-**6a**, B = Ade$^{Bz}$
*Sc*,*Sp*-**6b**, B = Cyt$^{Bz}$
*Sc*,*Sp*-**6c**, B = Gua$^{dmf}$
*Sc*,*Sp*-**6d**, B = Thy

(0.1 mmol) was reacted with the 5′-*O*-free thymidine **4** (0.1 mmol) in the presence of a promoter (0.1 mmol) and 3-Å molecular sieves in acetonitrile (0.5 mL) at 25 °C for 5–10 min. This reaction mixture was treated with acetic anhydride (0.2 mmol) in pyridine (1.0 mmol) followed by treatment with bis[3-(triethoxysilyl)propyl]tetrasulfide (TEST) [13] (0.1 mmol) at 25 °C for 5 min. The crude product was subjected to extractive workup and chromatography to obtain the desired dinucleoside phosphorothioate as a mixture of two diastereoisomers, *Rc*,*Rp*-**5** and *Sc*,*Sp*-**6**. Subsequently, the *p*-configuration of the two diastereoisomers was determined as follows. First, the protected TpsT compound, which was prepared from the phosphoramidite *Rc*-**1c** and includes two diastereoisomers in a 90:10 ratio, was selected among the obtained dinucleoside phosphorothioates and converted into the unprotected TpsT by treatment with dichloroacetic acid to remove the 5′-*O*-DMTr group, with aqueous ammonia to remove the *N*-acetylpyrrolidinylmethyl group on the phosphorothioate function, and with tetrabutylammonium fluoride to eliminate the 3′-*O*-TBDMS group. The resulting product, containing two components in a 90:10 ratio (analyzed by HPLC; the major and minor components have longer and shorter retention times, respectively), was subjected to enzymatic digestions with snake venom phosphodiesterase (SVPDE) and nuclease P1.[14] In these reactions, the major component was completely hydrolyzed by nuclease P1, but left intact by treatment with SVPDE. On the other hand, the minor component was completely decomposed in the reaction using SVPDE, but did not undergo hydrolysis in the case using nuclease P1. These experiments indicated that major and minor components in the product are *R*p-TpsT and *S*p-TpsT, respectively. Consequently, the major and minor diastereoisomers in the initial protected TpsT compound are *Rc*,*Rp*-**5d** and *Sc*,*Sp*-**6d**, respectively. The ratio of *Rc*,*Rp*- and *Sc*,*Sp*-isomers in the protected ApsT, CpsT, and GpsT products was estimated by comparing their $^{31}$P NMR spectroscopic data with the $^{31}$P NMR spectroscopic data of *Rc*,*Rp*-**5d** and *Sc*,*Sp*-**6d**. These estimated ratios are shown in Table 1 and Table 2. The chemical yields of the products listed in Table 1 and Table 2 were almost quantitative, unless otherwise noted. In all cases, regardless of the stereochemistry of the phosphoramidite, the azolium triflates showed higher stereoselectivity than 1*H*-tetrazole. For example, in the synthesis of the A$^{Bz}$psT derivative using *Rc*-**1a** as the phosphoramidite, *N*-PhIMT, which is the most effective promoter among the azolium triflates in this case, produced a >99:1 mixture of *Rc*,*Rp*-**5a** and *Sc*,*Sp*-**6a**. By contrast, the synthesis using 1*H*-tetrazole as the promoter afforded an 89:11 mixture of *Rc*,*Rp*-**5a** and *Sc*,*Sp*-**6a**. In the synthesis of the A$^{Bz}$psT derivative using the phosphoramidite *Sc*-**2a**, the use of *N*-PhIMT as the promoter afforded the target product consisting of *Rc*,*Rp*-**5a** and *Sc*,*Sp*-**6a** in a 3:97 ratio. Meanwhile, the use of 1*H*-tetrazole as the promoter afforded a 10:90 mixture of *Rc*,*Rp*-**5a** and *Sc*,*Sp*-**6a** as the product. *N*-CMPyrTFB, which showed excellent stereoselectivity in the Wada method, also served as an ef-

Table 1. Stereoselective synthesis of dithymidine phosphorothioates **5d** and **6d**.

