Stereoselective Synthesis of Dithymidine Phosphorothioates Using Xylose Derivatives as Chiral Auxiliaries

Yi Jin and George Just*

Department of Chemistry, McGill University, Montreal, Canada H3A 2K6

Received December 29, 1997

1,2-*O*-Cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **1** and its enantiomer **11** were used as chiral auxiliaries to form, respectively, Sp and Rp dithymidine phosphorothioates **9** and **10** in 98% diastereomeric excess, using phosphoramidite methodology and 2-bromo-4,5-dicyanoimidazole as catalyst. The stereochemistry of the coupling reaction is discussed.

Introduction

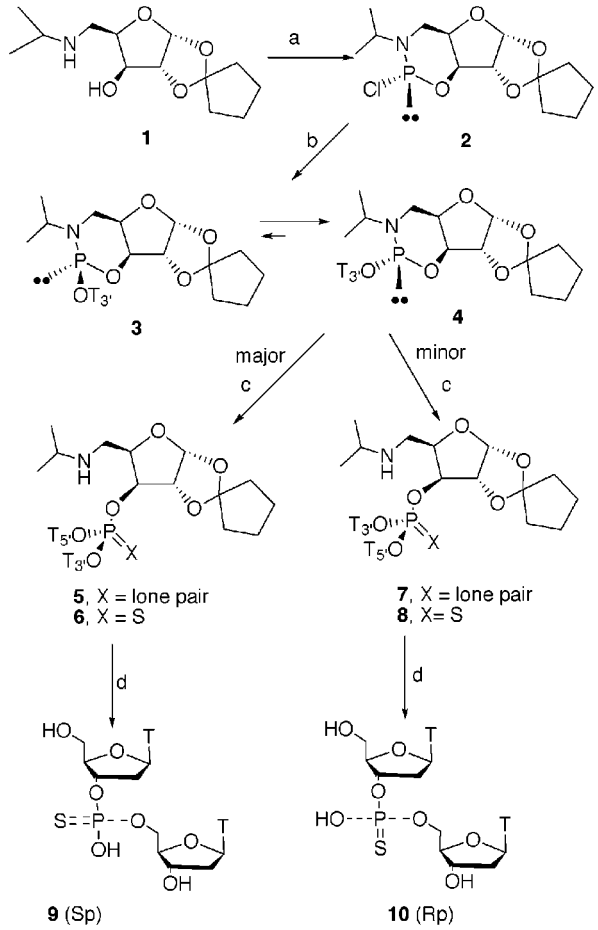
The effect of oligonucleotide phosphorothioates (PS-oligos) as antisense inhibitors of gene expression is well recognized,[1] and their therapeutic potential has been demonstrated in clinical trials against AIDS and cancer.[2] The PS-oligos currently employed in clinical studies and biological evaluations are obtained as mixtures of 2^n diastereomers, where *n* is equal to the number of internucleotidic phosphorothioate linkages. To date only one generally applicable stereoselective synthesis methodology of PS-oligos has been described by Stec et al.[3] It unfortunately cannot be scaled up due to a difficult chromatographic separation of the required chiral precursors. Therefore, there still exists a need to develop practical stereoselective syntheses of PS-oligos.

In a preliminary communication,[4] we have described the usefulness of xylose derivative **1** and **11** as chiral auxiliary for a stereocontrolled synthesis of phosphite triesters and therefore of phosphorothioates. In what follows, we give the full details of our work in this area.

Results and Discussion

The known 1,2-*O*-cyclopentylidene-5-tosyl-α-D-xylofuranose was synthesized from D-xylose by modification of a method described by Tronchet et al.[5] The tosylate function was then displaced with isopropylamine to provide 1,2-*O*-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **1**. The overall yield for the transformation from D-xylose to **1** was ~60%. Reaction of γ-amino alcohol **1** with PCl_3 and triethylamine in dry chloroform provided phosphorochloridite **2**. Its ^{31}P NMR showed a single peak at 148.42 ppm. By analogy with phosphoramidite **4**, it most likely has the Rp stereochemistry depicted in Scheme 1. Reaction of the phosphorochloridite **2** with 5′-O-*tert*-butyldimethylsilylthymidine (**$T_{3'}$OH**) in dry chloroform gave a mixture of phosphoramidites **4** and **3** (^{31}P NMR 129.8 and 138.7 ppm) in a ratio which was time and temperature dependent. At −78 °C, the two isomers were formed in a 3:2 ratio, whereas at rt, the ratio was >10:1. After overnight heating at 50 °C, the minor isomer **3** corresponding to the peak at 138.7 ppm was quantitatively isomerized to the thermodynamically more stable isomer **4** (129.8 ppm). Both isomers were configurationally stable after extraction and removal of triethylammonium chloride and not separable by flash chromatography.

Scheme 1

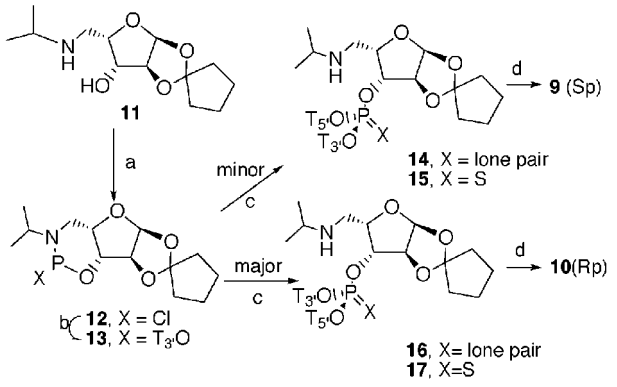


(a) PCl_3, NEt_3, $CHCl_3$, 0 °C - 50 °C. (b) $T_{3'}OH$, NEt_3, $CHCl_3$, 0 °C - 50 °C. (c) $T_{5'}OH$, 2-bromo-4,5-dicyanoimidazole, CH_3CN or $CHCl_3$; then Beaucage's reagent. (d) 70% TFA, RT

(1) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
(2) Zon, G.; Stec, W. J. Phosphorothioate Oligonucleotides. In *Oligonucleotides Analogues: A Practical Approach*; Eckstein, F. Ed.; The Practical Approach Series; IRL Press: 1991, pp 87−108.
(3) (a) Stec, W. J.; Wilk, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 709. (b) Stec, W. J.; Grajkowski, A.; Kobylanska, A.; Karwowski, B.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019.
(4) Jin, Y.; Biancotto, G.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 973.
(5) Tronchet, J. M. J.; Zosimo-Landolfo, G.; Villedon-Denaide, F.; Balkadjian, M.; Cabrini, D.; Barbalat-Rey, F. *J. Carbohydr. Chem.* **1990**, *9(6)*, 823.

S0022-3263(97)02318-9 CCC: $15.00
Published on Web 05/07/1998

Scheme 2



(a) PCl_3, NEt_3, $CHCl_3$, 0 ^{0}C - 50 ^{0}C. (b) $T_{3'}OH$, NEt_3, $CHCl_3$, 0 ^{0}C - 50 ^{0}C. (c) $T_{5'}OH$, 2-bromo-4,5-dicyanoimidazole, CH_3CN or $CHCl_3$; then Beaucage's reagent. (d) 70% TFA, RT

The coupling reaction between phosphoramidite **4** and 3′-*O*-*tert*-butyldimethylsilyl thymidine (**$T_{5'}OH$**) was first carried out at room temperature in acetonitrile, using 2-bromo-4,5-dicyanoimidazole as catalyst. It provided phosphite triesters **5** and **7** in a ratio of 6:1, as established by ^{31}P NMR (143.76 and 142.55 ppm for the major and minor isomer, respectively). Without purification, the mixture of triesters was sulfurized with Beaucage's reagent[6] to give a 6:1 mixture of phosphorothioates **6** and **8**, ^{31}P NMR 68.23 and 68.43 ppm. When a similar coupling reaction was carried out in $CHCl_3$ at 0 °C for 4 h or at −15 °C for 6 h, the ratio of **5** to **7** increased to 40:1 and 68:1, respectively. Chromatography of the reaction mixture obtained at −15 °C provided one isomer of **6** only. After hydrolysis of **6**/**8** with 70% trifluoroacetic acid at rt, the phosphorothioate dinucleosides **9** (^{31}P NMR 55.70 ppm) and **10** (^{31}P NMR 56.10 ppm) was obtained in 85% yield with an Sp:Rp diastereomer ratio the same as the ratio of **6** to **8**. The configurations of the major diastereomer **9** and minor diastereomer **10** were established as Sp and Rp, respectively by snake venom phosphodiesterase and P1 nuclease digestion and also by HPLC analysis.[7] It is opposite to the one we had tentatively and erroneously assigned[4] because the chemical shifts of ^{31}P NMR for the phosphorothioate dinucleosides **9** and **10** are opposite in CD_3OD and D_2O. In D_2O, the signal of ^{31}P NMR corresponding to the Sp diastereomer occurs at higher field (^{31}P NMR 55.70 ppm) than that of the *R*p diastereomer (^{31}P NMR 56.10 ppm),[8] while in CD_3OD the signal of ^{31}P NMR corresponding to the *S*p diastereomer occurs at lower field (^{31}P NMR 58.64 ppm) than that of the *R*p diastereomer (^{31}P NMR 58.57 ppm).

In a parallel run, L-xylose was transformed to the amino alcohol **11**. In a series of reactions identical to those described (Scheme 2), **11** was converted via phosphorochloridite **12** (^{31}P NMR 148.75 ppm) to phosphoramidite **13** (^{31}P NMR 129.34 ppm). Phosphoramidite **13** then underwent the coupling reaction with **$T_{5'}OH$** to give **14** and **16** which were then sulfurized with Beaucage's reagent to give **15** and **17** (^{31}P NMR 68.91 and 69.12 ppm). The diastereomeric ratio of **15** and **17** was 1:7 when the coupling reaction was performed at rt in acetonitrile. When the coupling reaction was performed at −15 °C in chloroform, the diastereomeric ratio of **15** and **17** was 1:70 as determined by ^{31}P NMR. Only isomer **17** could be isolated by chromatography. After hydrolysis of **15/17** with 70% TFA at rt, the phosphorothioate dinucleosides **9** and **10** were obtained with an *S*p:*R*p diastereomeric ratio identical to that of **15** to **17**.

