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Stereoselective synthesis of dithymidine phosphorothioates using D-xylose derived chiral auxiliaries

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Abstract—Different sized catalysts **1**, **2**, **5** and **7** were prepared and used as the activators in the coupling reactions of phosphoramidites with nucleosides. D-Xylose derived chiral auxiliary **22a** has been synthesized and applied for the stereoselective synthesis of dithymidine phosphorothioates **27**. The mechanisms of the coupling reactions are discussed.

1. Introduction

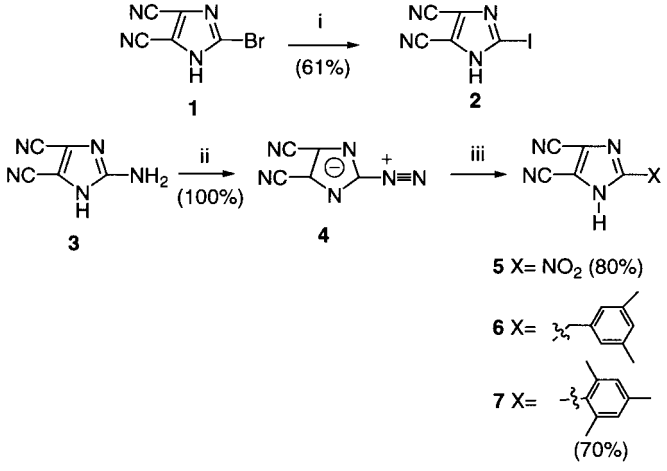
The therapeutic potential of oligonucleotide phosphorothioates (PS-Oligos) has been well established.[1] An unappreciated problem concerning the use of PS-Oligos in antisense-based therapy is their polydiastereoisomerism. Stec's oxathiaphospholane approach[2] is the most advanced method to date, however, it has not been applied for the large production of PS-Oligos.

In a preliminary communication,[3] we have described that the development of an easily removed xylose derived chiral auxiliary and a new catalyst for the stereoselective formation of dithymidine phosphorothioates. Full details of our studies in this area are given here.

2. Results and discussion

2.1. Synthesis of the catalysts for the coupling reactions

Stec and co-workers[4] attempted to obtain chiral phosphite triester by reaction of a single diastereomer of phosphoramidite with a nucleoside using tetrazole as catalyst. However, complete epimerization at the phosphorus center was observed. The epimerization mechanism proposed



Scheme 1. (i) Kl, CuI, HMPA, 120°C; (ii) $NaNO_2$, HCl; (iii) $NaNO_2$ or mesitylene.

Keywords: chiral auxiliaries; diastereoselection; nucleotides; phosphorothioates.

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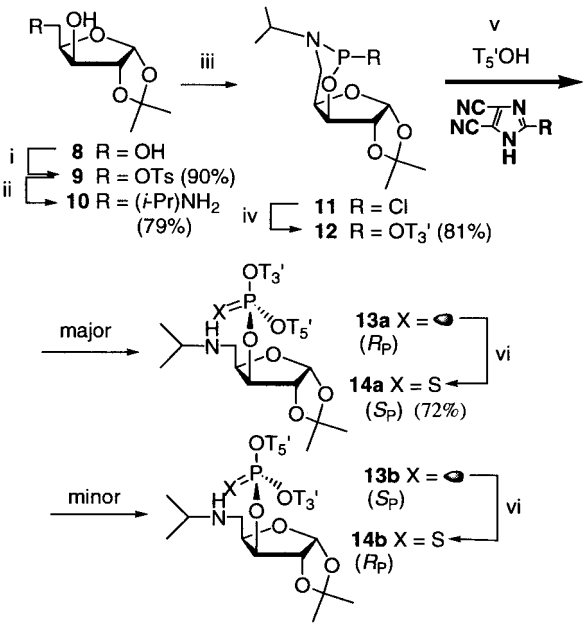


Scheme 2. (i) TsCl, pyridine, 0°C; (ii) *i*-$PrNH_2$, 55°C, overnight; (iii) PCl_3, Et_3N, CH_2Cl_2, 0–40°C; (iv) $T_3'OH$, Et_3N, CH_2Cl_2, 0–50°C; (v) R=I, Br, or mesityl; (vi) Beaucage's reagent.

involved rapid and multiple displacements of the intermediate tetrazolide by tetrazole, followed by a slow reaction of the intermediate tetrazolide with an alcohol.

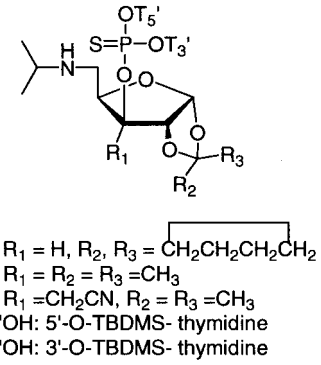
In the xylose-based cyclic phosphoramidite approach,[5] a reasonable degree of stereoselectivity was achieved when 2-bromo-4,5-dicyanoimidazole was used as a catalyst in the coupling reactions. We therefore reasoned if we used a more sterically hindered catalyst, it was reasonable to expect the epimerization process to slow down in the coupling reaction, therefore leading to a better selectivity. Recently,[6] Engels et al. reported the use of aryl or triarylmethyl substituted 4,5-dicyanoimidazoles as activators for R_P diastereoselective synthesis of dinucleotide methylphosphonates. Since 2-bromo-4,5-dicyanoimidazole is the most effective catalyst we had in our previous systems, we decided to keep cyano groups at the 4 and 5 positions of imidazole to confer the necessary acidity and install larger groups at the 2-position.

We initially tried to transform 2-bromo-4,5-dicyanoimidazole[7] **1** to 2-iodo-4,5-dicyanoimidazole **2** through a Finkelstein reaction. Refluxing **1** and excess potassium iodide in acetone for three days failed to produce any desired product. We then applied a copper(I)iodide-assisted halogen exchange[8] reaction by treating **1** with potassium iodide and copper(I)iodide in hexamethylphosphoric triamide at 120°C. The desired iodo derivative **2** was obtained (Scheme 1).

Diazotization of 2-amino-4,5-dicyanoimidazole **3** with

Table 1.

Catalysts	**1**	**2**	**5**	**7**
13a/13b	1/6	1/6	Hydrolyzed	1/5



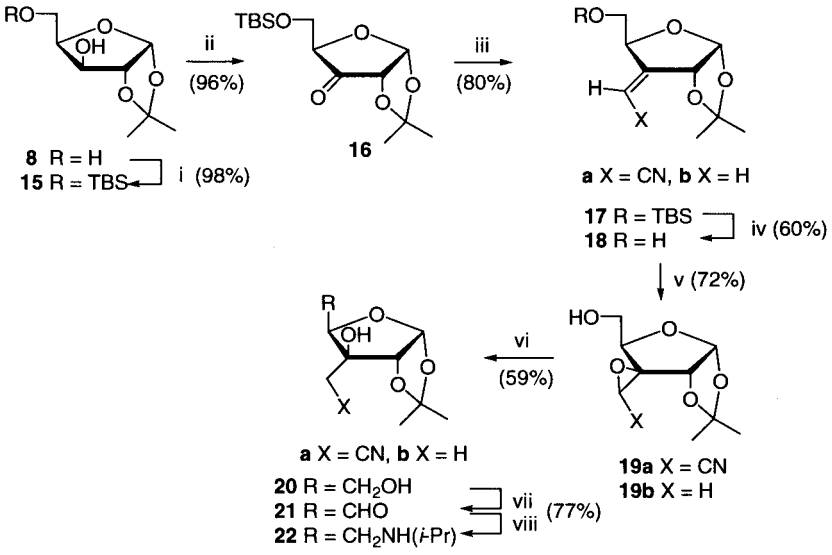
Scheme 3.

sodium nitrite in aqueous hydrochloric acid gave colorless 2-diazo-4,5-dicyanoimidazole **4** quantitatively. Intermediate **4** has been reported[9] to be shock sensitive, and in order to avoid an explosion, wet **4** was transferred carefully and dried under vacuum. Reaction of **4** with another equivalent of sodium nitrite yielded 2-nitro-4,5-dicyanoimidazole **5**. Refluxing **4** in mesitylene gave the product of the insertion. There was no precedent in the literature concerning the sites of the insertion, i.e. whether the insertion would happen at the methyl position to give a derivative of type **6** or on the aromatic ring directly to yield a derivative of type **7**. DEPT spectra of the product clearly indicate the insertion occurred at the aromatic ring to give **7** as the product.