| Phosphoramidite | Promoter | Ratio of isomers *Rp*-**5d**:*Sp*-**6d** |
|---|---|---|
| *Rc*-**1d** | *N*-phenylimidazolium triflate | 98:2 |
| *Rc*-**1d** | *N*-methylbenzimidazoium triflate | 97:3 |
| *Rc*-**1d** | benzimidazoium triflate | 97:3 |
| *Rc*-**1d** | *N*-(cyanomethyl)pyrrolidinium triflate | 98:2 |
| *Rc*-**1d** | 2,4-dinitrobenzoic acid | 94:6 |
| *Rc*-**1d** | 1*H*-tetrazole | 89:11 |
| *Sc*-**2d** | benzimidazoium triflate | 2:98 |
| *Sc*-**2d** | *N*-methylbenzimidazoium triflate | 2:98 |
| *Sc*-**2d** | *N*-phenylimidazolium triflate | 3:97 |
| *Sc*-**2d** | 1*H*-tetrazole | 4:96 |
| *Sc*-**2d** | *N*-(cyanomethyl)pyrrolidinium triflate | 5:95 |
| *Sc*-**2d** | trichloroacetic acid | 27:73 |
| *Sc*-**2d** | trichloroacetic acid | 29:71 |
| *Sc*-**2d** | 2,4-dinitrobenzoic acid | 29:71 |
| *Sc*-**2d** | 1-hydroxybenzotriazole | –[a] |

[a] Yield of the desired product was very low. Several byproducts were obtained.

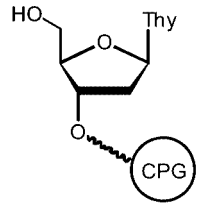
fective promoter similar to azolium triflates in the stereoselective synthesis of the dinucleoside phosphorothioates using both *R*c- and *S*c-phosphoramidites of adenosine, guanosine, and thymidine, but this promoter was not useful for the stereoselective synthesis of the $C^{Bz}$psT derivative *R*c,*R*p-**5b** or *S*c,*S*p-**6b** using the cytidine phosphoramidite *R*c-**1b** or *S*c-**2b**, respectively. For example, the stereoselectivities in the syntheses of *R*c,*R*p-**5b** and *S*c,*S*p-**4b** using *R*c-**1b** and *S*c-**2b** as the phosphoramidite, respectively, were 72% de and 48% *de*, respectively. These de values were lower than those obtained by the synthesis using benzimidazolium triflate (94% de for *R*c,*R*p-**5b** and 90% de for *S*c,*S*p-**6b**) and 1*H*-tetrazole (86% de for *R*c,*R*p-**5b** and 88% de for *R*c,*S*p-**6b**) as the promoter. According to the results of the stereoselective synthesis of *S*c,*S*p-**6d** using *S*c-**2d**, carboxylic acids are generally not effective for this purpose. The same synthesis indicated that HOBT is not useful as a promoter for the reaction of the Agrawal phosphoramidite; in this synthesis, a number of undesired products were obtained.

Table 2. Stereoselective synthesis of various dinucleoside phosphorothioates in solution phase.