In summary, the use of chiral auxiliary **1** derived from D-xylose leads to phosphorothioate dinucleoside **9** (*S*p), while use of the chiral auxiliary **11** derived from L-xylose leads to phosphorothioate dinucleoside **10** (*R*p).

The essential step in the synthesis of the phosphite triester and therefore of phosphorothioate is the azole-activated (tetrazole or, in our case, 2-bromo-4,5-dicyanoimidazole) coupling reaction of the phosphoramidite with the free 5′-hydroxylfunction of thymidine. The mechanism of this activation process still remains to be clarified. The main question is whether azole serves only as a proton donor yielding as primary product a protonated nucleoside phosphoramidite or whether it will attack as a nucleophile giving rise to the formation of an azolide intermediate.[9] In the first case, after protonation of phosphoramidite, the free 5′-hydroxyl group of thymidine (**$T_{5'}OH$**) reacts with inversion of configuration at the phosphorus center to give the desired phosphite triester. The second assumption involves the displacement of the amine function of the amidite by the azole after protonation, followed by either displacement of the azole by another azole leading to a diastereomeric mixture of phosphorazolides or by displacement of the azole by an alcohol which gives the desired phosphite triester. The phosphite triesters are then sulfurized. The latter reaction is known to proceed with retention of configuration.[10]

The stereochemistry of a reaction is one important clue to the reaction mechanism. With structure assignments for the intermediate phosphoramidites **3** and **4** being somewhat ambiguous, we first tried to obtain crystals of phosphoramidite **4** or of the corresponding thioate **4S** (^{31}P NMR 67.5 ppm) or **3S** (^{31}P NMR 72.4 ppm)[11] for X-ray crystallographic structure determination. Neither of them gave suitable crystals. The same was also true for phosphoramidites **18a**−**d** (^{31}P NMR 129.0, 128.2, 125.3, and 127.5 ppm, respectively), in which the exocyclic thymidine was replaced by 2-propanol, cholesterol, and *p*-nitrophenol, and for their sulfur derivatives **19a**−**d** (^{31}P NMR 67.5, 66.6, 62.0, and 66.8 ppm) (Scheme 3).

The structural assignment for the phosphoramidites **3** and **4** was therefore made as described by Huang et al. and others.[12−15] They showed that in bicyclic six-membered ring phosphites the 1,3,2-dioxaphosphorinane

(6) (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859−62. (b) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253−54. (c) For a review, see: Beaucage, S. L., Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223−2311.

(7) The snake venom phosphodiesterase and P1 nuclease digestion and HPLC analysis were carried out at ISIS Pharmaceuticals (Carlsbad, CA) by Dr. M. Manoharan.

(8) (a)Uznanski, B.; Niewiarowski, W.; Stec, W. J. *Tetrahedron Lett.* **1982**, *23*, 4289−4292. (b). Lesnikowski, Z. J.; Jaworska, M. M. *Tetrahedron Lett.* **1989**, *30*, 3821−3824.

(9) Berner, S.; Muhlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853.

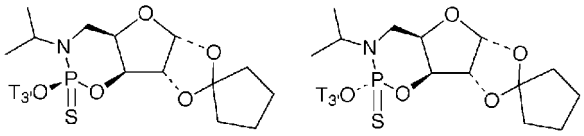
(10) (a) Fujii, M.; Ozaki, K.; Sekine, M. and Hata,T. *Tetrahedron* **1987,** *43*, 3395−3407. (b) Mikolajczyk, M. *J. Chem. Soc., Chem. Commun.* **1969**, 1221.

(11) Although the phosphoramidites **3** and **4** are not separable, the phosphorothioamidates **3S** and **4S** can be separated by a chromatography method.

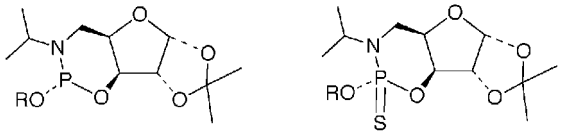
(12) Huang, Y.; Yu, J.; Bentrude, W. G. *J. Org. Chem.* **1995**, *60*, 4767−4773.

(13) (a) Hermans, R. J. M.; Buck, J. M. *Phosphorus Sulfur* **1987**, *31*, 255. (b) Nelson, K. A.; Sopchik, A. E.; Bentrude, W. G. *J. Am. Chem. Soc.* **1983**, *105*, 7752.

Scheme 3



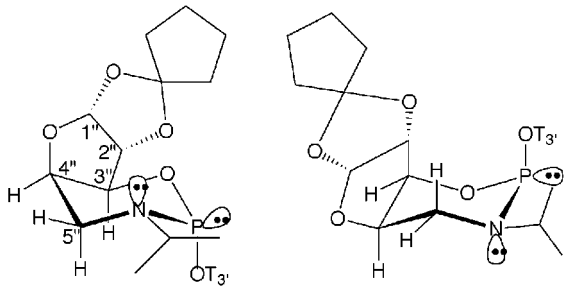
3S **4S**



18a: R = $OT_{3'}$
18b: R = isopropyl
18c: R = *p*-nitrophenyl
18d: R = cholesteryl

19a: R = $OT_{3'}$
19b: R = isopropyl
19c: R = *p*-nitrophenyl
19d: R = cholesteryl

Scheme 4



4a **4b**

Table 1. Selected ^{13}C NMR Coupling Constants[a]

comp	$J_{C3'-P}$	$J_{C2''-P}$	$J_{C3''-P}$	$J_{C4''-P}$	$J_{C5''-P}$	J_{CHN-P}
3S	4.6	12	6.3	1.8	5.5	6.4
4S	5.5	11	9.2	4.6	7.3	6.4
3	10	4.6	2	2.7	3.7	37.5
4	19.2	4.6	3.7	1.8	3.2	36.6

[a]Solvent: $CDCl_3$. The carbons of the branched-chain sugar units at the 3′-end of the molecule are denoted by C′ and the carbons of the sugar at the chiral auxiliary are denoted by C″.

ring preferentially adopts a chair conformation with the exocyclic OR attached to the phosphorus group in an axial position. The thermodynamically less stable isomers, however, showed a preference for a twist conformation of the dioxaphosphorinane ring in which the OR group occupies a pseudoaxial position[12] instead of a chair conformation in which the OR group would occupy an equatorial position. By analogy, we suggest that the thermodynamically more stable phosphoramidite **4** has the thymidine residue at the axial position of the six-membered ring, which has a chair conformation (Scheme 4). This is based on the ^{13}C NMR data: the large ^2J coupling constant between the $C_{3'}$ of thymidine and phosphorus of 19.2 Hz, the analogous coupling of 3.7 Hz for C−O−P of the oxazaphosphorinane carbon, and the small coupling constant of 1.8 Hz for $J_{C4''-P}$ in isomer **4** (Table 1).

Therefore, there are only two possible conformers, **4a** (Sp) and **4b** (Rp) for the isomer **4** (Scheme 4), of which **4a** should be strongly favored because of the far smaller 1,3-diaxial interactions of the P−$OT_{3'}$ bond.

The conformational analyses of the six-membered rings were also based on the values of $^3J_{POCH}$ and $^3J_{HCCH}$.

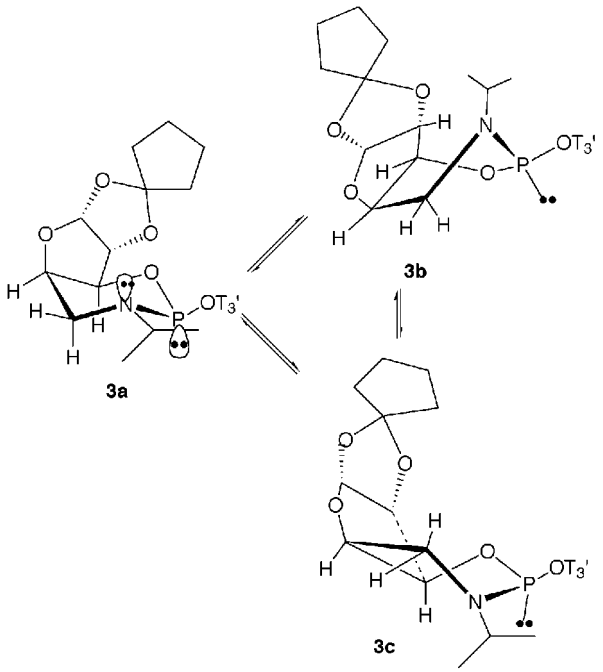
(14) Huang, Y.; Arif, A. M.; Bentrude, W. G. *J. Org. Chem.* **1993**, *58*, 6235.
(15) Van Heeswijk, W. A. R.; Goedhart, J. B.; Vliegenthart, J. F. G. *Carbohyd. Res.* **1977**, *58*, 337−344.

Table 2. Selected ^1H NMR Coupling Constants[a]

comp	$J_{H3''-H4''}$	$J_{H3''-P}$	$J_{H4''-H5''a}$	$J_{H4''-H5''e}$	$J_{H5''a-P}$	$J_{H5''e-P}$	$J_{H3'-P}$
4	2.4	1.0	2.4	2.4	2.4	7.1	10.5
3	3.9	3.2	7.3	7.3	4.7	6.1	9.0
4S	2.4	2.4	2.4	2.4	3.2	20	10
3S	4.1	5.3	6.6	6.4	12	19	10

[a]Solvent: $CDCl_3$. The protons of the branched-chain sugar units at the 3′-end of the molecule are denoted by H′ and the protons of the sugar at the chiral auxiliary are denoted by H″.

Scheme 5



3a **3b** **3c**

Coupling constants $^3J_{PNCH}$ have not been found to be useful in conformational analyses of three-coordinate phosphorus-containing heterocyclic six-membered rings.[12,14]

The data observed for conformer **4a** are similar to those found in the previously studied three-coordinate 1,3,2-dioxa- and oxazaphosphorinanes that populate analogous chair conformations with substituents attached axially to phosphorus. Thus the small values of $^3J_{H3''-P}$ (1.0 Hz), $^3J_{H3''-H4''}$ (2.4 Hz), $^3J_{H4''-H5a''}$ (2.4 Hz), $^3J_{H4''-H5e''}$ (2.4 Hz), and $^3J_{H5a''-P}$ (2.4 Hz) recorded are characteristic of gauche HCCH and HCOP arrangements, while the large value of $^3J_{H5e''-P}$ (7.1 Hz) observed is typical of the antiperiplanar HCOP geometry (Table 2).