2.2. Test of the catalysts in the coupling reactions

With catalysts 2-iodo, 2-nitro and 2-mesityl-4,5-dicyanoimidazoles **2**, **5**, **7** in hand, we then synthesized phosphoramidites **12**, which can be easily prepared from D-xylose, to find out the stereoselectivities of the different catalysts in the coupling reactions.

The phosphorothioate was synthesized as illustrated in Scheme 2. 1,2-Di-*O*-isopropylidene-D-xylofuranose **8** was tosylated selectively at the 5-position to yield **9**. Displacement of tosylate by isopropylamine afforded γ-amino alcohol **10**, which was treated with phosphorus trichloride in the presence of triethylamine to give **11**. Upon heating at 40°C to equilibrate, a single diastereomer of **11** having ^{31}P NMR resonance at around 150 ppm was obtained. Phosphorochloridite **11** was transformed into phosphoramidite **12** by reaction with 5′-*O*-TBDMS-thymidine ($T_3'OH$) in the presence of triethylamine. Again, a mixture of diastereomers of **12** obtained initially could be equilibrated by heating at 50°C to afford a single diastereomer with a ^{31}P NMR resonance at 130 ppm. The cyclic phosphoramidite was easily purified by flash chromatography. For the coupling reactions, phosphoramidite **12**, 3′-*O*-TBDMS-thymidine ($T_5'OH$) and different catalysts, i.e. dicyanoimidazoles **1**, **2**, **5** or **7** were dried under vacuum overnight. Acetonitrile was added to the NMR tubes. The diastereomerical ratios of two isomers were evaluated by ^{31}P NMR. Major and minor diastereomers were observed at 145 and 144 ppm and their configurations were assigned as R_P and S_P, respectively, by comparison with the literature data.[5b] Further sulfurization with Beaucage's reagent[10] gave the corresponding phosphorothioates. To conclude this work,



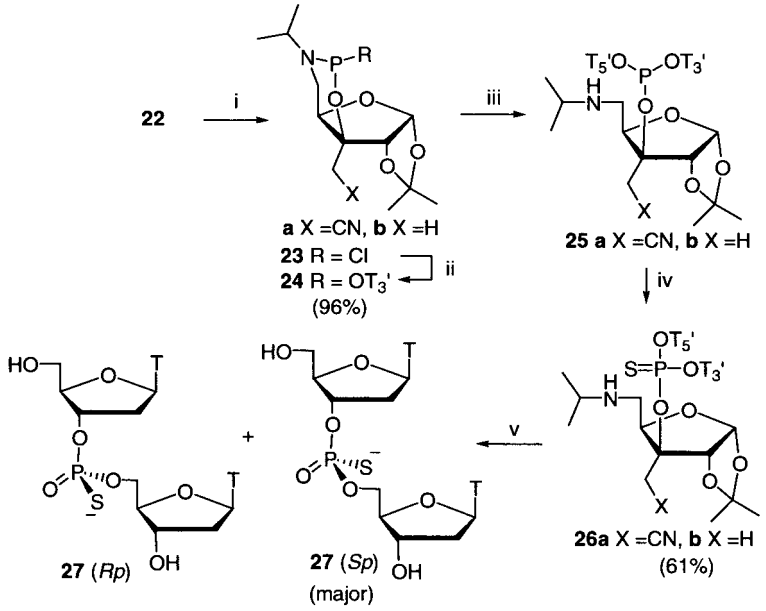
Scheme 4. (i) TBDMSCl, imidazole, DMF; (ii) CrO_3, Pyridine, Ac_2O, rt; (iii) a: $CNCH_2P(O)(OEt)_2$, NaH, THF, 0°C to rt; b: CH_3PPh_3Br, NaH, THF, 0°C to rt; (iv) TBAF, 0°C; (v) a: Triton-B, TBHP, THF; b: MCPBA, CH_2Cl_2; (vi) a: $LiBH_4$, diglyme; b: $LiAlH_4$; (vii) $(COCl)_2$, DMSO, CH_2Cl_2; (viii) *i*-$PrNH_2$, $Na(OAc)_3BH$, DCE, rt.

although more hindered catalysts were used, the selectivity did not improve. When iodo and mesitylimidazoles **2**, **7** were used, the reactions went more slowly, presumably due to their lower acidities. When nitroimidazole **5** was used, a major resonance at around 12 ppm was observed. This is probably due to hydrolysis to an H-phosphonate. Table 1 summarized these results.

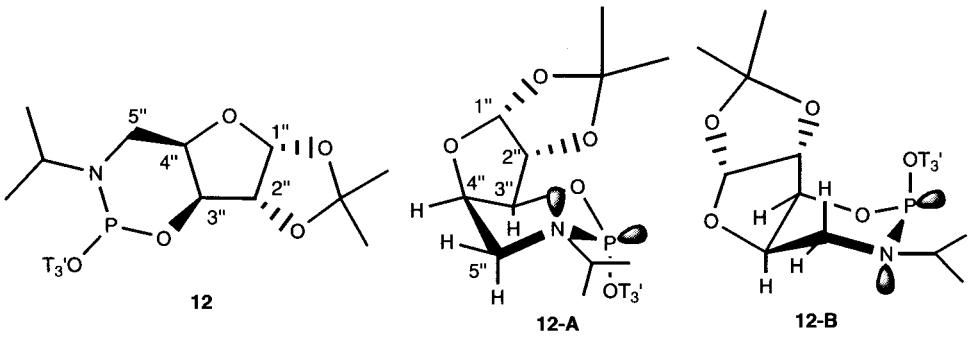
2.3. Easily removable chiral auxiliary

We reported[5a] the use of 1,2-di-*O*-cyclopentylidene-5-deoxy-5-isopropylamino-α-D-xylofuranose for the stereoselective synthesis of phosphorothioates (**A**) (Scheme 3). However, the removal of the chiral auxiliary at the end of the synthesis was only achieved by treatment with 70% aqueous trifluoroacetic acid, which seemed to be too harsh if bases other than thymidine are used. We wanted to modify the chiral auxiliary so that it could be easily removed under mild conditions. Chiral auxiliaries type **B** or **C** seemed to be good candidates. A S_N1 type displacement or β-elimination should allow the easy removal of the chiral auxiliaries.

We then synthesized chiral auxiliary type **B** and **C** as illustrated in Scheme 4. Ketone **16** was transformed into alkenes **17** via Wittig reactions. The silyl group was then removed to furnish 5-hydroxy alkene **18a** or **18b**, which were treated with either *N*-benzyltrimethylammonium hydroxide (triton-B) and *tert*-butyl hydroperoxide (TBHP) or MCPBA to yield the desired epoxide **19a** or **19b**. The epoxide was reduced to afford diol **20**, whose *cis*-configuration was



Scheme 5. (i) PCl_3, Et_3N, CH_2Cl_2, 0°C to rt; (ii) $T_{3'}OH$, Et_3N, CH_2Cl_2, 0°C to rt; (iii) 1.1 equiv. $T_{5'}OH$, 1.5 equiv. 2-bromo-4,5-dicyanoimidazole, $CDCl_3$, 0°C; (iv) Beaucage's reagent; (v) conc. ammonia, rt; TBAF.



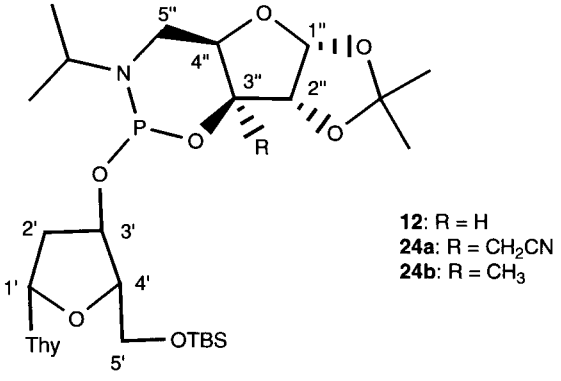
Scheme 6.

confirmed by 2D-NOSEY spectra. Swern oxidation, followed by reductive amination employing sodium triacetoxyborohydride[11] and isopropylamine furnished *cis*-γ-aminoalcohol **22a** or **22b**.