| Phosphoramidite | Promoter | Main product | Ratio of isomers *R*p:*S*p |
|---|---|---|---|
| *R*c-**1a** | *N*-phenylimidazolium triflate | *R*c-**5a** | >99:<1 |
| *R*c-**1a** | *N*-(cyanomethyl)pyrrolidinium triflate | *R*c-**5a** | >99:<1 |
| *R*c-**1a** | benzimidazoium triflate | *R*c-**5a** | 98:2 |
| *R*c-**1a** | *N*-methylbenzimidazoium triflate | *R*c-**5a** | 98:2 |
| *R*c-**1a** | 1*H*-tetrazole | *R*c-**5a** | 89:11 |
| *S*c-**2a** | *N*-phenylimidazolium triflate | *S*c-**6a** | 3:97 |
| *S*c-**2a** | *N*-methylbenzimidazoium triflate | *S*c-**6a** | 4:96 |
| *S*c-**2a** | benzimidazoium triflate | *S*c-**6a** | 5:95 |
| *S*c-**2a** | 1*H*-tetrazole | *S*c-**6a** | 10:90 |
| *S*c-**2a** | *N*-(cyanomethyl)pyrrolidinium triflate | *S*c-**6a** | 13:87 |
| *R*c-**1b** | benzimidazoium triflate | *R*c-**5b** | 97:3 |
| *R*c-**1b** | *N*-phenylimidazolium triflate | *R*c-**5b** | 96:4 |
| *R*c-**1b** | *N*-methylbenzimidazoium triflate | *R*c-**5b** | 95:5 |
| *R*c-**1b** | 1*H*-tetrazole | *R*c-**5b** | 93:7 |
| *R*c-**1b** | *N*-(cyanomethyl)pyrrolidinium triflate | *R*c-**5b** | 86:14 |
| *S*c-**2b** | benzimidazoium triflate | *S*c-**6b** | 5:95 |
| *S*c-**2b** | *N*-phenylimidazolium triflate | *S*c-**6b** | 8:92 |
| *S*c-**2b** | 1*H*-tetrazole | *S*c-**6b** | 11:89 |
| *S*c-**2b** | *N*-methylbenzimidazoium triflate | *S*c-**6b** | 12:88 |
| *S*c-**2b** | *N*-(cyanomethyl)pyrrolidinium triflate | *S*c-**6b** | 26:74 |
| *R*c-**1c** | benzimidazoium triflate | *R*c-**5c** | 99:1 |
| *R*c-**1c** | *N*-phenylimidazolium triflate | *R*c-**5c** | 97:3 |
| *R*c-**1c** | *N*-methylbenzimidazoium triflate | *R*c-**5c** | 96:4 |
| *R*c-**1c** | *N*-(cyanomethyl)pyrrolidinium triflate | *R*c-**5c** | 96:4 |
| *R*c-**1c** | 1*H*-tetrazole | *R*c-**5c** | 88:12 |
| *S*c-**2c** | *N*-phenylimidazolium triflate | *S*c-**6c** | 1:99 |
| *S*c-**2c** | *N*-methylbenzimidazoium triflate | *S*c-**6c** | 1:99 |
| *S*c-**2c** | benzimidazoium triflate | *S*c-**6c** | 2:98 |
| *S*c-**2c** | *N*-(cyanomethyl)pyrrolidinium triflate | *S*c-**6c** | 2:98 |
| *S*c-**2c** | 1*H*-tetrazole | *S*c-**6c** | 5:95 |

### Stereoselectivity in the Solid-Phase Synthesis of Dinucleoside Phosphorothioates

Subsequently, we investigated the stereoselectivity in the solid-phase synthesis of *R*p-TpsT and *S*p-TpsT through the condensation (5 min) of the thymidine **7** attached to controlled pore glass (CPG) and the phosphoramidite *R*c-**1d** or *S*c-**2d**, respectively, using BIT, *N*-MeBIT, *N*-PhIMT, 1*H*-tetrazole, or *N*-CMPyrTFB as the promoter. After the condensation, the resulting product was treated with (1) acetic anhydride in pyridine (0.3 min) to block the amino group in the pyrrolidinylmethyl substituent on the phosphite moiety, (2) TEST in the presence of *N*-methylimidazole[13] (10 min) to convert the phosphite to the phosphorothioate, (3) dichloroacetic acid (1.3 min) to remove the 5′-*O*-DMTr protecting group, and (4) conc. ammonia at 55 °C (16 h) to eliminate the *N*-acetylpyrrolidinylmethyl group on the phosphorothioate moiety and to detach the product from the solid support. This sequence of reactions gave TpsT including *R*p- and *S*p-isomers, which were identified by comparison of the HPLC retention times and $^{31}$P NMR chemical shifts with those of authentic samples of *R*p-TpsT and *S*p-TpsT. The ratios of the products obtained by several syntheses are summarized in Table 3. In the solid-phase synthesis, the azolium triflates generally showed greater ability than 1*H*-tetrazole as a promoter for the stereoselective synthesis of nucleoside phosphorothioates via the Agrawal strategy.




**7**

Table 3. Stereoselective synthesis of TpsT on solid phase.

| Phosphoramidite | Promoter | Ratio of isomers in the product *R*p:*S*p |
|---|---|---|
| *R*c-**1d** | *N*-phenylimidazolium triflate | 90:10 |
| *R*c-**1d** | *N*-methylbenzimidazoium triflate | 89:11 |
| *R*c-**1d** | benzimidazoium triflate | 90:10 |
| *R*c-**1d** | *N*-(cyanomethyl)pyrrolidinium triflate | 92:8 |
| *R*c-**1d** | 1*H*-tetrazole | 89:11 |
| *S*c-**2d** | benzimidazoium triflate | 6:94 |
| *S*c-**2d** | *N*-methylbenzimidazoium triflate | 7:93 |
| *S*c-**2d** | *N*-phenylimidazolium triflate | 7:93 |
| *S*c-**2d** | *N*-(cyanomethyl)pyrrolidinium triflate | 14:86 |
| *S*c-**2d** | 1*H*-tetrazole | 20:80 |
| *S*c-**2d** | trichloroacetic acid | 50:50 |