The conformation of **4** was also confirmed by a 2D NOE experiment in which $H_{5''a}$ showed NOE with the $H_{3''}$. The 2D NOE experiment also showed a cross-peak between $H_{3''}$ and $H_{4'}$ which tells us that $H_{3''}$ and $OT_{3'}$ should be on the same side of the six-membered ring. No detectable NOE's with $H_{2''}/H_{5''a}$ or $H_{1''}/H_{5''a}$ were observed.

Using a similar line of reasoning, twist conformation **3c** is the most likely conformation for **3** (Scheme 5).

When we used a 3:2 mixture of **4** and **3** to carry out the coupling reaction with thymidine derivative **$T_{3'}$OH** under the same coupling reaction conditions as for diastereomer **4**, we obtained the phosphite triesters **5** and **7** in a ratio of 3:2. Treatment with Beaucage's reagent gave a 3:2 mixture of phosphorothioates **6** and **8** (^{31}P NMR 68.23 and 68.43 ppm). We can therefore conclude that the two isomers gave reversed diastereoselectivity in the coupling reaction, with **3** giving a phosphorothioate with the *R*p configuration. We also observed that the

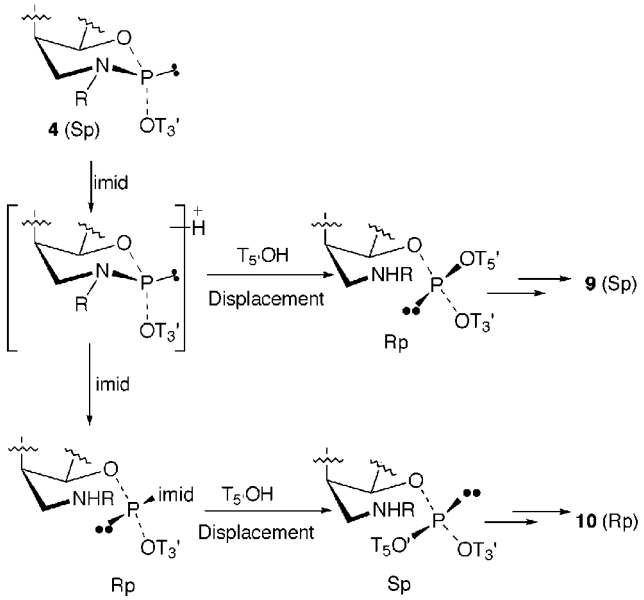
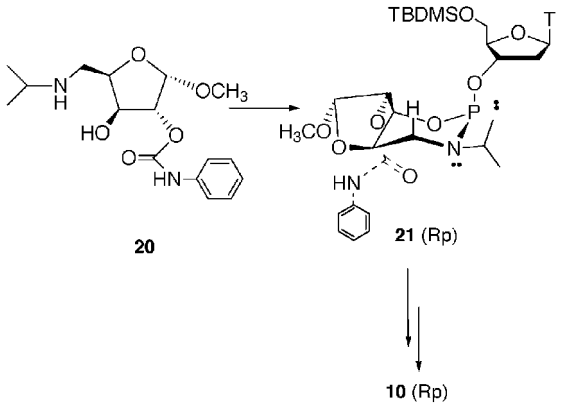


Figure 1. The mechanism of the coupling reaction (imid = 2-bromo-4,5-dicyanoimidazole or its anion).

Scheme 6



thermodynamically less favored diastereomer **3** is considerably more reactive than diastereomer **4**.

Analysis of the stereochemistry of phosphoramidite **4** (*S*p) and phosphorothioate **9** (*S*p) indicates that the transformation involves one inversion only for this particular system (Figure 1). This excludes the double inversion postulated by Stec and Zon[16] and by Seliger et al.[9] Indeed, when bromoacetic acid ($pK_a = 2.86$) was used as activator in the coupling reaction, the same phosphite triesters **5** and **7** were obtained in a ratio of 4:1. This is to be compared to a 6:1 ratio when 2-bromo-4,5-dicyanoimidazole was used as catalyst, using identical reaction conditions. This change in reaction path is particularly puzzling because the use of a closely related chiral auxiliary **20** (Scheme 6) gave the opposite *R*p stereochemistry.[17] If the mechanism we suggested here is general, the phosphoramidite **21** formed from chiral auxiliary **20** should have opposite configuration (*R*p).

Effect of the Acidity of the Catalyst on the Stereoselectivity and Rate of the Reaction. When trying to apply the 2-bromo-4,5-dicyanoimidazole-catalyzed coupling reaction of the phosphoramidite to 5′-dimethoxytritylthymidine, we realized that the reaction conditions were too acidic, resulting in hydrolysis of the dimethoxytrityl group. The same was true when the thymidine was substituted at 5′ with a monomethoxytrityl function. A trityl group appeared to be stable but is not suitable for automated DNA synthesis. We therefore investigated other less acidic catalysts to see if 2-bromo-4,5-dicyanoimidazole could be replaced without loss of stereoselectivity of the reaction.

First, we tried to examine the pK_a effect of catalyst. Since 1*H*-tetrazole ($pK_a = 4.8$) is a commonly used efficient activator for the coupling reactions,[9] we tried using it in our coupling reaction. ^{31}P NMR showed the coupling reaction to be very slow and without good stereoselectivity. We also tried other catalysts with $pK_a > 4.8$, such as 4,5-dicyanoimidazole ($pK_a = 5.2$), 2-benzyl-4,5-dicyanoimidazole ($pK_a = 5.3$), 2-bromo-4,5-diethylcarboxylimidazole ($pK_a = 6.4$), and 2-nitroimidazole ($pK_a = 6.4$). The ^{31}P NMR showed that the coupling reactions were very slow and without stereoselectivity. Then we tried using a catalyst with $pK_a < 4.8$. Benzimidazolium triflate ($pK_a = 4.5$) and *p*-nitrophenyltetrazole ($pK_a = 3.7$) have been reported to be better activators than 1*H*-tetrazole,[18] but the coupling reaction was slow and without stereoselectivity in our system.

Since increasing the size of the substituents of imidazole seemed to give higher stereoselectivity,[19] we synthesized 2-iodo-4,5-dicyanoimidazole and found that it did not give better stereoselectivity than 2-bromo-4,5-dicyanoimidazole. Also it is not a good catalyst choice because of its high acidity.

Conclusion

We have shown that diastereomerically pure cyclic phosphoramidites **4** and **13** obtained without chromatographic purification can lead diastereoselectively to Sp and Rp dinucleoside phosphorothioates **9** and **10**, respectively. The conditions employed here were suitable for solution synthesis but are for the time being not applicable to solid phase synthesis. The stereochemistry of the coupling reaction suggested involves only one inversion at phosphorus in the case of 1,2-*O*-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose **1** or 1,2-*O*-cyclopentylidene-5-deoxy-5-isopropylamino-α-L-xylofuranose **11** derived phosphoramidites, if our analysis of the conformers of the intermediate phosphoramidites is correct. It should however be noticed that a closely related system described in Scheme 6 gives the opposite stereochemistry.

Experimental Section

Materials and Methods. NMR spectra were recorded at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, and 202 MHz for ^{31}P NMR.[20] Chloroform was distilled from P_2O_5; triethylamine and acetonitrile were distilled from CaH_2. Tetrazole and Beaucage's reagent were gifts from Isis Pharmaceuticals. All reactions involving trivalent phosphorus compounds were performed under dry argon.

1,2-*O*-Cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose (1). A solution of 1,2-*O*-cyclopentylidene-5-

(16) Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279.
(17) Marsault, E.; Just, G. *Tetrahedron* **1997**, *53*, 16945−16958.
(18) (a) Froehler, B. C.; Matteucci, M. *D. Tetrahedron Lett.* **1983**, *24*, 3171. (b) Pon, R. T. *Tetrahedron Lett.* **1987**, *28*, 3643. (c) Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996.
(19) Xin, Z.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 969.

tosyl-α-D-xylofuranose (2.64 g, 7.1 mmol) in isopropylamine (15 mL) was stirred at 55 °C overnight in a pressure bottle. The solvent was removed by rotary evaporator, and the remaining yellow syrup was taken up with chloroform, washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. After removal of the solvent, the mixture was chromatographed on silica gel (ethyl acetate-3% triethylamine) to furnish 1.33 g of white solid **1** (73%): mp 44−45 °C; $[\alpha]^{20}_D$ = 31.06 (c = 2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.0 (b, NH), 5.90 (1H, d, J = 3.9 Hz, H-1), 4.38 (1H, d, J = 3.9 Hz, H-2), 4.27 (1H, m, H-3), 4.20 (1H, m, H-4), 3.36−2.92 (2H, ABX, 2 × H-5), 2.74−2.70 (1H, septet, NCH), 1.95−1.63 (8H, m, cyclopentylidene protons), 1.04−1.03 (6H, dd, **Me**$_2$CH); ^{13}C NMR ($CDCl_3$) δ 121.06 (O**C**O), 104.82 (C-1), 86.14 (C-2), 78.30 (C-3), 77.07 (C-4), 48.64 (NCH), 45.90 (C-5), 36.85, 36.32 (**C**H$_2$C**C**H$_2$), 23.51, 22.84 (**C**H$_2$CH$_2$CCH$_2$**C**H$_2$), 22.64 (**CH$_3$**CHN), 22.34 (**CH$_3$**CHN); HRMS (EI) *m/e* calcd for $C_{13}H_{23}NO_4$ [M$^+$] 257.1627, found 257.1630.