The synthesis of phosphoramidites and phosphorothioates is depicted in Scheme 5. γ-Aminoalcohol **22a** was treated with phosphorus trichloride in the presence of triethylamine to afford **23a**. Further in situ treatment with $T_3'OH$ then yielded cyclic phosphoramidite **24a**, which was obtained as a single diastereomer after chromatographic purification.

Phosphoramidite **24a** was coupled with $T_5'OH$ using 2-bromo-4,5-dicyanoimidazole as the catalyst. Two isomers of phosphite triesters **25a** (^{31}P NMR=138.5 and 137.5 ppm) were obtained in a ratio of 1 to 6. Further sulfurization gave phosphorothioates **26a** with a ^{31}P NMR at 59.05 and 59.13 ppm in the same ratio. The phosphorus NMR resonances originally at 59 ppm shifted to 57.3 and 56.8 ppm, in a ratio of 1 to 6, after treatment with concentrated ammonia solution at room temperature for 5 min. Removal of the silyl groups furnished dithymidine phosphorothioates **27** (56.8 ppm/56.6 ppm=1/6). The minor and major isomers of **27** were assigned with R_P and S_P configuration, by comparison of ^{31}P NMR[5b] and HPLC data.

Table 2. Selected carbon–phosphorus coupling constants of phosphoramidites



Compounds	$J_{C3'-P}$ (Hz)	$J_{C3''-P}$ (Hz)	$J_{C5''-P}$ (Hz)	J_{CHN-P} (Hz)
12	19.2	3.7	2.8	44.0
24a	23.8	8.3	3.6	38.5
24b	21.1	8.2	4.5	37.6

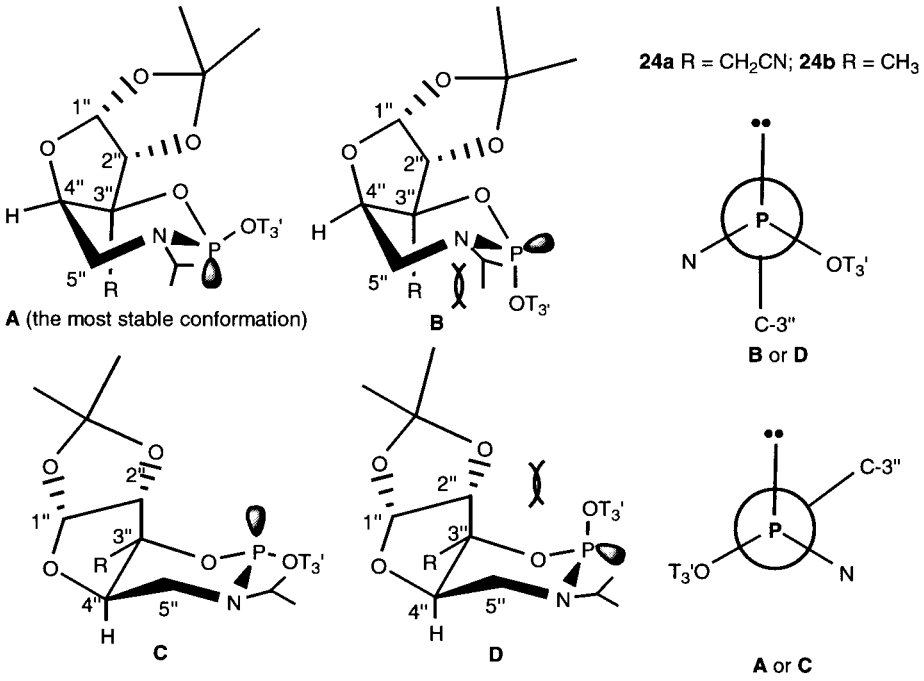
Similarly, **22b** was transformed into phosphorothioate **26b** using similar sequence of reactions described above. Species **26b** has a ^{31}P NMR resonance at 60 ppm. This is not a typical ^{31}P NMR resonance value for a phosphorothioate, and ^{1}H NMR and mass spectrum proved the presence of the sugar chiral auxiliary moiety in **26b** or an isomer. We then proceeded to try to remove chiral auxiliary using either concentrated ammonia or 3% TFA aqueous solution. However, we were not able to form free phosphorothioate **27**. The attachment of the sugar moiety was confirmed by ^{31}P NMR, ^{1}H NMR and mass spectrum. We suspect that a rearrangement took place to an isomeric species, the structure of which we could not elucidate.

2.4. The mechanism of the coupling reaction

The mechanism of the coupling reaction remains to be clarified. The main question is whether azole serves only as a proton donor yielding as a protonated nucleoside phosphoramidite or whether it will attack as a nucleophile giving rise to the formation of an azolide intermediate.[12] In the first case, after protonation of phosphoramidite, $T_5'OH$ reacts to give a phosphite triester with inversion of configuration. The second process involves first displacement of the protonated amino part of phosphoramidite by azole, followed by displacement by an alcohol that gives the desired phosphite triester with retention of configuration.

2.4.1. The structural assignments for the phosphoramidites. We first tried to obtain crystals of **12**, **24a** or **24b** for X-ray crystallographic structure determination. However, none of them gave suitable crystals. The structural assignment for the phosphoramidite **12** was then made according to the literature.[13–15] It was shown by Bentrude and coworkers that in bicyclic six-membered ring phosphites, the 1,3,2-dioxaphosphorinane ring preferentially adopted a chair conformation with the exocyclic OR group attached to the phosphorus in an axial position. Therefore, the thermodynamically more stable isomer of phosphoramidite **12** would adopt a chair form with thymidine attached axially at the exocyclic position. Between the two possible isomers **12-A** and **12-B**, the former one should be strongly favored due to the far smaller 1,3-diaxial interaction (Scheme 6).

The axial position of OT_3' was based on ^{13}C NMR data: the large coupling constant (19.2 Hz) between C-3′ of thymidine and phosphorus, a small coupling constant (3.7 Hz) for C–O–P of the oxazaphosphorinane carbon and a small coupling constant (1.8 Hz) for C-4″ of the chiral auxiliary



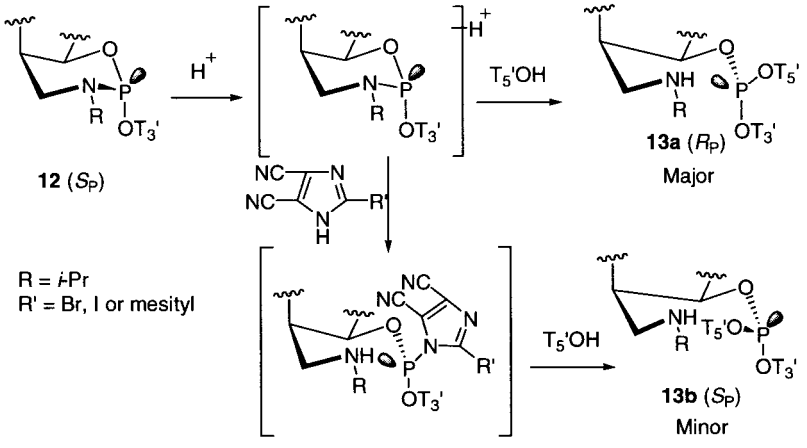
Scheme 7.

and the phosphorus. Table 2 lists the selected carbon and phosphorus coupling constants for phosphoramidites **12**, **24a** and **24b**.

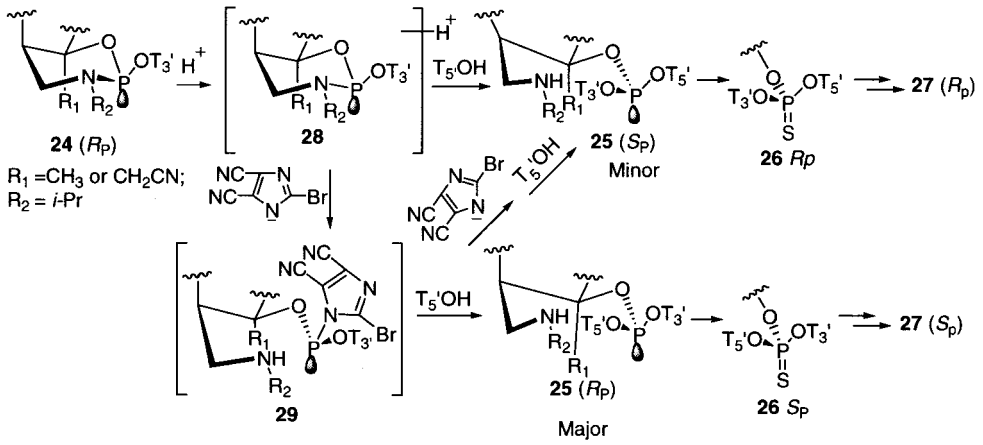
The C-3″ and phosphorus ($J_{C3''-P}$) coupling constants for **24a** and **24b** are much larger than that of **12**, which indicates a different configuration for the 3″-alkylphosphoramidites **24a** and **24b** as compared to **12**, which has a hydrogen at the C-3″ position.