## Conclusions

We have revealed that certain azolium triflates, such as *N*-phenylimidazolium triflate, benzimidazolium triflate, and *N*-methylbenzimidazolium triflate, serve as more effective promoters than the conventionally used 1*H*-tetrazole for the stereoselective condensation of *R*c-**1** or *S*c-**2** and a nu-

cleoside, which is a key reaction for the synthesis of stereodefined nucleoside phosphorothioates according to the Agrawal method. Compared with the related synthetic methods developed by Just and Wada, the Agrawal method has a great advantage in terms of the ease of preparation of the phosphoramidite building blocks, but also the disadvantage of inferior stereoselectivity of the condensation of the nucleoside phosphoramidite and the nucleoside. The present study improved this defect and enhanced the value of the Agrawal method. Further, we suggested that $^{1}H$ and $^{31}P$ NMR analyses are not suitable for conclusive determination the *p*-configurations of the Agrawal phosphoramidites, *R*c-**1** or *S*c-**2**, and alternative, absolutely reliable methods, such as X-ray analysis, are necessary for this purpose.

## Experimental Section

**General Remarks:** NMR spectra were taken on a JEOL JNM-α400 or ECA-500 instrument. The $^{1}H$, $^{13}C$, and $^{31}P$ NMR chemical shifts are described as $\delta$ values in ppm relative to $(CH_3)_4Si$ (for $^{1}H$ and $^{13}C$) and 85% $H_3PO_4$, respectively. ESI-TOF high resolution mass (HRMS) spectra were obtained on Applied Biosystems Voyager MDE and Mariner spectrometers, respectively. HPLC analysis was carried out using a COSMOSIL $5C_{18}$-MS column (Nacalai Tesque, ODS-5 mm, 4.6 × 250 mm) on a Waters 2695 Separations Module chromatograph with a Waters 2996 Photodiode Array detector. Column chromatography was performed using Nacalai Tesque silica gel 60 (neutrality, 75 μm). Unless otherwise noted, synthetic reactions were carried out at ambient temperature. The reactions requiring anhydrous conditions were achieved under an argon atmosphere in flasks dried by heating at 400 °C under 133–400 Pa, or by washing with a 5% solution of dichlorodimethylsilane in dichloromethane, followed by washing with anhydrous dichloromethane, and then heating at 100 °C. The phosphoramidites **1**[2] and **2**[2] and nucleoside **4**[15] were prepared by reported methods. CPG-anchored nucleoside **7** was purchased from Applied Biosystems. *N*-Phenylimidazolium triflate,[10] *N*-methylbenzimidazolium triflate,[10] benzimidazoium triflate·[9] *N*-(cyanomethyl)pyrrolidinium triflate[5] were prepared by reported methods. Acetonitrile, DMF, and dichloromethane were distilled from calcium hydride. Other organic reagents were used as commercially supplied without any purification. Solid and amorphous organic substances were used after drying over $P_2O_5$ at 50–60 °C for 8–12 h under 133–400 Pa. Powdered 3-Å molecular sieves (MS) were employed after drying the commercially supplied product (Nacalai tesque) at 200 °C for 12 h under 133–400 Pa.