Cyclic Phosphorochloridite (2). To a solution of freshly distilled phosphorus trichloride (96 mL, 1,1 mmol) in 2.5 mL of anhydrous chloroform stirred at 0 °C under Ar was added a mixture of **1** (257 mg, 1.0 mol) and triethylamine (278 mL, 2.2 mmol) in 3.5 mL of anhydrous $CHCl_3$. The mixture was heated at 50 °C, and the reaction was followed by ^{31}P NMR until a single peak was obtained. The product was not isolated and was directly used in the following step: ^{1}H NMR ($CDCl_3$) δ 5.75 (1H, d, J = 3.9 Hz, H-1), 4.51 (1H, m, H-3), 4.37 (1H, d, J = 3.9 Hz, H-2), 4.19−4.17 (1H, m, H-4), 3.42−3.34 (1H, septet, NCH), 3.33−3.00 (2H, **AB**X, 2 × H-5), 1.81−1.51 (8H, m, cyclopentylidene protons), 1.05 (6H, d, J = 6.8 Hz, **Me$_2$**-CH); ^{13}C NMR ($CDCl_3$) δ 121.48 (O**C**O), 104.26 (C-1), 83.85 (d, J = 3.7 Hz, C-2), 73.01 (d, J = 6.4 Hz, C-3), 72.35 (C-4), 50.28 (d, J = 35.7 Hz, C-5), 36.93 (d, J = 5.5 Hz, NCH), 36.62, 35.91 (**C**H$_2$C**C**H$_2$), 23.22, 22.50 (**C**H$_2$CH$_2$CCH$_2$**C**H$_2$), 20.84 (d, J = 12.8 Hz, **CH$_3$**CHN), 19.62 (d, J = 5.5 Hz, **CH$_3$**CHN); ^{31}P NMR ($CDCl_3$) δ 148.42.

Oxazaphosphorinane (4). The solution of **2** was cooled to 0 °C under Ar; then a solution of **T$_{3'}$OH** (356 mg, 1.0 mmol) in 4.5 mL of anhydrous $CHCl_3$ and triethylamine (140 mL, 1.1 mmol) was added slowly. The mixture was heated to 50 °C until a single peak corresponding to a new product was found in the ^{31}P NMR spectrum. The solution was taken up in ethyl acetate (prewashed with a saturated solution of $NaHCO_3$), washed with saturated $NaHCO_3$, and dried over $MgSO_4$. The crude product was flash chromatographyed on a silica gel column (hexane−ethyl acetate−triethylamine = 5:3:2) to furnish 510 mg of **4** as a white foam (80%): mp 68−70 °C; $[\alpha]^{20}_D$ = +62.91 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.19 (1H, bs, NH), 7.47 (1H, d, $J_{H6-CH3C=C}$ = 1.4 Hz, H-6), 6.34−6.31 (1H, dd, $J_{H1'-H2a'}$ = 5.6 Hz, $J_{H1'-H2b'}$ = 8.6 Hz, H-1′), 5.89 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-1″), 4.57−4.54 (1H, ddt, $J_{H3'-H4'}$ = $J_{H3'-H2a'}$ = 2.2 Hz, $J_{H3'-H2b'}$ = 6.6 Hz, $J_{H3'-P}$ = 10.5 Hz, H-3′), 4.41 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-2″), 4.35 (1H, dd, $J_{H3''-H4''}$ = 2.4 Hz, $J_{H3''-P}$ = 1.0 Hz, H-3″), 4.17 (1H, q, $J_{H4''-H3''}$ = $J_{H4''-H5a''}$ = $J_{H4''-H5e''}$ = 2.4 Hz, H-4″), 4.06 (1H, q, $J_{H4'-H3'}$ = $J_{H4'-H5a'}$ = $J_{H4'-H5b'}$ = 2.2 Hz, H-4′), 3.90−3.77 (2H, ABX, 2 × H-5′), 3.47−3.41 (1H, d septet, $J_{NCH-CH3}$ = 6.6 Hz, J_{NCH-P} = 11.7 Hz, NCH), 3.47−3.44 (1H, dt, $J_{H5a''-H5e''}$ = 14.2 Hz, $J_{H5a''-H4''}$ = $J_{H5a''-P}$ = 2.4 Hz, H-5a″), 3.06−3.01 (1H, ddd, $J_{H5e''-H4''}$ = 2.4 Hz, $J_{H5e''-H5a''}$ = 14.2 Hz, $J_{H5e''-P}$ = 7.1 Hz, H-5e″), 2.39−2.35 (1H, ddd, $J_{H2a'-H1'}$ = 5.9 Hz, $J_{H2a'-H3'}$ = 2.2 Hz, $J_{H2a'-H2b'}$ = 13.7 Hz, H-2′), 2.11−2.06 (1H, ddd, $J_{H2b'-H1'}$ = 8.4 Hz, $J_{H2b'-H3'}$ = 6.6 Hz, $J_{H2b'-H2a'}$ = 14 Hz, H-2′), 1.90 (3H, d, J = 1.5 Hz, MeC=C), 1.97−1.62 (8H, m, cyclopentylidene protons), 1.13−1.10 (6H, dd, J = 6.8 Hz, J = 7.8 Hz, **Me$_2$CH**), 0.91 (9H, s, *t*-BuSi), 0.10 (6H, d, J = 1.5 Hz, **Me$_2$**Si); ^{13}C NMR ($CDCl_3$) δ 163.38 (C-4), 150.10 (C-2), 135.24 (C-6), 121.46 (O**C**O), 110.94 (C-5), 104.63 (C-1″), 86.44 (d, J = 2.8 Hz, C-4′), 84.83 (C-1′), 84.82 (d, J = 7.3 Hz, C-2″), 73.75 (d, J = 19.2 Hz, C-3′), 73.07 (d, J = 1.8 Hz, C-4″), 71.85 (d, J = 3.7 Hz, C-3″), 63.21 (C-5′), 50.0 (d, J = 36.6 Hz, NCH), 40.28 (d, J = 5.5 Hz, C-2′), 36.92, 36.24 (**C**H$_2$C**C**H$_2$), 36.12 (d, J = 3.2 Hz, C-5″), 25.94 (SiC**CH$_3$**), 23.49, 22.80 (**C**H$_2$CH$_2$CCH$_2$**C**H$_2$), 22.03 (d, J = 9.2 Hz, **CH$_3$**CHN), 21.70 (d, J = 6.4 Hz, **CH$_3$**CHN), 18.36 (Si**C**CH$_3$), 12.52 (**CH$_3$**C=C), −5.37, −5.44 (**Me$_2$**Si); ^{31}P NMR ($CDCl_3$) δ 129.80; HRMS (FAB, glycerol) *m/e* calcd for $C_{29}H_{49}N_3O_9PSi$ [MH$^+$] 642.2973, found 642.2976.

Oxazaphosphorinane (3). The reaction procedure and workup were analogous to those of **4** except the reaction was done at −78 °C and workup was done at 0 °C. After purification with silica gel, a mixture of **3** and **4** was obtained in a ratio of 2:3. The NMR spectrum of **3** was obtained by subtracting the spectrum of **4** from the spectrum of the mixture of **3** and **4**: ^{1}H NMR ($CDCl_3$) δ 8.19 (1H, bs, NH), 7.45 (1H, d, J = 1.4 Hz, H-6), 6.34 (1H, dd, $J_{H1'-H2a'}$ = 5.1 Hz, $J_{H1'-H2b'}$ = 8.3 Hz, H-1′), 5.97 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-1″), 4.71, 4.68 (1H, ddt, $J_{H3'-4'}$ = $J_{H3'-2a'}$ = 2.2 Hz, $J_{H3'-2b'}$ = 6.1 Hz, $J_{H3'-P}$ = 9.0 Hz, H-3′), 4.56 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-2″), 4.09 (1H, dd, $J_{H3''-H4''}$ = 3.4 Hz, $J_{3''-P}$ = 3.2 Hz, H-3″), 4.51 (1H, dt, $J_{H4''-H3''}$ = 3.9 Hz, $J_{H4''-H5a''}$ = $J_{H4''-H5e''}$ = 7.3 Hz, H-4″), 4.04 (1H, q, $J_{H4'-H3'}$ = $J_{H4'-H5a'}$ = $J_{H4'-H5b'}$ = 2.2 Hz, H-4′), 3.86−3.73 (2H, ABX, 2 × H-5′), 3.50 (1H, d.septet, $J_{NCH-CH3}$ = 6.8 Hz, J_{NCH-P} = 10.7 Hz, NCH), 3.21 (1H, ddd, $J_{H5e''-H4''}$ = 7.8 Hz, $J_{H5e''-H5b''}$ = 13.7 Hz, $J_{H5e''-P}$ = 6.1 Hz, H-5e″), 3.08 (1H, ddd, $J_{H5a''-H4''}$ = 6.9 Hz, $J_{H5a''-H5e''}$ = 13.7 Hz, $J_{H5a'-P}$ = 4.7 Hz, H-5a″), 2.37 (1H, m, H-2′), 2.02 (1H, m, H-2′), 1.88 (3H, d, J = 1.5 Hz, **Me**C=C), 1.97−1.62 (8H, m, cyclopentylidene protons), 1.13−1.10 (6H, m, **Me$_2$**CH), 0.89 (9H, s, t-BuSi), 0.08 (6H, s, **Me$_2$**Si); ^{13}C NMR ($CDCl_3$) δ 163.57 (C-4), 150.08 (C-2), 135.33 (C-6), 121.42 (OCO), 110.78 (C-5), 105.76 (C-1″), 86.78 (d, J = 3.7 Hz, C-4′), 84.80 (C-1′), 84.53 (d, J = 4.6 Hz, C-2″), 73.03 (d, J = 10 Hz, C-3′), 78.72 (d, J = 2.7 Hz, C-4″), 75.82 (d, J = 2.0 Hz, C-3″), 63.06 (C-5′) 48.16 (d, J = 37.5 Hz, NCH), 40.30 (d, J = 4.5 Hz, C-2′), 37.03, 36.62 (**CH$_2$C**C**H$_2$**), 38.91 (d, J = 3.7 Hz, C-5″), 25.90 (SiC**Me$_3$**), 23.39, 23.09 (**CH$_2$**CH$_2$CCH$_2$**CH$_2$**), 22.58 (d, J = 8.0 Hz, **CH$_3$**CHN), 22.29 (d, J = 3.7 Hz, **CH$_3$**-CHN) 18.33 (Si**C**Me$_3$), 12.51 (**CH$_3$**C=C), −5.35, −5.47 (**Me$_2$**-Si); ^{31}P NMR ($CDCl_3$) δ 138.70.