As shown in Scheme 7, there are two possible configurations and four possible conformations for **24a** or **24b**, depending on whether OT_3' is axially or equatorially disposed. The two conformations (**B** or **D**) where OT_3' is axially disposed are unfavorable due to the strong 1,3-dipolar interactions. Such interactions would be avoided if OT_3' adopted an equatorial or pseudoequatorial positions (**A** or **C**).

The equatorial disposition of the OT_3' group was confirmed by C-3″ and phosphorus coupling constants. It is well documented in the literature[16–18] that the carbon to phosphorus coupling constants (J_{C-P}) in trivalent phosphorus derivatives are highly dependent upon the relative spatial orientation of the lone pair on phosphorus and the carbon atom. Haemers et al. reported[19,20] the amplitudes of the J_{C-P} coupling constants in a series of rigid dioxaphosphorinanes depend on the dihedral angle between the phosphorus lone pair and the O–C bond. A dihedral angle close to 0° will induce a large coupling constant, whereas a dihedral angle close to 180° will correspond to a small coupling constant. In the Newman projection of configuration **B** or **D**, the dihedral angle between the phosphorus lone pair and the O–C-3″ bond is close to 180°, and a smaller coupling constant is expected for these two conformers. When the dihedral angle is 60° as illustrated for **A** or **C**, larger C-3″ and phosphorus coupling constants (8.2 Hz) are observed.



Scheme 8.



Scheme 9.

Between two conformers **A** and **C**, **A** seems to be favorable due to smaller interaction between N-isopropyl and OT_3' groups. To confirm this, we carried our molecular modeling to differentiate **A** and **C**. Calculations were done by applying the MacroModel program (version 5.5). Monte-Carlo search was carried out to find out the global minimum for **A** or **C**. The calculations show that configuration **A** is about 3.5 kcal/mol more stable than **C**. Therefore, the most likely configuration for **24a** or **24b** is **A**.

2.5. The coupling mechanisms

2.5.1. Coupling reactions of 12. An examination of the stereochemistry of **12** (S_P) and **13** (R_P) indicated there was only one inversion during the displacement process (Scheme 8). The mechanism we therefore propose is that after the protonation of phosphoramidite **12**, $T_5'OH$ attacks directly the phosphorus center to yield phosphite triester **13** as a major diastereomer. This result is in agreement with a similar system.[5b] The mechanism is further confirmed by comparing the diastereoselectivities of the coupling reactions when the different catalysts (**1**, **2**, and **7**) were used. Although the catalyst sizes were different, the selectivity remained about the same. This can be understood if the sole role of the catalysts had been to act as a proton source.

2.5.2. Coupling reaction of 24. Analysis of the stereochemistry of **24** (R_P) and the major isomer of dithymidine phosphorothioate **27** (S_P) indicated that the transformation involved a double inversion (Scheme 9). The following mechanism is proposed. The R_P-phosphoramidite **24** is first protonated by the catalyst to give **28**. The catalyst then displaces the protonated amine to form imidazolide intermediate **29**, which is displaced by thymidine to give the desired R_P-phosphite triester **25**. Sulfurization and the deprotection then yield dithymidine phosphorothioate **27** (S_P) as the major isomer. Imidazolide **29** can be displaced by another molecule of the catalyst and lead to epimerization. The competing reaction of $T_5'OH$ with **28** can be another source of epimerization. The double displacement mechanism is further confirmed when a bigger catalyst, 2-mesityl-4,5-dicyanoimidazole was used as the activator in the coupling reaction of **24b**. The same phosphite triesters were obtained in a ratio of 15 to 1, as compared to a ratio of 6 to 1 when 2-bromo-4,5-dicyanoimidazole was used as a catalyst under the same conditions.

3. Summary

We have synthesized cyclic phosphoramidite **24a**, which is stable enough to be isolated and led to the stereoselective formation of phosphorothioates. The removal of the chiral auxiliary can be easily realized by rapid treatment with concentrated ammonia at room temperature. Different sized catalysts were prepared and used as the activators for the coupling reactions between phosphoramidites and nucleosides. When larger catalysts are used as activators, the stereoselectivity is improved in tertiary alcohol derived phosphoramidite (**24**) system, but remains about the same in the system using phosphoramidite **12**. Analysis of the stereochemistry of the phosphoramidites and the phosphorothioates suggested both the single protonation mechanism and the double inversion mechanism are possible pathways for the coupling reactions. It is not clear which factors are responsible for the change of the mechanism.

4. Experimental

4.1. General methods

Mass spectra were recorded on MS25RFA and ZAB 2F HS mass spectrometers. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR were recorded on a JOEL 270 or a Varian Unity 500 spectrometer and are referenced with respect to the residual signals of the solvent. The assignments of the proton spectra are based on COSY experiments. ^{31}P NMR spectra are referenced to the external 85% H_3PO_4 signal as 0 ppm. THF was distilled from sodium benzophenone ketyl, triethylamine from calcium hydride, dichloromethane from phosphorus pentoxide and methanol from magnesium. Phosphorus trichloride was first degassed by refluxing for 2 h under argon followed by fractional distillation and was stored under argon. Beaucage's reagent was a gift from ISIS Pharmaceuticals, Carlsbad, CA. All other reagents were purchased from Aldrich.

4.1.1. 2-Iodo-4,5-dicyanoimidazole 2. A mixture of 2-bromo-4,5-dicyanoimidazole **1** (0.20 g, 1 mmol), potassium

iodide (1.66 g, 10 mmol), copper(I)iodide (0.95 g, 5 mmol) in hexamethylphosphorus triamide (HMPA) (5 ml) was heated to 120°C for 2 days. The reaction was quenched by the addition of 2N HCl solution (20 ml), followed by EtOAc (20 ml). The organic layer was washed successively with aqueous Na_2CO_3, 2N HCl and water, and dried over $MgSO_4$. The solvent was removed in vacuo to give the desired product as a yellow solid (0.15 g, 61%). ^{13}C NMR (75.3 MHz, CD_3COCD_3) δ 130.6, 124.3, 116.5; HRMS (FAB) *m/e* calcd for $C_5H_2IN_4$ $[M+H]^+$ 244.9324, found: 244.9323.

4.1.2. 2-Diazo-4,5-dicyanoimidazole 4. *CAUTION*! Dry **4** is highly shock-sensitive! To avoid an explosion, wet **4** should be transferred carefully into a reaction vessel for the next reaction.

To a suspension of 2-amino-4,5-dicyanoimidazole (1.33 g, 10 mmol) in water (30 ml) at room temperature was added concentrated HCl (7.5 ml). The imidazole was dissolved. The solution was cooled down by ice bath and a solution of sodium nitrite (1 g, 14 mmol) in water (3 ml) was added dropwise while keeping the temperature of the solution below 0°C. The precipitate was formed quantitatively. The mixture was stirred at 0°C for a few more minutes and the solid was collected by filtration. Wet **4** was transferred and dried under vacuum. White solid was obtained as the desired product (1.44 g, 100%), which was used directly in the following reactions without further purification.

4.1.3. 2-Nitro-4,5-dicyanoimidazole 5. To **4** (0.14 g, 1 mmol) obtained previously was added a solution of sodium nitrite (0.07 g, 1 mmol) in water at room temperature. The mixture was stirred until TLC showed the disappearance of starting material. The product was taken up in EtOAc, washed and dried. Purification by flash chromatography (eluting solvent: EtOAc) afforded the desired product as a yellow solid (0.19 g, 80%). HRMS (FAB) *m/e* calcd for $C_5H_2N_5O_2$ $[M+H]^+$ 164.0208, found: 164.0210.