**Synthesis of (*R*)-*N*-Acetyl-2-pyrrolidinylmethyl $N^6$-(Benzoyl)-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyadenin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*R*)-Phosphorothioate (*R*c,*R*p-5a) with Benzimidazolium Triflate as a Promoter:** A Typical Procedure for the Synthesis of Dinucleoside Phosphorothioate in a Solution Phase: The phosphoramidite *R*c-**1a** (79 mg, 0.1 mmol), nucleoside **4** (35 mg, 0.1 mmol), and 3-Å molecular sieves (20 mg) were added to acetonitrile (10 mL) and stirred for 30 min. To the resulting mixture was added benzimidazolium triflate (27 mg, 0.1 mmol), and after 5 min pyridine (79 mg, 79 μL, 1.0 mmol) and acetic anhydride (20 mg, 19 μL, 0,2 mol) were added. Stirring was continued for 5 additional min. To this mixture was added TEST (108 mg, 0.1 mL, 0.2 mmol) and the resulting mixture was further stirred for 5 min. The insoluble material was removed by filtration. The filtrate was diluted with dichloromethane (50 mL). The resulting organic solution was washed with a saturated aqueous sodium hydrogen carbonate solution (10 mL) followed by brine (10 mL), then dried and concentrated to give an amorphous solid. This crude product was subjected to silica gel (25 g) column chromatography using a 30:1, 20:1 to 10:1 mixture of dichloromethane and methanol as the eluent to provide the desired nucleotide *R*c,*R*p-**5a** containing a small amount of its *R*c,*S*p-isomer (*R*c,*R*p-**5a**:the *R*c,*S*p-isomer = 98:2, 117 mg, 96% yield) as an amorphous solid. $^{1}H$ NMR of *R*c,*R*p-**5a**: $\delta$ = 0.09 (s, 6 H), 0.89 (s, 9 H), 1.91–2.25 (m, 12 H), 3.35–3.49 (m, 4 H), 3.70–3.79 (m, 8 H), 4.03–4.41 (m, 7 H), 5.41–5.45 (m, 1 H), 6.16–6.25 (m, 1 H), 6.46–6.56 (m, 1 H), 6.77–6.81 (m, 4 H), 7.18–7.59 (m, 13 H), 8.04 (d, *J* = 7.8 Hz, 2 H), 8.20 (s, 1 H), 8.72 (s, 1 H), 9.29 (br. s, 2 H) ppm. $^{13}C$ NMR: $\delta$ = –4.77, –4.62, 12.48, 18.28, 20.78, 22.84, 24.07, 25.69, 27.28, 38.74, 40.50, 48.04, 53.41, 55.20, 55.23, 56.00, 56.10, 58.49, 63.29, 67.10, 67.35, 67.42, 71.64, 80.06, 80.10, 84.67, 85.10, 85.22, 85.28, 86.86, 111.14, 113.26, 123.59, 127.04, 128.09, 128.78, 129.12, 130.11, 132.75, 133.54, 135.36, 135.83, 141.39, 144.27, 149.75, 150.20, 151.61, 152.48, 158.61, 164.81, 169.87 ppm. $^{31}P$ NMR: $\delta$ = 67.19 ppm. HRMS ($ESI^+$): *m*/*z* calcd. for [M + $H^+$]. $C_{61}H_{74}N_8O_{13}PSSi$ 1217.4598, found 1217.5411.

**(*R*)-*N*-Acetyl-2-pyrrolidinylmethyl $N^4$-(Benzoyl)-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxycytidin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*R*)-Phosphorothioate (*R*c,*R*p-5b):** $^{1}H$ NMR: $\delta$ = 0.09 (s, 6 H), 0.89 (s, 9 H), 1.91–2.40 (m, 15 H), 2.85–2.99 (m, 1 H), 3.39–3.52 (m, 4 H), 3.74–4.46 (m, 9 H), 5.17–5.22 (m, 1 H), 6.22–6.34 (m, 2 H), 6.83–6.88 (m, 4 H), 7.21–7.68 (m, 14 H), 7.90 (d, *J* = 7.8 Hz, 2 H), 8.13–8.18 (m, 1 H) ppm. $^{13}C$ NMR: $\delta$ = –4.65, –4.52, 12.56, 16.42, 17.99, 18.42, 20.92, 22.94, 24.15, 25.80, 27.36, 40.60, 48.13, 53.51, 55.34, 58.57, 62.41, 62.54, 67.20, 71.79, 79.34, 85.05, 85.30, 85.46, 85.67, 87.01, 87.33, 111.17, 113.24, 113.48, 123.90, 127.34, 127.79, 128.19, 129.07, 129.23, 130.10, 130.17, 133.29, 135.06, 135.22, 136.02, 143.97, 149.71, 150.19, 158.84, 163.72, 169.96, 175.31 ppm. $^{31}P$ NMR: $\delta$ = 67.48 ppm. HRMS ($ESI^+$): *m*/*z* calcd. for [M + $Na^+$]. $C_{60}H_{73}N_6O_{14}PSSiNa$ 1215.4305, found 1215.5332.