Phosphorothioates (6/8). A mixture of phosphoramidite **4** (15 mg, 0.0234 mmol), **T$_{5'}$OH** (10 mg, 1.2 equiv) and 2-bromo-4,5-dicyanoimidazole (9.2 mg, 2.0 equiv) was dried at high vacuum overnight. Anhydrous acetonitrile (0.6 mL) was added at 0 °C under Ar. The solid dissolved instantly. The reaction was warmed to rt, and its course was followed by ^{31}P NMR. Within 5 min, the peak corresponding to the phosphoramidite had disappeared and two new peaks corresponding to **5** and **7** (143.76 ppm, 142.55 ppm = 6:1) appeared: MS (FAB, nitrobenzyl alcohol) [M + H$^+$] calcd for $C_{45}H_{77}N_5PO_{14}Si_2$ phosphite triester 998, found 998.4. This product was used in the following sulfurization without isolation. To this solution was added Beaucage's reagent (5.6 mg, 1.2 equiv) in 140 μL of acetonitrile (0.2 M). Instantaneously the ^{31}P NMR showed another two peaks corresponding to **6** and **8** (68.23 ppm, 68.43 ppm = 6:1), while the peaks corresponding to the starting material disappeared. The solvent was evaporated, and the mixture was redissolved in ethyl acetate, washed with saturated $NaHCO_3$ and water, and dried over $MgSO_4$. The crude product was purified on a silica gel column (ethyl acetate:methanol = 95:5) to give 22 mg of a white solid (91%). When a similar reaction was carried out at 0 °C for 4 h and at −15 °C for 6 h, using chloroform as the solvent, the ratio of **6** and **8** increased to 40:1 and 68:1, respectively. Chromatography of the reaction mixture obtained at −15 °C only provided one isomer (**6**). ^{1}H NMR ($CDCl_3$) δ 7.46 (1H, d, J = 1.5 Hz, ^{5}H-6), 7.23 (1H, d, J = 1.5 Hz, ^{3}H-6), 6.37−6.35 (1H, dd, J = 5.4 Hz, J = 9.3 Hz, ^{5}H-1′), 6.18 (1H, t, J = 6.4 Hz, ^{3}H-1′), 5.87 (1H, d, J = 3.9 Hz, H-1″), 5.14 (1H, dd, J = 5.4 Hz, J = 9.3 Hz, ^{5}H-3′), 4.84 (1H, dd, J = 2.9 Hz, J = 12.2 Hz, H-3″), 4.59 (1H, d, J = 3.9 Hz, H-2″), 4.35 (m, 1H, H-4″), 4.26−4.20 (4H, m, ^{3}H-3′, 2 × ^{3}H-5′, ^{5}H-4′), 4.00 (1H, m, ^{3}H-4′), 3.86 (2H, m, 2 × ^{5}H-5′), 2.83 (2H, m, 2 × H-5″), 2.77 (1H, septet, NCH), 2.45 (1H, m, ^{5}H-2′), 2.25 (2H, m, 2 × ^{3}H-2′), 2.15 (1H, m, ^{5}H-2′), 1.92

(20) ^{1}H and ^{13}C NMR chemical shifts are expressed in ppm relative to the internal tetramethylsilane (TMS). Positive ^{31}P NMR chemical shifts are expressed in ppm downfield from external 85% H_3PO_4 with proton-decoupling ({^{1}H}). ^{1}H NMR peak assignments for all the compounds were derived from 2D COSY and NOE (compounds **3**, **4, 3S, 4S, 13**) experiments. ^{13}C NMR peak assignments for all compounds are derived from 2D HMQC experiments.

(3H, d, J = 1.0 Hz, $^3CH_3C{=}C$), 1.90 (3H, d, J = 1.0 Hz, $^5CH_3C{=}C$), 1.97−1.61 (8H, m, cyclopentylidene protons), 1.02 (6H, dd, J = 5.9 Hz, Me_2CHN), 0.92 (9H, s, ^{3}t-BuSi), 0.87 (9H, s, ^{5}t-BuSi), 0.13 (6H, s, Me_2Si), 0.07 (6H, s, Me_2Si); ^{13}C NMR ($CDCl_3$) δ 163.97, 163.66 (^{5}C-4, ^{3}C-4), 150.39, 150.04 (^{5}C-2, ^{3}C-2), 135.58, 134.49 (^{5}C-6, ^{3}C-6), 121.77 (OCO), 111.26, 111.04 (^{5}C-5, ^{3}C-5), 104.00 (C-1″), 85.57 (d, J = 6.4 Hz, ^{5}C-4′), 85.48 (^{3}C-1′), 84.65 (d, J = 9.2 Hz, ^{3}C-4′), 84.39 (^{5}C-1′), 83.47 (C-2″), 80.70 (d, J = 4.6 Hz, C-3″), 80.68 (d, J = 4.6 Hz, ^{5}C-3′), 79.00 (d, J = 7.3 Hz, ^{3}C-3′), 71.49 (C-4″), 67.66, 67.52 (d, J = 4.6 Hz, ^{3}C-5′), 63.47 (^{5}C-5′), 48.93 (NCH), 45.23 (C-5″), 40.37 (^{3}C-2′), 39.12 (d, J = 6.4 Hz, ^{5}C-2′), 37.06, 36.16 (**$CH_2C\mathbf{CH_2}$**), 25.88, 25.60 (^{5}Si**Me_3**, ^{3}Si**Me_3**), 23.52, 22.85 (**$CH_2CH_2CCH_2\mathbf{CH_2}$**), 22.65 22.39 (NCH**$Me_2$**), 18.28, 17.81 (^{5}Si**C**Me_3, ^{3}Si**C**Me_3), 12.51, 12.46 ($^5C{=}C\mathbf{CH_3}$, $^3C{=}C\mathbf{CH_3}$), −4.66, −4.83, −5.40, −5.46 (^{5}-Si**Me2**, ^{3}Si**Me_2**); ^{31}P NMR ($CDCl_3$) δ 68.23; HRMS (FAB, glycerol-CsI) *m/e* calcd for $C_{45}H_{77}N_5O_{14}PSSi_2$ [MH^+] 1030.4460, found 1030.4464.

Phosphorothioate Dithymidine (9). The protected phosphorothioate dinucleoside **6** (14 mg, 0.0136 mmol) was dissolved in 1.0 mL of 70% TFA−H_2O at 0 °C and allowed to stir at rt for 2 h. Evaporation of the solvent and coevaporation with methanol furnished a white solid which was purified by chromatography to give 7.2 mg of white solid **9** (94%): ^{1}H NMR (CD_3OD) δ 7.87 (s, 1H, ^{5}H-6), 7.85 (1H, s, ^{3}H-6), 6.36−6.33 (1H, dd, J = 6.4 Hz, J = 7.8 Hz, ^{5}H-1′), 6.30−6.27 (1H, dd, J = 6.4 Hz, J = 7.3 Hz ^{3}H-1′), 5.06−5.03 (1H, m, ^{5}H-3′), 4.53−5.52 (1H, m, ^{3}H-3′), 4.21−4.06 (4H, m, ^{5}H-4′, 2 × ^{3}H-5′, ^{3}H-4′), 3.84−3.80 (2H, m, 2 × ^{5}H-5′), 2.50−2.46 (1H, m, ^{5}H-2′), 2.31−2.24 (2H, m, 2 × ^{3}H-2′), 2.21−2.17 (1H, m, ^{5}H-2′), 1.96 (3H, s, ^{3}-$CH_3C{=}C$), 1.87 (3H, s, $^5CH_3C{=}C$); ^{31}P NMR (D_2O) δ 55.70; HRMS (FAB, glycerol) *m/e* calcd for $C_{20}H_{28}N_4O_{11}PS$ [MH^+] 563.11347, found 563.11350. HPLC showed only a single peak. When the mixture of protected phosphorothioates **6**/**8** was hydrolyzed in the same reaction condition, the phosphorothioate dinucleosides **9**/**10** were obtained with an *S*p:*R*p diastereomer ratio the same as the ratio of **6** to **8**.

1,2-*O*-Cyclopentylidene-5-deoxy-5-isopropylamino-α-L-xylofuranose (11). Using the same procedure as described for **1**, **11** was obtained in a 72% yield from 1,2-*O*-cyclopentylidene-5-tosyl-α-L-xylofuranose: mp 39−41 °C; $[\alpha]^{20}_D$ = −31.37 (C = 2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.0 (bs, NH), 5.90 (1H, d, J = 3.9 Hz, H-1), 4.38 (1H, d, J = 3.9 Hz, H-2), 4.27 (1H, m, H-3), 4.20 (1H, m, H-4), 3.36−2.92 (2H, ABX, 2 × H-5), 2.74−2.70 (1H, septet, NCH), 1.94−1.62 (8H, m, cyclopentylidene protons), 1.03−1.01 (6H, dd, J = 2.4 Hz, J = 6.4 Hz, **Me_2**CH); ^{13}C NMR ($CDCl_3$) δ 121.01 (O**C**O), 104.79 (C-1), 86.10 (C-2), 78.26 (C-3), 77.04 (C-4), 48.62 (NCH), 45.87 (C-5), 36.81, 36.28 (**$CH_2C\mathbf{CH_2}$**), 23.49, 22.81 (**$CH_2CH_2CCH_2\mathbf{CH_2}$**), 22.62 (**$CH_3$**CHN), 22.32 (**$CH_3$**CHN); HRMS (EI) *m/e* calcd for $C_{13}H_{23}NO_4$ [M^+] 257.16270, found 257.16250.

Cyclic phosphorochloridite (12) was obtained by the procedure as described for **2**. The product was not isolated, and it was used directly in the following step. **12:** ^{31}P NMR ($CDCl_3$) δ 148.75.