4.1.4. 2-Mesityl-4,5-dicyanoimidazole 7. Diazonium salt **4** (0.14 g, 1 mmol) was dissolved in mesitylene (10 ml). The mixture was brought to reflux overnight. After cooling, yellow solid precipitated out. Recrystallization from ethyl acetate/hexanes afforded the desired product as yellow needle crystals (0.16 g, 70%). ^1H NMR (270 MHz, $CDCl_3$) δ 6.78 (s, 1H, aromatic H), 6.65 (s, 1H, aromatic H), 1.91–2.19 (3 s's, 9H, 3×CH_3); ^{13}C NMR (75.3 MHz, $CDCl_3$) δ 138.1, 128.5, 126.7, 125.4, 124.4, 110.8, 63.9, 20.4, 19.1; HRMS (FAB) *m/e* calcd for $C_{14}H_{13}N_4$ $[M+H]^+$ 237.11402, found: 237.11397.

4.1.5. 1,2-Di-*O*-isopropylidene-5-*O*-tosyl-D-xylofuranose 9. *p*-Toluenesulfonyl chloride (0.21 g, 1.1 mmol) was added to a solution of 1,2-di-*O*-isopropylidene-D-xylofuranose (0.19 g, 1 mmol) in pyridine (5 ml) at 0°C under argon. The mixture was stirred at room temperature for 3 h. Water was then added to quench the reaction and the solvent was removed under high vacuum, co-evaporated with toluene twice. The residue was taken up in EtOAc, washed with water and brine, and dried over $MgSO_4$. Recrystallization from THF/ether furnished **9** (0.31 g, 90%) as a white solid. ^1H NMR (270 MHz, $CDCl_3$) δ 7.26–7.82 (m, 4H, Ph), 5.88 (d, 1H, H-1, J_{1-2}=3.6 Hz), 4.51 (d, 1H, H-2, J_{1-2}=3.6 Hz), 4.28–4.35 (m, 3H, H-5, H-3), 4.15 (m, 1H, H-4), 2.45 (s, 3H, OCH_3), 2.22 (d, 1H, OH, *J*=4.8 Hz), 1.46 (s, 3H, CCH_3), 1.30 (s, 3H, CCH_3); MS (CI) *m/z* 345 $(M+1)^+$.

4.1.6. 1,2-Di-*O*-isopropylidene-5-deoxy-5-isopropylamino-D-xylofuranose 10. 1,2-Di-*O*-isopropylidene-5-*O*-tosyl-D-xylofuranose **9** (0.33 g, 1 mmol) was dissolved in isopropylamine (4 ml) and heated to 55°C for 36 h in a pressure bottle. The solvent was removed in vacuo. The residue was taken up in CH_2Cl_2, washed with saturated $NaHCO_3$, water and brine. The organic layer was dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Et_3N=9/1) afforded the desired product **10** as a white solid (0.18 g, 79%).

^1H NMR (270 MHz, $CDCl_3$) δ 8.0 (br, 1H, NH), 5.91 (d, 1H, H-1, J_{1-2}=3.7 Hz), 4.44 (d, 1H, H-2, J_{1-2}=3.7 Hz), 4.18–4.25 (m, 2H, H-3, H-4), 2.90–3.39 (m, 2H, H-5), 2.66–2.79 (1H, septet, NCH), 1.44 (s, 3H, CCH_3), 1.28 (s, 3H, CCH_3), 1.03 (d, 6H, $CH(CH_3)_2$, *J*=6.4 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.4, 105.1, 86.1, 78.2, 76.9, 48.8, 45.9, 26.8, 26.2, 22.6, 22.3; MS (CI) *m/z* 232 ($(M+1)^+$, 100%).

4.1.7. Cyclic phosphoramidite 12. To a solution of freshly distilled phosphorus trichloride (96 μl, 0.11 mmol) in dry dichloromethane (0.2 ml) in a NMR tube at 0°C was added a solution of **10** (23 mg, 0.10 mmol) in dichloromethane (0.45 ml) containing triethylamine (30.7 μl, 0.22 mmol). The NMR tube was then sealed and heated at 40°C overnight. A solution of $T_3'OH$ (39 mg, 0.11 mmol) in dichloromethane (0.5 ml) containing triethylamine (15.3 μl, 0.11 mmol) was added. The NMR tube was re-sealed and heated at 50°C overnight. The reaction mixture was diluted with dichloromethane, washed with saturated $NaHCO_3$ and water, and dried over $MgSO_4$. Flash chromatographic purification (eluting system: Hexanes/EtOAc/Et_3N=5/3/2) afforded the desired product as a white solid (50 mg, 81%).

^1H NMR (500 MHz, $CDCl_3$) δ 7.48 (s, 1H, H-6), 6.32–6.33 (dd, 1H, H-1′, *J*=8.3 Hz, 5.4 Hz), 5.91 (d, 1H, H-1″, *J*=3.5 Hz), 4.56 (m, 1H, H-3′), 4.49 (d, 1H, H-2″, J=4.0 Hz), 4.34 (s, 1H, H-3″), 4.16 (d, 1H, H-4″, *J*=2.0 Hz), 4.06 (m, 1H, H-4′), 3.77–3.89 (2H, H-5′), 3.39–3.47 (m, 2H, H-5″, NCH), 3.01–3.01 (m, 1H, H-5″), 2.35–2.38 (m, 1H, H-2′), 2.05–2.10 (m, 1H, H-2′), 1.89 (s, 3H, $CH_3C{=}C$), 1.47 (s, 3H, CH_3CCH_3), 1.29 (s, 3H, CH_3CCH_3), 1.09–1.12 (m, 6H, $(CH_3)_2CHN$, 0.90 (s, 9H, $SiC(CH_3)_3$), 0.10 (s, 6H, $Si(CH_3)_2$; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 130.2 ppm; ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.7, 150.3, 135.2, 111.7, 111.0, 104.8, 86.4, 84.8, 73.8, 73.7, 72.9, 71.8, 63.2, 50.1, 49.8, 40.3, 36.1, 26.7, 26.2, 25.9, 22.1, 22.0, 21.7, 21.7,18.3, 12.5, −5.4, −5.5; HRMS (FAB) *m/e* calcd for $C_{28}H_{49}N_3O_9PSi$ $[M+H]^+$ 630.2976, found: 630.2974.

4.1.8. Phosphorothioate 14. To a mixture of **12** (20 mg, 0.03 mmol), $T_5'OH$ (21 mg, 0.06 mmol) and 2-bromo-4,5-dicyanoimidazole (8.8 mg, 0.045 mmol) in a NMR tube was added dry acetonitrile (0.60 ml) at room temperature. The NMR tube was then sealed. ^{31}P NMR indicated the formation of two new resonances at 144.8 and 143.7 ppm in

a ratio of 6 to 1 within 10 min. Beaucage's reagent (7.2 mg, 0.036 mmol) was directly introduced to the reaction mixture. Instantaneously, the ^{31}P NMR showed peaks at 68.5 ppm and 68.7 in a ratio of 6 to 1. The mixture was purified by flash chromatography (eluting solvents: EtOAc to acetone) to afford the product as a white solid (24 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (m, 1H, ^{3}H-6), 7.23 (m, 1H, ^{5}H-6), 6.34–6.37 (m, 1H, ^{5}H-1′), 6.18 (m, 1H, ^{3}H-1′), 5.90 (d, 1H, H-1″, J=3.42 Hz), 5.11–5.14 (m, 1H, ^{5}H-3′), 4.82–4.85 (dd, 1H, H-3″, J=2.44 and 12.2 Hz), 4.65 (d, 1H, H-2″, J=3.91 Hz), 4.33–4.38 (m, 2H, ^{3}H-3′, H-4″), 4.22–4.24 (m, 3H, 2× ^{3}H-5′, ^{5}H-4′), 3.99 (m, 1H, ^{3}H-4′), 3.85 (s, 2H, ^{5}H-5′), 2.80–2.88 (m, 3H, H-5″, CHN), 2.43–2.46 (m, 1H, ^{5}H-2′), 2.10–2.27 (m, 3H, 2× ^{3}H-2′, ^{5}H-2′), 1.91 (s, 3H, CH_3C═C), 1.89 (s, 3H, CH_3C═C), 1.27 (s, 3H, C(CH_3)$_2$, 1.25 (s, 3H, C(CH_3)$_2$,1.14 (m, 6H, NHCH(CH_3)$_2$, 0.91 (s, 9H, SiC(CH_3)$_3$), 0.86 (s, 9H, SiC(CH_3)$_3$), 0.11 (s, 6H, Si(CH_3)$_2$), 0.06 (s, 6H, Si(CH_3)$_2$); HRMS (FAB) *m/e* calcd for $C_{43}H_{75}N_5O_{14}PSSi_2$ $[M+H]^+$ 1004.4307, found: 1004.4309.