**(*R*)-*N*-Acetyl-2-pyrrolidinylmethyl $N^2$-(Dimethylaminomethylene)-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyguanosin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*R*)-Phosphorothioate (*R*c,*R*p-5c):** $^{1}H$ NMR: $\delta$ = 0.07 (s, 6 H), 0.88 (s, 9 H), 1.88–2.26 (m, 10 H), 2.61–2.79 (m, 2 H), 3.06 (s, 3 H), 3.13 (s, 3 H), 3.31–3.44 (m, 4 H), 3.77 (s, 6 H), 4.01–4.40 (m, 7 H), 5.28–5.29 (m, 1 H), 6.19 (t, *J* = 6.8 Hz, 1 H), 6.29–6.33 (m, 1 H), 6.79 (d, *J* = 7.8 Hz, 4 H), 7.19–7.40 (m, 11 H), 7.70 (s, 1 H), 8.56 (s, 1 H), 9.01 (br., 1 H) ppm. $^{13}C$ NMR: $\delta$ = –4.70, –4.54, 12.64, 17.99, 18.37, 22.91, 24.16, 25.76, 27.30, 29.01, 35.26, 39.09, 40.64, 41.42, 48.09, 55.34, 56.06, 56.13, 63.43, 67.42, 71.72, 79.71, 83.15, 84.88, 85.09, 85.68, 86.90, 111.16, 113.36, 127.10, 128.04, 128.17, 130.11, 130.15, 135.42, 135.49, 135.73, 135.92, 144.39, 150.10, 150.23, 156.97, 157.86, 158.16, 158.71, 163.49, 169.93 ppm. $^{31}P$ NMR: $\delta$ = 67.48 ppm. HRMS ($ESI^+$): *m*/*z* calcd. for [M + $Na^+$]. $C_{57}H_{74}N_9O_{13}PSSiNa$ 1206.4526, found 1206.5835.

**(*R*)-*N*-Acetyl-2-pyrrolidinylmethyl 5′-*O*-(*p,p*′-Dimethoxytrityl)thymidin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*R*)-Phosphorothioate (*R*c,*R*p-5d):** $^{1}H$ NMR: $\delta$ = 0.09 (s, 6 H), 0.89 (s, 9 H), 1.89–2.58 (m, 12 H), 3.36–3.48 (m, 4 H), 3.75–3.83 (m, 8 H), 3.96–4.42 (m, 7 H), 5.34–5.39 (m, 1 H), 6.20 (t, *J* = 6.4 Hz, 1 H), 6.37–6.41 (m, 1 H), 6.83 (d, *J* = 8.8 Hz, 4 H), 7.23–7.38 (m, 10 H), 7.57 (s, 1 H), 9.00 (br., 1 H), 9.09 (br., 1 H) ppm. $^{13}C$ NMR: $\delta$ = –4.80, –4.68, 12.48, 17.87, 18.26, 22.78, 22.93, 23.70, 24.02, 25.67, 27.24, 28.89, 30.33, 38.71, 39.05, 39.11, 40.48, 48.00, 48.28, 55.24, 55.91, 56.00, 58.46, 63.52, 66.95, 67.01, 67.31, 67.37, 68.12, 71.56,

80.07, 80.11, 84.46, 84.83, 84.91, 84.99, 85.65, 87.31, 111.09, 111.60, 113.36, 127.19, 128.04, 128.77, 130.02, 130.82, 135.08, 135.19, 135.82, 144.13, 150.24, 150.32, 158.78, 163.60, 169.74 ppm. $^{31}$P NMR: $\delta$ = 67.34 ppm. HRMS (ESI$^+$): *m*/*z* calcd. for [M + Na$^+$]. $C_{54}H_{70}N_5O_{14}PSSiNa$ 1126.4039, found 1126.4496.