Oxazaphosphoranine (13) was obtained from **12** by the procedure described for **4**; mp 99−101 °C; $[\alpha]^{20}_D$ = −72.00 (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.77 (bs, 1H, NH), 7.46 (s, 1H, H-6), 6.33−6.30 (dd, J = 5.9 Hz, J = 7.8 Hz, 1H, H-1′), 5.88 (d, J = 3.9 Hz, 1H, H-1″), 4.56−4.53 (m, 1H, H-3′), 4.43 (d, J = 3.9 Hz, 1H, H-2″), 4.35 (m, 1H, H-3″), 4.18 (q, J = 2.0 Hz, 1H, H-4″), 4.05 (m, 1H, H-4′), 3.90−3.76 (ABX, 2H, 2 × H-5′), 3.45−3.42 (m, 2H, H-5″, NCH), 3.03−2.99 (m, 1H, H-5″), 2.38−2.35 (m, 1H, H-2′), 2.12−2.06 (m, 1H, H-2′), 1.89 (s, 3H, MeC=C), 1.96−1.62 (m, 8H, cyclopentylidene protons), 1.11−1.08 (dd, 6H, J = 6.4 Hz, J = 11.2 Hz, **Me_2**CH), 0.90 (s, 9H, t-BuSi), 0.09 (d, J = 2.0 Hz, 6H, Me_2Si); ^{13}C NMR ($CDCl_3$) δ 163.74 (C-4), 150.31 (C-2), 135.18 (C-6), 121.44 (O**C**O), 110.93 (C-5), 104.59 (C-1″), 86.59 (d, J = 5.5 Hz, C-4′), 84.74 (d, J = 2.8 Hz, C-2″), 84.70 (C-1′), 73.96 (d, J = 17.4 Hz, C-3′), 73.06 (d, J = 1.8 Hz, C-4″), 71.80 (d, J = 4.6 Hz, C-3″), 62.98 (C-5′), 49.91 (d, J = 36.6 Hz, NCH), 39.93 (d, J = 2.8 Hz, C-2′), 36.86, 36.19 (**$CH_2C\mathbf{CH_2}$**), 36.09 (d, J = 3.1 Hz, C-5″), 25.92 (Si**C**Me_3), 23.45, 22.76 (**$CH_2CH_2CCH_2\mathbf{CH_2}$**), 21.95 (d, J = 9.2 Hz, **CH_3**-CHN), 21.66 (d, J = 6.4 Hz, **CH_3**CHN), 18.35 (Si**C**Me_3), 12.52 (**CH_3**C=C), −5.39, −5.45 (**Me_2**Si); ^{31}P NMR ($CDCl_3$) δ 129.34; HRMS (FAB, glycerol) *m/e* calcd for $C_{29}H_{49}N_3O_9PSi$ [MH^+] 642.2973, found 642.2976.

Phosphorothioates (15 and 17). A mixture of **13** (15 mg, 0.0234 mmol), **$T_{5'}$OH** (10 mg, 1.2 equiv), and 4,5-dicyano-2-bromo-imidazole (9.17 mg, 2.0 equiv) was dried in high vacuum overnight. Anhydrous acetonitrile (0.6 mL) was added at 0 °C under Ar. The solid was dissolved instantly. The reaction was followed by ^{31}P NMR. Within 5 min, the peak corresponding to the phosphoramidite at 129.34 ppm was replaced by two new peaks corresponding to phosphite triesters **14** and **16** (142.14 ppm:140.67 ppm = 1:7). To this solution was added Beaucage's reagent (5.6 mg, 1.2 equiv) in 140 μL of acetonitrile (0.2 M) Instantaneously the ^{31}P NMR showed another two peaks corresponding to **15** and **17** (67.44 ppm:68.60 ppm = 1:7). The workup and purificatin step is the same as that of **6**/**8** to give 23 mg of white solid **15**/**17** (91%). When a similar reaction was carried out at −15 °C for 6 h, using chloroform as the solvent, the ratio of **15** and **17** increased to 1:70. After purification, only one product (**17**) was obtained: ^{1}H NMR ($CDCl_3$) δ 7.46 (d, J = 1.5 Hz, 1H, ^{5}H-6), 7.27 (d, J = 1.5 Hz, 1H, ^{3}H-6), 6.34−6.31 (dd, 1H, J = 5.2 Hz, J = 9.3 Hz, ^{5}H-1′), 6.12−6.09 (t, 1H, J = 6.6 Hz, ^{3}H-1′), 5.86 (d, J = 3.4 Hz, 1H, H-1″), 5.16 (dd, 1H, J = 5.6 Hz, J = 9.8 Hz, ^{5}H-3′), 4.82 (dd, J = 2.7 Hz, J = 11.7 Hz, 1H, H-3″), 4.55 (d, J = 3.9 Hz, 1H, H-2″), 4.39−4.37 (m, 2H, ^{3}H-3′, H-4″), 4.25−4.21 (m, 3H, 2 × ^{5}H-5′, ^{5}H-4′), 3.98 (m, 1H, ^{3}H-4′), 3.91−3.85 (m, 2H, 2 × ^{5}H-5′), 2.84 (d, J = 6.4 Hz, 2H, 2 × H-5″), 2.81 (m, 1H, NCH), 2.52−2.47 (m, 1H, ^{5}H-2′), 2.25−2.23 (m, 2H, 2 × ^{3}H-2′), 2.08−2.02 (m, 1H, ^{5}H-2′), 1.91 (d, 3H, J = 1.0 Hz, $^3CH_3C{=}C$), 1.89 (d, 3H, J = 1.0 Hz, $^5CH_3C{=}C$), 1.92−1.65 (m, 8H, cyclopentylidene protons), 1.05 (d, J = 5.9 Hz, 6H, Me_2CHN), 0.90 (s, 9H, ^{3}t-BuSi), 0.86 (s, 9H, ^{5}t-BuSi), 0.11 (s, 6H, Me_2Si), 0.05 (s, 6H, Me_2Si); ^{13}C NMR ($CDCl_3$) δ 163.80, 163.59 (^{5}C-4, ^{3}C-4), 150.33, 150.15 (^{5}C-2, ^{3}C-2), 136.13, 134.64 (^{5}C-6, ^{3}C-6), 122.03 (OCO), 111.43, 111.11 (^{5}C-5, ^{3}C-5), 104.28 (C-1″), 86.29 (^{3}C-1′), 85.93 (d, J = 6.4 Hz, ^{5}C-4′), 84.78 (d, J = 10 Hz, ^{3}C-4′), 84.69 (^{5}C-1′), 83.59 (C-2″), 80.83 (d, J = 5.5 Hz, C-3″), 80.68 (d, J = 4.6 Hz, ^{5}C-3′), 79.00 (d, J = 8.2 Hz, ^{3}C-3′), 71.30 (C-4″), 67.36 (d, J = 5.5 Hz, ^{3}C-5′), 63.37 (^{5}C-5′), 48.91 (NCH), 45.18 (C-5″), 40.35 (^{3}C-2′), 39.11 (d, J = 3.7 Hz, ^{5}C-2′), 37.14, 36.23 (**$CH_2C\mathbf{CH_2}$**), 25.88, 25.65 (^{5}Si**C**$\mathbf{Me_3}$, ^{3}Si**C**$\mathbf{Me_3}$), 23.55, 22.89 (**$CH_2CH_2CCH_2\mathbf{CH_2}$**), 22.80, 22.55 (NCH**$Me_2$**), 18.28, 17.86 (^{5}Si**C**Me_3, ^{3}Si**C**Me_3), 12.52, 12.49 ($^3C{=}C\mathbf{CH_3}$, $^5C{=}CCH_3$), −4.66, −4.83, −5.40, −5.46 (^{5}Si**Me2**, ^{3}Si**Me_2**); ^{31}P NMR ($CDCl_3$) δ 68.60; HRMS (FAB, glycerol-CsI) *m/e* calcd for $C_{45}H_{77}N_5O_{14}PSSi_2$ [MH^+] 1030.4460, found 1030.4464.

Phosphorothioate Dithymidine (10). The protected phosphorothioate dinucleoside **17** (15 mg, 0.014 mmol) was dissolved in 1.0 mL of 70% TFA−H_2O at 0 °C and allowed to stir at rt for 2 h. Evaporation of the solvent and coevaporation with methanol furnished a white solid which was purified by chromatography to give 7.3 mg of white solid **10** (94%): ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, ^{5}H-6), 7.86 (s, 1H, ^{3}H-6), 6.36−6.33 (dd, J = 6.4 Hz, J = 7.8 Hz, 1H, ^{5}H-1′), 6.29−6.26 (dd, J = 5.9 Hz, J = 7.8 Hz, ^{3}H-1′), 5.08−5.05 (m, 1H, ^{5}H-3′), 4.52−5.51 (m, 1H, ^{3}H-3′), 4.21 (m, 1H, ^{5}H-4′), 4.14−4.11 (m, 2H, 2 × ^{3}H-5′), 4.04 (m, 1H, ^{3}H-4′), 3.86−3.79 (m, 2H, 2 × ^{5}H-5′), 2.48−2.44 (m, 1H, ^{3}H-2′), 2.31−2.24 (m, 2H, 2 × ^{5}H-2′), 2.23−2.16 (m, 1H, ^{3}H-2′), 1.97 (s, 3H, $^3CH_3C{=}C$), 1.87 (s, 3H, $^5CH_3C{=}C$); ^{31}P NMR (D_2O) δ 56.10; HRMS (FAB, glycerol) *m/e* calcd for $C_{20}H_{28}N_4O_{11}PS$ [MH^+] 563.11347, found 563.11350.