When catalyst **5** or **7** was used, the reactions were carried out in a similar fashion.

4.1.9. 1,2-Di-*O*-isopropylidene-5-*O*-TBDMS-D-xylofuranose 15. To 1,2-di-*O*-isopropylidene-D-xylofuranose (10 g, 52.6 mmol) in dry DMF (100 ml) at 0°C were added TBDMSCl (8.71 g, 57.8 mmol) and imidazole (5.36 g, 78.9 mmol). The resulting mixture was stirred at 0°C for 45 min and white precipitate was formed. The precipitate was filtered off, and the filtrate was evaporated to dryness under high vacuum. The residue was taken up in EtOAc, washed with water and brine, and dried over $MgSO_4$. Removal of the solvent afforded the desired product as a colorless oil (15.8 g, 98.6%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (d, 1H, H-1, J_{1-2}=3.5 Hz), 4.49 (d, 1H, H-2, J_{2-1}=3.5 Hz), 4.32 (m, 1H, H-3), 4.10–4.13 (m, 3H, H-4 and H-5), 1.47 (s, 3H, C(CH_3)$_2$), 1.31 (s, 3H, C(CH_3)$_2$), 0.88 (s, 9H, Si(CH_3)$_3$), 0.09 (s, 6H, Si(CH_3)$_2$(*t*-Bu)); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.5, 104.9, 85.6, 78.1, 77.1, 62.4, 26.8, 26.1, 25.7, 18.1, −5.5, −5.6; HRMS (FAB) *m/e* calcd for $C_{14}H_{25}O_5Si$ $[M+H]^+$ 305.1784, found: 305.1783.

4.1.10. 3-Deoxy-3-cyanoethylene-1,2-*O*-isopropylidene-5-*O*-TBDMS-D-xylofuranose 17a. Chromium oxide (6.83 g, 68.3 mmol) was added in portions into a stirred solution of pyridine (11 ml, 136.6 mmol) in sufficient amount of dichloromethane to dissolve CrO_3·2Py complex. The mixture was stirred for 15 min to produce a deep red solution. 1,2-Di-*O*-isopropylidene-5-*O*-TBDMS-D-xylofuranose **15** (5.2 g, 17.1 mmol) in a small amount of dichloromethane was added with stirring at room temperature. Acetic anhydride (6.45 ml, 68.3 mmol) was added at once. After 30 min, the reaction mixture was concentrated to a small volume. The residue was loaded directly to the top of a column and eluted with ethyl acetate. The solvent was removed in vacuo, co-evaporated with toluene several times to remove pyridine and acetic anhydride. Further drying under high vacuum provided the crude ketone **16** (4.7 g) as a sticky white solid.

To a suspension of sodium hydride (0.68 g, 17.1 mmol) in dry THF (15 ml) at 0°C was added slowly diethylcyanomethyl phosphonate (2.76 ml, 17.1 mmol). The resulting mixture was allowed to stand at 0°C for 45 min. Ketone **16** (4.7 g, 15.6 mmol) in THF was added slowly. The reaction mixture was stirred at room temperature overnight. The mixture was then concentrated. The residue was extracted with EtOAc and washed with saturated $NaHCO_3$ and water, and dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Hexanes=1/5) afforded the desired product **17a** (3.5 g, 62.0%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (d, 1H, H-1, J_{1-2}=3.5 Hz), 5.65 (m, 1H, CNCH), 5.18 (m, 1H, H-2), 4.83 (m, 1H, H-4), 3.71–3.73 (m, 2H, H-5), 1.48 (s, 3H, C(CH_3)$_2$), 1.42 (s, 3H, C(CH_3)$_2$), 0.87 (s, 9H, Si(CH_3)$_3$), 0.05 (s, 6H, Si(CH_3)$_2$(*t*-Bu)); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 164.4, 115.0, 113.8, 105.1, 96.1, 80.5, 80.1, 65.0, 27.4, 27.3, 25.7, 18.1, −5.5, −5.6; HRMS (FAB) *m/e* calcd for $C_{16}H_{27}NO_4KSi$ $[M+K]^+$ 364.1346, found: 364.1346.

4.1.11. 3-Deoxy-3-cyanoethylene-1,2-di-*O*-isopropylidene-D-xylofuranose 18a. 1 M Tetrabutylammonium fluoride (TBAF) (6.71 ml, 6.71 mmol) was added slowly to a solution of **17a** (2.0 g, 6.1 mmol) in THF (10 ml) at 0°C. The colorless solution turned dark during the addition. The dark solution was stirred at 0°C for 10 min. The solvent was removed and the residue was taken up in EtOAc, washed with water and brine, and dried over $MgSO_4$. The crude was purified by flash chromatography (eluting system: EtOAc/Hexanes=1/1) to afford the desired product **18a** (0.63 g, 49.2%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 (m, 1H, H-1), 5.53 (m, 1H, CNCH), 5.21 (m, 1H, H-2), 4.88 (m, 1H, H-4), 3.87 (m, 1H, H-5), 3.71 (m, 1H, H-5), 1.48 (s, 3H, C(CH_3)$_2$), 1.40 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 161.9, 114.6, 113.7, 104.4, 96.7, 79.8, 79.7, 62.7, 27.1, 27.0; HRMS (FAB) *m/e* calcd for $C_{10}H_{14}NO_4$ $[M+H]^+$ 212.0923, found: 212.0924.

4.1.12. Epoxide 19a. *N*-Benzyltrimethylammonium hydroxide (triton-B) (45.6 μl, 0.1 mmol) was added to *tert*-butyl hydroperoxide (TBHP) (0.29 ml, 1.5 mmol) in THF (5 ml) at 0°C. After 1 min, alkene **18a** (0.21 g, 1 mmol) in a small amount of THF was added. The resulting mixture was stirred for half an hour. Saturated NH_4Cl was added to the reaction mixture. The solvent was then removed, and the residue was taken up in EtOAc, washed with brine and dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Hexanes=1/1) yielded the desired product as a colorless oil (0.16 g, 72%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.02 (d, 1H, H-1, J=3.5 Hz), 4.52–4.54 (m, 2H, H-2 and H-4), 3.89 (s, 1H, CNCH), 3.54–3.69 (m, 2H, H-5), 1.59 (s, 3H, C(CH_3)$_2$), 1.38 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 114.6, 113.8, 104.0, 82.4, 74.6, 70.3, 59.4, 41.4, 26.8, 26.5; MS (EI) *m/z* 227 $[M]^+$.

4.1.13. *cis*-Diol 20a. Lithium borohydride (13.5 mg, 0.62 mmol) was added to epoxide **19a** (140 mg, 0.62 mmol) in 2-methoxylethyl ether (diglyme) (5 ml). The mixture was heated to 100°C and stirred for 15 h. The reaction mixture was then allowed to cool down to room temperature. Saturated NH_4Cl was added to quench the reaction. The solvent was removed under high vacuum,

and the residue was taken up in EtOAc, washed and dried. Purification by flash chromatography (eluting system: EtOAc/Hexanes=2/1) afforded the desired product as a white solid (82 mg, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 (d, 1H, H-1, J=3.5 Hz), 5.32 (br, 1H, OH), 4.39 (d, 1H, H-2, J=3.5 Hz), 4.19 (m, 2H, H-5), 3.89 (m, 1H, H-4), 2.73–2.85 (2d's, 2H, CH_2CN, J=16.5 Hz, AB system), 1.49 (s, 3H, C(CH_3)$_2$), 1.33 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 116.7, 112.9, 104.1, 85.9, 80.1, 78.9, 60.5, 26.8, 26.3, 23.1; HRMS (FAB) *m/e* calcd for $C_{10}H_{15}KNO_5$ $[M+K]^+$ 268.05873, found: 268.05865.