**(*S*)-*N*-Acetyl-2-pyrrolidinylmethyl *N*$^6$-(Benzoyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*S*)-Phosphorothioate (*Sc*,*Sp*-6a):** $^1$H NMR: $\delta$ = 0.08 (s, 6 H), 0.89 (s, 9 H), 1.92 (s, 3 H), 2.01 (s, 3 H), 1.88–2.26 (m, 8 H), 2.77 (dd, *J* = 6.1, 13.9 Hz, 1 H), 3.08–3.15 (m, 1 H), 3.39–3.46 (m, 1 H), 3.76 (s, 6 H), 3.99–4.29 (m, 4 H), 4.38 (d, *J* = 3.3 Hz, 2 H), 5.43–5.47 (m, 1 H), 6.26 (t, *J* = 6.8 Hz, 1 H), 6.50–6.53 (m, 1 H), 6.78–6.81 (m, 4 H), 7.18–7.62 (m, 13 H), 8.14 (d, *J* = 7.8 Hz, 2 H), 8.21 (s, 1 H), 8.69 (s, 1 H), 9.26 (br. s, 2 H) ppm. $^{13}$C NMR: $\delta$ = –4.68, –4.55, 12.64, 18.00, 22.95, 24.09, 25.79, 27.35, 38.38, 40.71, 48.12, 53.52, 55.32, 56.15, 56.23, 63.27, 67.22, 67.25, 67.39, 71.91, 80.26, 84.86, 85.22, 85.28, 85.38, 86.91, 111.32, 113.33, 123.73, 127.10, 128.02, 128.13, 128.87, 129.14, 130.11, 132.85, 133.65, 135.48, 135.70, 141.80, 144.38, 149.76, 150.36, 151.75, 152.53, 158.70, 163.77, 170.04 ppm. $^{31}$P NMR: $\delta$ = 67.56 ppm. HRMS (ESI$^+$): *m*/*z* calcd. for [M + H$^+$]. $C_{61}H_{74}N_8O_{13}PSSi$ 1217.4598, found 1217.5351.

**(*S*)-*N*-Acetyl-2-pyrrolidinylmethyl *N*$^4$-(Benzoyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*S*)-Phosphorothioate (*Sc*,*Sp*-6b):** $^1$H NMR: $\delta$ = 0.07 (s, 6 H), 0.88 (s, 9 H), 1.88–2.26 (m, 15 H), 2.89–2.95 (m, 1 H), 3.39–3.50 (m, 4 H), 3.78, 3.79 (2 s, 6 H), 3.96–4.39 (m, 9 H), 5.29–5.34 (m, 1 H), 6.24 (t, *J* = 6.8 Hz, 1 H), 6.30 (t, *J* = 6.4 Hz, 1 H), 6.83–6.87 (m, 4 H), 7.21–7.71 (m, 14 H), 7.90 (d, *J* = 7.3 Hz, 2 H), 8.13 (d, *J* = 7.8 Hz, 1 H), 8.73 (br., 1 H) ppm. $^{13}$C NMR: $\delta$ = –4.70, –4.57, 12.62, 17.98, 22.96, 23.05, 23.83, 24.21, 24.29, 25.77, 27.42, 29.01, 30.44, 38.81, 40.57, 40.66, 48.20, 48.28, 48.33, 53.51, 56.13, 56.21, 56.41, 62.21, 62.81, 67.20, 67.25, 67.54, 67.59, 68.23, 71.88, 79.12, 85.13, 85.20, 85.35, 85.53, 85.58, 87.24, 87.35, 111.28, 113.48, 127.30, 128.18, 128.88, 129.07, 130.11, 130.20, 130.96, 132.54, 133.25, 134.98, 135.19, 135.70, 143.95, 150.21, 158.83, 163.65, 167.84, 170.00 ppm. $^{31}$P NMR: $\delta$ = 67.17 ppm. HRMS (ESI$^+$): *m*/*z* calcd. for [M + Na$^+$]. $C_{60}H_{73}N_6O_{14}PSSiNa$ 1215.4305, found 1215.5270.

**(*S*)-*N*-Acetyl-2-pyrrolidinylmethyl *N*$^2$-(Dimethylaminomethylene)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl (*S*)-Phosphorothioate (*Sc*,*Sp*-6c):** $^1$H NMR: $\delta$ = 0.06 (s, 6 H), 0.88 (s, 9 H), 1.88–2.24 (m, 10 H), 2.53–2.82 (m, 2 H), 3.07 (s, 3 H), 3.13 (s, 3 H), 3.28–3.43 (m, 4 H), 3.77 (s, 6 H), 3.96–4.41 (m, 7 H), 5.38–5.42 (m, 1 H), 6.23 (t, *J* = 6.7 Hz, 1 H), 6.33 (t, *J* = 7.3 Hz, 1 H), 6.78–6.81 (m, 4 H), 7.19–7.40 (m, 11 H), 7.67 (s, 1 H), 8.57 (s, 1 H), 9.11 (br., 1 H) ppm. $^{13}$C NMR: $\delta$ = –4.81, –4.67, 12.55, 17.86, 18.27, 22.81, 23.95, 25.64, 25.66, 27.19, 35.11, 35.18, 40.55, 41.28, 41.34, 47.98, 55.21, 58.45, 63.35, 67.19, 71.74, 79.24, 83.05, 85.46, 86.79, 111.03, 113.19, 113.24, 120.57, 126.98, 127.90, 128.06, 130.00, 130.04, 135.20, 135.30, 135.72, 135.80, 136.05, 144.25, 150.04, 155.78, 156.84, 157.77, 157.84, 158.02, 158.58, 163.48, 169.91 ppm. $^{31}$P NMR: $\delta$ = 69.44 ppm. HRMS (ESI$^+$): *m*/*z* calcd. for [M + Na$^+$]. $C_{57}H_{74}N_9O_{13}PSSiNa$ 1206.4526, found 1206.5649.