Phosphorothioamidate (4S). To a solution of phosphoramidite **4** (46 mg, 0.075 mmol) in dry methanechloride (1 mL) was added Beaucage's reagent (1.2 equiv) at room temperature. The reaction mixture was stirred for 5 min. Afer flash chromatography (ethyl acetate:hexane = 1:2), white solid phosphorothioamidate **4S** was obtained in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 8.94 (br, 1H, NH), 7.51 (d, 1H, J = 1.2 Hz, H-6), 6.36−6.33 (dd, $J_{H1'-H2a'}$ = 5.1 Hz, $J_{H1'-H2b'}$ = 9.3 Hz, 1H, H-1′), 5.94 (d, $J_{H1''-H2''}$ = 3.9 Hz, 1H, H-1″), 5.05−5.02 (dd, $J_{H3'-H2'}$ = 5.6 Hz, $J_{H3'-P}$ = 10 Hz, 1H, H-3′), 4.67 (t, $J_{H3''-H4''}$ = $J_{H3''-P}$ = 2.4 Hz, 1H, H-3″), 4.57 (d, $J_{H2''-H1''}$ = 3.9 Hz, 1H, H-2″), 4.30−4.29 (pentet, $J_{H4''-H3''}$ = $J_{H4''-H5a''}$ = $J_{H4''-H5e''}$ = $J_{H4''-P}$ = 2.4 Hz, 1H, H-4″), 4.27 (m, 1H, H-4′), 4.25 (septet,

1H, CHN), 3.38 (AB, 2H, 2 × H-5′), 3.46−3.39 (ddd, $J_{H5e''-H4''}$ = 2.7 Hz, $J_{H5e''-H5a''}$ = 14.3 Hz, $J_{H5e''-P}$ = 20 Hz, 1H, H-5e″), 3.35−3.30 (dt, $J_{H5a''-H5e''}$ = 14.3 Hz, $J_{H5a''-H4''}$ = $J_{H5a''-P}$ = 3.2 Hz, 1H, H-5a″), 2.41 (m, 1H, H-2a′), 2.09 (m, 1H, H-2b′), 1.89 (d, *J* = 1.5 Hz, 3H, CH_3C═C), 1.96−1.62 (m, 8H, cyclopentylidene protons), 1.11 (d, *J* = 6.8 Hz, 3H, **CH_3**CHN), 1.05 (d, *J* = 6.8 Hz, 3H, **CH_3**CHN), 0.90 (s, 9H, t-BuSi), 0.11 (s, 6H, Me_2-Si); ^{13}C NMR ($CDCl_3$) δ 163.81 (C-4), 150.53 (C-2), 134.92 (C-6), 121.91 (OCO), 111.23 (C-5), 104.68 (C-1″), 85.91 (d, *J* = 1.8 Hz, C-4′), 84.80 (C-1′), 84.00 (d, *J* = 11.0 Hz, C-2″), 80.95 (d, *J* = 9.2 Hz, C-3″), 78.25 (d, *J* = 5.5 Hz, C-3′), 72.60 (d, *J* = 4.6 Hz, C-4″), 63.55 (C-5′), 47.90 (d, *J* = 6.4 Hz, CHN), 39.40 (d, *J* = 7.3 Hz, C-5″), 39.25 (C-2′), 36.85, 36.20 (**$CH_2C$$CH_2$**), 25.86 (SiC**$Me_3$**), 23.35, 22.78 (**$CH_2CH_2CCH_2$$CH_2$**), 20.60 (d, *J* = 6.4 Hz, NCH**Me**), 20.20 (d, *J* = 2.7 Hz, NCH**Me**), 18.25 (Si**C**Me_3), 12.44 (C═C**CH_3**), −5.47, −5.50 (Si**Me_2**); ^{31}P NMR ($CDCl_3$) δ 67.54; HRMS (FAB, glycerol) *m/e* calcd for $C_{29}H_{49}N_3O_9PSSi$ [MH^+] 674.2693, found 674.2696.

Phosphorothioamidate (3S). To a solution of a mixture of phosphoramidite **3** and **4** in a ratio of 2:3 (45 mg, 0.075 mmol) in dry chloroform (1.0 mL) was added Beaucage's reagent (1.2 equiv) at room temperature. The reaction mixture was stirred for 5 min. After flash chromatography (ethyl acetate:hexane = 1:3), the slow eluent (25 mg) is **4S** according to ^{31}P NMR (67.54 ppm), while the fast eluent (18 mg) is **3S** by ^{31}P NMR (72.35 ppm): ^{1}H NMR ($CDCl_3$) δ 8.41 (br,, 1H, NH), 7.52 (d, 1H, *J* = 1.2 Hz, H-6), 6.36−6.33 (dd, $J_{H1'-H2'a}$ = 5.3 Hz, $J_{H1'-H2'b}$ = 9.3 Hz, 1H, H-1′), 6.00 (d, 1H, $J_{H1''-H2''}$ = 4 Hz, H-1″), 5.20−5.17 (ddd, 1H, $J_{H3'-H2'}$ = 5.6 Hz, $J_{H3'-4'}$ = 1 Hz, $J_{H3'-P}$ = 10 Hz, H-3′), 4.65−4.63 (dd, 1H, $J_{H3''-H4''}$= 4.1 Hz, $J_{H3''-H2''}$ = 5.3 Hz, H-3″), 4.63 (d, 1H, $J_{H2''-H1''}$ = 4 Hz, H-2″), 4.56−4.52 (ddd, 1H, $J_{H4''-H3''}$ = 4.1 Hz, $J_{H4''-H5a''}$ = $J_{H4''-H5b''}$ = 6.6 Hz, H-4″), 4.16 (q, 1H, $J_{H4'-H3'}$ = $J_{H4'-H5a'}$ = $J_{H4'-H5b'}$ = 1 Hz, H-4′), 3.95 (septet, 1H, CHN), 3.88 (d, 2H, 2 × H-5′), 3.45−3.37 (ddd, 1H, $J_{H5a''-H4''}$ = 6.4 Hz, $J_{H5a''-H5b''}$ = 14, $J_{H5a''-P}$ = 19 Hz, H-5a″), 3.13−3.07 (ddd, 1H, $J_{H5b''-H4''}$ = 6.5 Hz, $J_{H5b''-H5a''}$ = 14 Hz, $J_{5b''-P}$ = 12 Hz, H-5b″), 2.49−2.46 (dd, 1H, $J_{H2a'-H2b'}$ = 14 Hz, $J_{H2a'-H3'}$ = 5.4 Hz, H-2a′), 2.10−2.04 (ddd, 1H, $J_{H2b'-H2a'}$ = 15 Hz, $J_{H2b'-H1'}$ = 9 Hz, $J_{H2b'-H3'}$ = 5.3 Hz, H-2b′), 1.89 (d, 3H, *J* = 1 Hz, CH_3C═C), 1.96−1.62 (m, 8H, cyclopentylidene protons), 1.11 (d, 3H *J* = 6.8 Hz, CH_3CHN), 1.07 (d, 3H, *J* = 6.8 Hz, CH_3CHN), 0.91 (s, 9H, t-BuSi), 0.12 (s, 6H, Me_2Si); ^{13}C NMR ($CDCl_3$) δ 163.43 (C-4), 150.13 (C-2), 135.07 (C-6), 121.87 (OCO), 111.15 (C-5), 105.65 (C-1″), 86.25 (d, *J* = 6.4 Hz, C-4′), 84.72 (C-1′), 83.97 (d, *J* = 12 Hz, C-2″), 80.83 (d, *J* = 6.3 Hz, C-3″), 79.13 (d, *J* = 4.6 Hz, C-3′), 77.80 (d, *J* = 1.8 Hz, C-4″), 63.46 (C-5′), 46.90 (d, *J* = 6.4 Hz, CNH), 40.74 (C-2′), 39.36 (d, *J* = 5.5 Hz, C-5″), 37.04, 36.62 (**$CH_2C$$CH_2$**), 25.93 (SiC**$Me_3$**), 23.37, 23.11 (**$CH_2CH_2CCH_2$$CH_2$**), 20.97 (d, *J* = 2.7 Hz, NCH**Me**), 20.57 (d, *J* = 4.6 Hz, NCHMe), 18.34 (Si**C**Me_3), 12.49 (C═C**CH_3**), −5.39 (Si**Me_2**); HRMS (FAB, glycerol) *m/e* calcd for $C_{29}H_{49}N_3O_9PSSi$ [MH^+] 674.2693, found 674.2696.

Synthesis of 18a−d and 19a−d. 1,2-*O*-Isopropylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose. Using the procedure described for **1**, 1,2-*O*-isopropylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose was obtained in 80% yield from 1,2-*O*-isopropylidene-5-tosyl-α-L-xylofuranose: ^{1}H NMR ($CDCl_3$) δ 8.0 (bs, NH), 5.91 (1H, d, *J* = 3.9 Hz, H-1), 4.44 (1H, d, *J* = 3.9 Hz, H-2), 4.25−4.18 (2H, m, H-3, H-4), 3.39−2.90 (2H, ABX, 2 × H-5), 2.79−2.66 (1H, septet, NCH), 1.44 (3H, s, CCH_3), 1.03 (6H, d, *J* = 6.4 Hz, **Me_2**CH); ^{13}C NMR ($CDCl_3$) δ 111.43 (O**C**O), 105.12 (C-1), 86.14 (C-2), 78.23 (C-3), 76.91 (C-4), 48.84 (NCH), 45.90 (C-5), 26.81, 26.17 (**C**H_3C**C**H_3), 22.64 (**CH_3**CHN), 22.34 (**CH_3**CHN); MS (CI) *m/e* 232 ([M + H^+], 100%).

Cyclic phosphorochloridite was obtained by the procedure described for **2**. The product was not isolated and it was directly used in the following step: ^{31}P NMR ($CDCl_3$) δ 148.31.

Oxazaphosphorinane (18a). Using the procedure described for **4, 18a** was obtained from the above cyclic phosphorochloridite in 84% yield: ^{1}H NMR ($CDCl_3$) δ 8.19 (1H, bs, NH), 7.50 (1H, d, $J_{H6-CH3C=C}$ = 1.0 Hz, H-6), 6.35−6.33 (1H, dd, $J_{H1'-H2a'}$ = 5.4 Hz, $J_{H1'-H2b'}$ = 8.3 Hz, H-1′), 5.94 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-1″), 4.60−4.57 (1H, m, H-3′), 4.51 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-2″), 4.36 (1H, m, H-3″), 4.18 (1H, m, H-4″), 4.09 (1H, m, H-4′), 3.91−3.79 (2H, ABX, 2 × H-5′), 3.49−3.47 (1H, m, H-5a″), 3.47−3.43 (1H, septet, NCH), 3.08−3.03 (1H, m, H-5e″), 2.41−2.37 (1H, m, H-2′), 2.13−2.07 (1H, m, H-2′), 1.92 (3H, d, *J* = 1.0 Hz, MeC═C), 1.49 (3H, s, **CH_3**CCH_3), 1.31 (3H, s, CH_3C**CH_3**), 1.14−1.11 (6H, m, **Me_2**CH), 0.93 (9H, s, t-BuSi), 0.12 (6H, d, *J* = 1.5 Hz, **Me_2**Si); ^{13}C NMR ($CDCl_3$) δ 164.38 (C-4), 150.50 (C-2), 135.24 (C-6), 111.61 (OCO), 110.94 (C-5), 104.78 (C-1″), 86.34 (C-4′), 84.73 (C-1′), 84.82 (C-2″), 73.64 (d, *J* = 18.1 Hz, C-3′), 72.32 (C-4″), 71.77 (C-3″), 63.11 (C-5′), 49.92 (d, *J* = 36.6 Hz, NCH), 40.24 (C-2′), 36.01 (C-5″), 26.62, 26.09 (**Me_2**C), 25.94 (SiC**CH_3**), 22.03, 21.80 (**Me_2**CHN), 18.27 (Si**C**CH_3), 12.46 (**CH_3**C═C), −5.37, −5.44 (**Me_2**Si); ^{31}P NMR ($CDCl_3$) δ 129.0; MS (FAB, nitrobenzyl alcohol) *m/e* [MH^+] 616.