4.1.14. 1,2-Di-*O*-isopropylidene-3-C-cyanoethyl-5-deoxy-5-isopropylamino-xylofuranose 22a. A solution of oxalyl chloride (12.8 μl, 0.14 mmol) in dichloromethane (5 ml) was cooled down to −78°C and treated dropwise with DMSO (19 μl, 0.26 mmol) under argon. After 15 min, a solution of diol **20a** (28 mg, 0.12 mmol) in dry dichloromethane (5 ml) was introduced slowly. The mixture was stirred for 30 min at −78°C. Triethylamine (85 μl, 0.6 mmol) was added and the reaction mixture was stirred for an additional 30 min. The mixture was then allowed to warm up to room temperature. The solvent was removed in vacuo to give the crude aldehyde **21a**. Aldehyde **21a**, excess isopropylamine and sodium triacetoxyborohydride were dissolved in dichloroethane (DCE) (10 ml). The mixture was stirred at room temperature for 20 h. Aqueous NaOH (2N) was introduced to quench the reaction. Aqueous layer was extracted with ether, the combined extracts were washed with brine and dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Hexanes=1/1 followed by EtOAc/Hexanes/Et_3N=5/5/1) afforded the desired aminoalcohol **22a** as a white solid (25 mg, 77% for two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (d, 1H, H-1, J=3.5 Hz), 4.29 (d, 1H, H-2, J=3.5 Hz), 3.92 (m, 1H, H-4), 3.35–3.39 (m, 2H, H-5), 2.78 (sept, 1H, NCH(CH_3)$_2$), 2.63–2.78 (2d's, 2H, CH_2CN, J=16.5 Hz, AB system), 1.46 (s, 3H, C(CH_3)$_2$), 1.30 (s, 3H, C(CH_3)$_2$),1.05–1.09 (2d's, 6H, NCH(CH_3)$_2$, J=6.5 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 117.3, 112.6, 104.2, 86.6, 80.2, 77.9, 48.7, 45.0, 26.9, 26.3, 23.7, 22.6, 22.3; HRMS (FAB) *m/e* calcd for $C_{13}H_{23}N_2O_4$ $[M+H]^+$ 271.16578, found: 271.16577.

4.1.15. Cyclic phosphoramidite 24a. To a cooled solution of phosphorus trichloride (6.78 μl, 0.077 mmol) in dry dichloromethane (0.20 ml) in a NMR tube at 0°C was added slowly a solution of **22a** (21 mg, 0.077 mmol) in dry dichloromethane (0.45 ml) containing triethylamine (23.8 μl, 0.17 mmol). The NMR tube was then sealed. ^{31}P NMR showed a major resonance at 147.6 ppm right after the addition. A solution of 5′-*O*-TBDMS-thymidine (30.5 mg, 0.085 mmol) in dry dichloromethane (0.4 ml) containing triethylamine (11.8 μl, 0.085 mmol) was then added. The NMR tube was re-sealed. A single resonance appeared at 133 ppm within 5 min. The solvent was removed and the residue was purified by flash column chromatography (eluting system: EtOAc/Hexanes/Et_3N=3/6/1) to afford the desired phosphoramidite **24a** as a white solid (40 mg, 81%). ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 133.08; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (s, 1H, H-6), 6.29 (m, 1H, H-1′ of thymidine), 5.84 (d, 1H, H-1″, J=3.5 Hz), 4.66 (m, 1H, H-3′ of thymidine), 4.41 (d, 1H, H-2″, J=3.5 Hz), 4.20 (m, 1H, H-4′), 3.88–3.94 (m, 3H, H-4″ and H-5′), 3.48 (sept, 1H, NCH(CH_3)$_2$), 3.27–3.31(m, 1H, H-5″), 3.05–3.10 (m, 2H, H-5″, CH_2CN), 2.81 (d, 1H, CH_2CN, J=16 Hz), 2.36 (m, 1H, H-1′), 2.09 (m, 1H, H-2′), 1.90 (s, 3H, CH=C(CH_3)), 1.48 (s, 3H, C(CH_3)$_2$), 1.32 (s, 3H, C(CH_3)$_2$), 1.12 (d, 6H, NCH(CH_3)$_2$, J=7 Hz), 0.90 (s, 9H, SiC(CH_3)$_3$); 0.10 (s, 6H, Si(CH_3)$_2$(*t*-Bu); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.5, 150.2, 135.3, 116.3, 113.0, 110.9, 104.5, 86.7 (J=4.5 Hz), 84.9, 84.8, 78.8,78.8, 75.1, 74.9, 74.3, 63.3, 50.3, 50.0, 47.5, 45.9, 40.6 (d, J=4.5 Hz), 39.9, 34.4 (d, J=3.6 Hz), 26.9, 26.4, 25.9, 23.2, 22.0 (d, J=9.1 Hz), 21.7 (d, J=5.4 Hz), 18.3, 12.5, −5.3, −5.4; HRMS (FAB) *m/e* calcd for $C_{30}H_{50}N_4O_9PSi$ $[M+H]^+$ 669.3085, found: 669.3090.

4.1.16. Dithymidine phosphorothioates 27. Phosphoroamide **24a** (30 mg, 0.046 mmol), T_5′OH (19.6 mg, 0.055 mmol) and 2-bromo-4,5-dicyanoimidazole (18 mg, 0.09 mmol) were dried under vacuum overnight. Dry dichloromethane (0.45 ml) was added slowly to the mixture at 0°C and the mixture was kept at that temperature under argon overnight. Beaucage's reagent (11 mg, 0.055 mmol) was added to the mixture. After 5 min, concentrated ammonium hydroxide (1 ml) was added. Tetrabutylammonium fluoride (TBAF) (1 ml) was introduced after 10 min and the reaction mixture was stirred at room temperature for 1 h. The crude mixture was purified by flash chromatography (eluting system: acetone to H_2O/acetone=1/50) to afford the desired phosphorothioates **27** (R_P/S_P=1/6) as a white solid (12.2 mg, 33%).

27 (S_P isomer): ^{31}P NMR (202.3 MHz, CD_3OD) δ 58.86; ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (s, 1H, ^{5}H-6), 7.78 (s, 1H, ^{3}H-6), 6.29 (dd, 1H, ^{5}H-1′, J=8.0 and 6.5 Hz), 6.22 (dd, 1H, ^{3}H-1′, J=7.8 and 6.0 Hz), 4.98 (m, 1H, ^{3}H-3′), 4.45 (m, 1H, ^{5}H-3′), 4.12 (m, 1H, ^{3}H-4′), 4.05 (m, 2H, ^{5}H-5′), 3.98 (m, 1H, ^{5}H-4′), 3.74 (m, 2H, ^{3}H-5′), 3.17 (m, 8H), 2.40 (m, 1H, ^{3}H-2′), 2.20 (m, 2H, ^{5}H-2′), 2.12 (m, 1H, ^{5}H-2′), 1.90 (s, 3H, CH=C(CH_3)), 1.80 (s, 3H, CH=C(CH_3)); 1.59 (m, 8H), 1.35 (m, 8H), 0.94 (m, 12H); MS (FAB-, NBA) *m/z* 561 (dTP(S)(O)dT)$^-$); HPLC: Retention time: 14.62 min; (Phenomenex C8 Column; solvent A: water; solvent B: acetonitrile; flow rate: 1.5 ml/min; 3% B increases linearly to 7% for the first 30 min, then increases to 40% during the next 20 min).

27 (R_P isomer): ^{31}P NMR (202.3 MHz, CD_3OD) δ 58.81; HPLC: Retention time: 10.04 min, conditions same as the above.

4.1.17. 3-Deoxy-3-methylene-1,2-di-*O*-isopropylidene-D-xylofuranose 18b. To a suspension of methyl triphenylphosphoniumbromide (0.36 g, 1 mmol) in THF at −78°C was added a 1.6 M *n*-butyllithium solution in hexane (0.69 ml, 1.1 mmol). The mixture was kept at −78°C for half an hour and then allowed to warm up to 0°C. Ketone **16** (0.43 g, 1 mmol) in THF was added. The mixture was allowed to warm up slowly to room temperature and stirred for one additional hour. The solvent was removed and the residue was taken up in EtOAc, washed with water, brine and dried over $MgSO_4$. Solvent was removed to provide crude **17b**, which was dissolved in THF and treated with TBAF (1 mmol). After 10 min, the solvent was removed and the residue was taken up in ethyl acetate, washed with water

and brine, and dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Hexanes=1/1) afforded the desired product **18b** (93 mg, 50.1%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84 (d, 1H, H-1, J_{1-2}=4.0 Hz), 5.45 (m, 1H, CH_2=C), 5.16 (m, 1H, CH_2=C), 4.88 (d, 1H, H-2, J_{2-1}=4.0 Hz), 4.79 (m, 1H, H-4), 3.85 (m, 1H, H-5), 3.64 (m, 1H, H-5), 1.97 (t, 1H, OH), 1.49 (s, 3H, C(CH_3)$_2$), 1.35 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 145.4 ($C(CH_3)_2$), 112.6 (CH_2=C), 112.2 (CH_2=C), 104.3 (C-1), 81.9 (C-2), 79.9 (C-4), 63.4 (C-5), 27.4 ($C(CH_3)_2$), 27.1 ($C(CH_3)_2$); MS (CI) *m/z* 187 ((M+1)$^+$, 22.6%), 155 (100%).