**(*S*)-*N*-Acetyl-2-pyrrolidinylmethyl 5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidin-3′-yl 3′-*O*-(*tert*-Butyldimethylsilyl)-thymidin-5′-yl (*S*)-Phosphorothioate (*Sc*,*Sp*-6d):** $^1$H NMR: $\delta$ = 0.05 (s, 6 H), 0.89 (s, 9 H), 1.40 (s, 3 H), 1.89–2.60 (m, 12 H), 3.36–3.46 (m, 4 H), 3.75–3.76 (m, 1 H), 3.78 (s, 6 H), 3.94–4.35 (m, 8 H), 5.38–5.41 (m, 1 H), 6.22 (t, *J* = 6.9 Hz, 1 H), 6.40–6.43 (m, 1 H), 6.83 (d, *J* = 9.2 Hz, 4 H), 7.22–7.38 (m, 10 H), 7.56 (s, 1 H), 9.12 (br., 1 H), 9.09 (br., 1 H) ppm. $^{13}$C NMR: $\delta$ = –4.71, –4.56, 11.76, 12.63, 17.98, 22.95, 24.12, 25.77, 27.39, 39.16, 40.65, 48.15, 53.53, 55.36, 56.11, 56.19, 63.51, 67.28, 67.45, 71.81, 80.01, 84.47, 84.93, 85.07, 85.41, 87.38, 111.31, 111.78, 113.48, 127.31, 128.16, 130.15, 135.12, 135.31, 135.73, 144.21, 150.35, 150.54, 158.86, 163.81, 163.86, 170.04 ppm. $^{31}$P NMR: $\delta$ = 69.23 ppm. HRMS (ESI$^+$): *m*/*z* calcd. for [M + Na$^+$]. $C_{54}H_{70}N_5O_{14}PSSiNa$ 1126.4039, found 1126.4176.

**Solid-Phase Synthesis of *R*p- and *S*p-TpsT:** Synthesis of *R*p-TpsT and *S*p-TpsT was carried out according to the reaction cycle shown in Table 4 using the phosphoramidites ***R*p-1d** and ***S*p-2d**, respectively, as monomer units. After chain elongation was completed, the resulting solid material was treated with concd. ammonia at 55 °C for 16 h to detach the target product.

Table 4. Reaction sequence of the solid-phase synthesis.

| Step | Operation | Reagent(s) | Time [min] |
|---|---|---|---|
| 1 | washing | $CH_3CN$ | 0.4 |
| 2 | detritylation | 3% $Cl_3CCOOH$ | 1.3 |
| 3 | washing | $CH_3CN$ | 0.8 |
| 4 | coupling | 0.1 M amidite/$CH_3CN$ | 5.0 |
| 5 | washing | $CH_3CN$ | 0.2 |
| 6 | capping | $Ac_2O$/2,6-lutidine/THF(1:1:8) + *N*-methylimidazole/THF | 0.3 |
| 7 | washing | $CH_3CN$ | 0.2 |
| 8 | sulfurization | 0.1 M TEST/0.5 M *N*-methylimidazole/DMF | 10.0 |
| 9 | washing | $CH_3CN$ | 0.6 |

## Acknowledgments

This study was partly supported by Grants-in-Aid for Scientific Research (Nos. 16011223 and 16350086) from the Ministry of Education, Culture, Science, Sports and Technology of Japan. We also acknowledge the contributions of Ms. Mari Morimura, who carried out some of the preliminary examinations, and Dr. Takeshi Wada, The University of Tokyo, who provided valuable input on the determination of the *p*-configurations of the phosphoramidites.

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Wada method, which is the best existing method for gaining high stereoselectivity, gave this target product in >98% *de*.

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Received: June 7, 2005
Published Online: September 23, 2005

  