Oxazaphosphorinane (19a). Using the procedure described for **4S**, **19a** was obtained in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 9.02 (1H, bs, NH), 7.52 (1H, d, $J_{H6-CH3C=C}$ = 1.0 Hz, H-6), 6.36−6.33 (1H, dd, $J_{H1'-H2a'}$ = 5.1 Hz, $J_{H1'-H2b'}$ = 9.0 Hz, H-1′), 5.96 (1H, d, $J_{H1''-H2''}$ = 3.7 Hz, H-1″), 5.03 (1H, dd, *J* = 5.6 Hz, *J* = 10.0 Hz, H-3′), 4.66 (1H, m, H-3″), 4.51 (1H, d, $J_{H1''-H2''}$ = 3.9 Hz, H-2″), 4.27−4.23 (3H, m, H-4′, H-4″, NCH), 3.92−3.86 (2H, m, 2 × H-5″), 3.47−3.32 (2H, m, 2 × H-5″), 2.43−2.39 (1H, m, H-2′), 2.12−2.06 (1H, m, H-2′), 1.89 (3H, s, MeC═C), 1.47 (3H, s, **CH_3**CCH_3), 1.29 (3H, s, CH_3C**CH_3**), 1.10 (3H, d, *J* = 6.8 Hz, **Me_2**CH), 1.05 (3H, d, *J* = 6.8 Hz, **Me_2**CH), 0.90 (9H, s, t-BuSi), 0.11 (6H, s, **Me_2**Si); ^{13}C NMR ($CDCl_3$) δ 163.65 (C-4), 150.41 (C-2), 134.97 (C-6), 112.32 (OCO), 111.25 (C-5), 104.94 (C-1″), 85.96 (d, *J* = 2.8 Hz, C-4′), 84.85 (C-1′), 84.12 (d, *J* = 11.0 Hz, C-2″), 80.95 (d, *J* = 9.2 Hz, C-3″), 78.29 (d, *J* = 5.5 Hz, C-3′), 72.45 (d, *J* = 4.6 Hz, C-4″), 63.61 (C-5′), 48.00 (d, *J* = 6.4 Hz, NCH), 39.46 (d, *J* = 7.3 Hz, C-5″), 39.23 (C-2′), 26.64, 26.60 (**Me_2**C), 25.90 (SiC**CH_3**), 20.65(d, *J* = 5.5 Hz, CH_3CHN), 20.47 (d, *J* = 2.8 Hz, CH_3-CHN), 18.30 (Si**C**CH_3), 12.49 (**CH_3**C═C), −5.42, −5.45 (**Me_2**-Si); ^{31}P NMR ($CDCl_3$) δ 67.50; HRMS (FAB, glycerol): *m/e* calcd. for $C_{27}H_{47}N_3O_9PSSi$ [MH^+]: 648.25422, found 648.25399.

Oxazaphosphorinane (18b). By the same procedure as described for **18a**, **18b** was obtained with yield of 60%. ^{1}H NMR ($CDCl_3$) δ 5.93 (1H, d, *J* = 3.7 Hz, H-1), 4.51 (1H, d, *J* = 3.7 Hz, H-2), 4.41 (1H, m, H-3), 4.17−4.13 (2H, m, H-4, OCH), 3.49−3.46 (1H, m, H-5), 3.40 (1H, septet, NCH),), 3.01−2.99 (1H, m, H-5), 1.47 (3H, s, CCH_3), 1.30 (3H, s, CCH_3), 1.23 (6H, m, OCH**Me_2**), 1.10 (6H, m, NCH**Me_2**); ^{13}C NMR ($CDCl_3$) δ 111.48 (OCO), 104.91 (C-1), 84.94 (d, *J* = 2.8 Hz, C-2), 73.48 (C-4), 71.15 (d, *J* = 2.8 Hz, C-3), 67.14 (d, *J* = 18.3, OCH), 49.74 (d, *J* = 35.7 Hz, NCH), 36.24 (d, *J* = 3.7 Hz, C-5), 26.68, 26.19 (**Me_2**C), 24.56 (d, *J* = 4.6 Hz, OCH**CH_3**), 24.33 (d, *J* = 2.7 Hz, OCH**CH_3**), 21.91 (d, *J* = 10.1 Hz, NCH**CH_3**), 21.39 (d, *J* = 6.4 Hz, NCH**CH_3**); ^{31}P NMR ($CDCl_3$) δ 128.18.

Phosphorothioamidate (19b). By the same procedure as described for **4S**, **19b** was obtained in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 5.97 (1H, d, *J* = 3.7 Hz, H-1), 4.71−4.66 (1H, m, OCH), 4.65 (1H, d, *J* = 3.7 Hz, H-2),4.60 (1H, m, H-3), 4.28 (1H, septet, NCH), 4.24 (1H, m, H-4), 3.42−3.32 (2H, m, 2 × H-5), 1.47 (3H, s, CCH_3), 1.31 (3H, d, *J* = 6.4 Hz, OCH**CH_3**), 1.30(3H, s, CCH_3), 1.27 (3H, d, *J* = 6.1 Hz, OCH**CH_3**), 1.10 (3H, d, *J* = 6.6 Hz, NCHC**H_3**), 1.04 (3H, d, *J* = 6.4 Hz, NCHC**H_3**) ^{13}C NMR ($CDCl_3$) δ 112.08 (OCO), 105.04 (C-1), 84.20 (d, *J* = 11.0 Hz, C-2), 80.33 (d, *J* = 9.1 Hz, C-3), 71.94 (d, *J* = 4.6 Hz, C-4), 71.87 (d, *J* = 6.4 Hz, OCH), 47.60 (d, *J* = 6.4 Hz, NCH), 39.20 (C-5), 26.68, 26.20 (**Me_2**C), 23.59 (d, *J* = 6.4 Hz, OCH**CH_3**), 23.40 (d, *J* = 3.7 Hz, OCH**CH_3**), 20.69 (d, *J* = 7.3 Hz, NCH**CH_3**), 20.10 (d, *J* = 1.8 Hz, NCH**CH_3**); ^{31}P NMR ($CDCl_3$) δ 67.08; HRMS (FAB, glycerol): *m/e* calcd. for $C_{14}H_{27}NO_5PS$ [MH^+]: 352.13485, found 352.13476.

Oxazaphosphorinane (18c). Using the procedure described for **18a**, **18c** was obtained with a yield of 78%: ^{31}P NMR ($CDCl_3$) δ 125.31.

Phosphorothioamidate (19c). By the procedure described for **19a**, **19c** was obtained in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 8.24−7.34 (4H, AA′BB′, aromatic protons), 6.02 (1H, d, *J* = 3.4 Hz, H-1), 4.71 (2H, m, H-2, H-3), 4.41 (1H, septet, NCH), 4.30 (1H, m, H-4), 3.60−3.43 (2H, m, 2 × H-5), 1.48 (3H, s, CCH_3), 1.31 (3H, s, CCH_3), 1.16 (3H, d, *J* = 6.6 Hz, NCHC**H_3**), 1.07 (3H, d, *J* = 6.4 Hz, NCHC**H_3**); ^{13}C NMR

($CDCl_3$) δ 156.11, 144.39, 125.57, 120.77, 120.73 (aromatic carbons), 112.42 (OCO), 104.90 (C-1), 84.12 (d, J = 11.0 Hz, C-2), 81.43 (d, J = 10.1 Hz, C-3), 71.90 (d, J = 5.5 Hz, C-4), 48.43 (d, J = 7.3 Hz, NCH), 39.38 (C-5), 26.58, 26.15 (**Me_2**C), 20.86 (d, J = 6.4 Hz, NCH**CH_3**), 20.26 (d, J = 2.8 Hz, NCH**CH_3**); ^{31}P NMR ($CDCl_3$) δ 61.58; HRMS (FAB, glycerol) *m/e* calcd for $C_{17}H_{24}N_2O_7PS$ [MH^+] 431.10426, found 431.10419.

Oxazaphosphorinane (18d). By the procedure described for **18a**, **18d** was synthesized and its ^{31}P NMR showed one peak at 127.50 ppm, but we cannot purify the product by chromatography. Therefore, the reaction mixture was used in the next step.

Phosphorothioamidate (19d). By the procedure described for **19a**, **19c** was synthesized and its ^{31}P NMR showed one peak at 66.8 ppm. Unfortunately we also cannot purify the product by chromatography.

Acknowledgment. We thank the Natural Science and Engineering Research Council of Canada and ISIS Pharmaceuticals, Carlsbad, CA, for generous financial support and Dr. Orval Mamer, McGill University Biomedical Mass Spectrometry Unit, for the measurement of mass spectra. We also thank Dr. Eric Marsault for his valuable comments on the paper. Y.J. thanks McGill University for an Alexander McFee Memorial fellowship, an FCAR fellowship, a J. W. McConnell McGill Major fellowship, and a Beijing Memorial McGill fellowship.

Supporting Information Available: Synthetic details for **1** and **11** and ^{1}H and ^{13}C NMR spectra for all compounds (35 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO972318O