4.1.18. 3-Epoxy-1,2-di-*O*-isopropylidene-D-xylofuranose 19b. To 3-deoxy-3-methylene-1,2-di-*O*-isopropylidene-D-xylofuranose **18b** (0.20 g, 1 mmol) in dichloromethane at room temperature was added MCPBA (0.45 g, 1.5 mmol). The mixture was then stirred at room temperature for 30 h and washed with 5% NaS_2O_3 solution, saturated $NaHCO_3$ solution and brine, respectively. The aqueous layer was re-extracted with chloroform several times. The combined organic extracts were dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Hexanes=1/1) afforded the desired product **19b** as a colorless oil (0.17 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.02 (d, 1H, H-1, J_{1-2}=3.5 Hz), 4.50 (m, 1H, H-4), 4.33 (d, 1H, H-2, J_{1-2}=3.5 Hz), 3.62 (m, 2H, H-5), 3.16 (d, 1H, CH_2C(O)C, J=3.5 Hz), 2.97 (d, 1H, CH_2C(O)C, J=3.5 Hz), 1.52 (s, 3H, C(CH_3)$_2$), 1.32 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 133.5 ($C(CH_3)_2$), 104.0 (C-1), 84.0 (C-2), 76.4 (C-4), 65.3 (C-3), 60.2 (C-5), 46.4 (C(O)C), 26.9 ($C(CH_3)_2$), 26.6 ($C(CH_3)_2$); MS (EI) *m/z* 202 (M^+).

4.1.19. 3-*C*-methyl-1,2-di-*O*-isopropylidene-D-xylofuranose 20b. To a suspension of lithium aluminum hydride (31 mg, 0.82 mmol) in dry THF at 0°C was added slowly epoxide **19b** (0.17 g, 0.82 mmol) in THF. The reaction mixture was allowed to warm up to room temperature and stirred for 10 min. A few drops of EtOAc were added to the reaction mixture to destroy excess lithium aluminum hydride. A few drops of water (*n*) were added slowly, followed by 15% NaOH (*n*) and water (3*n*). The precipitate was washed with chloroform several times, the combined organic washings were dried over $MgSO_4$. The solvent was removed to afford **20b** as white crystalline (0.13 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 (d, 1H, H-1, J_{1-2}=3.5 Hz), 4.24 (d, 1H, H-2, J_{1-2}=3.5 Hz), 3.95–4.12 (m, 2H, H-5), 4.04 (br, 1H, OH), 2.24 (br, 1H, OH), 1.48 (s, 3H, CH_3C(OH)), 1.35 (s, 3H, C(CH_3)$_2$), 1.32 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 112.3, 104.5, 87.0, 80.7, 80.7, 60.2, 27.0, 26.3, 18.9; MS (CI) *m/z* 222 ((M+1+NH_3)$^+$, 33.8%), 205 ((M+1)$^+$, 23.3%), 100 (100%); HRMS (FAB) *m/e* calcd for $C_{18}H_{33}O_{10}$ $[M+H]^+$ 409.2074, found: 409.2073.

4.1.20. 3-*C*-methyl-1,2-di-*O*-isopropylidene-5-oxo-D-xylofuranose 21b. Dess–Martin reagent (0.138 g, 0.32 mmol) was added to the diol **20b** (55 mg, 0.27 mmol) in dichloromethane at room temperature. The solution was stirred overnight. Silica gel was then added directly into the reaction mixture. Evaporation of the solvent yielded the white solid mixture that was loaded directly on the top of the column and eluted with the mixture of ethyl acetate and hexane (1/1). The desired product was obtained as a colorless oil (46.4 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.68 (s, 1H, CHO), 6.08 (d, 1H, H-1, J_{1-2}=3.5 Hz), 4.27 (d, 1H, H-2, J_{1-2}=3.5 Hz), 4.19 (s, 1H, H-4), 2.13 (br, 1H, OH), 1.51 (s, 3H, C(OH)CH_3), 1.49 (s, 3H, C(CH_3)$_2$), 1.34 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 201.5, 113.1, 105.6, 86.9, 86.5, 82.4, 27.2, 26.4, 18.9; MS (CI) *m/z* 203 ((M+1)$^+$, 12.3%), 173 (100%).

4.1.21. 1,2-Di-*O*-isopropylidene-3-*C*-methyl-5-deoxyl-5-isopropylamino-D-xylofuranose 22b. To a solution of aldehyde **21b** (45 mg, 0.22 mmol) and isopropylamine (40 μl, 0.44 mmol) in dichloroethane (10 ml) was added sodium triacetoxyborohydride (70 mg, 0.33 mmol). The mixture was stirred at room temperature overnight. Aqueous NaOH (1N) was added to quench the reaction. The product was extracted by ether, washed with brine and dried over $MgSO_4$. Purification by flash chromatography (eluting system: EtOAc/Hexanes/Et_3N=5/5/1) furnished **22b** as a light amber oil (50 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (d, 1H, H-1, J_{1-2}=3.5 Hz), 4.18 (d, 1H, H-2, J_{1-2}=3.5 Hz), 3.84 (m, 1H, H-4), 3.25–3.28 (m, 1H, H-5), 2.83 (m, 1H, H-5); 2.71 (m, 1H, CH(CH_3)$_2$), 1.46 (s, 3H, C(OH)CH_3), 1.30 (s, 3H, C(CH_3)$_2$), 1.28 (s, 3H, C(CH_3)$_2$), 1.04 (s, 3H, CH(CH_3)$_2$), 1.03 (s, 3H, CH(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.9, 104.8, 87.3, 80.6, 79.7, 48.6, 44.2, 27.1, 26.3, 22.7, 22.3, 19.6; MS (CI) *m/z* 246 ((M+1)$^+$, 93.9%).

4.1.22. Cyclic phosphoramidite 24b. To a cooled solution of phosphorus trichloride (4.63 μl, 0.053 mmol) in dry dichloromethane (0.20 ml) in a NMR tube at 0°C was added slowly a solution of **22b** (13 mg, 0.053 mmol) in dry dichloromethane (0.45 ml) containing triethylamine (15.5 μl, 0.11 mmol). The NMR tube was then sealed. ^{31}P NMR showed a single resonance at around 149 ppm right after the addition. T_5'OH (20 mg, 0.058 mmol) in dry dichloromethane (0.4 ml) was then added. The NMR tube was re-sealed. A single resonance appeared at 132 ppm within 5 min. The reaction mixture was poured into a flask, the solvent was removed and purification by flash chromatography (eluting system: EtOAc/Hexanes/Et_3N=1/5/1) afforded the desired product as a white solid (32 mg, 96%). ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 133.36; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (s, 1H, H-6), 6.30 (m, 1H, H-1′ of thymidine), 5.84 (d, 1H, H-1′, $J_{1'-2'}$=3.5 Hz), 4.56 (m, 1H, H-3′ of thymidine), 4.18 (d, 1H, H-2′, $J_{1'-2'}$=3.0 Hz), 4.12 (m, 1H, H-4′), 3.93 (m, 1H, H-4′ of thymidine), 3.77–3.90 (AB of ABX, 2H, H-5′), 3.43 (m, 1H, NCH(CH_3)$_2$), 3.33 (d, 1H, H-5′ of thymidine, $J_{5'eq-5'ax}$=13.5 Hz), 3.03 (m, 1H, H-5′ of thymidine), 2.35 (m, 1H, H-2′ of thymidine), 2.05 (m, 1H, H-2′ of thymidine), 1.89 (s, 3H, CH=C(CH_3)), 1.47 (d, 6H, C(CH_3)$_2$), 1.09 (2d, 6H, NCH(CH_3)$_2$, J=6.8 and 7.8 Hz), 0.90 (s, 9H, SiC(CH_3)$_3$), 0.10 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 177.6, 163.7, 150.3, 135.3, 122.2, 110.9, 104.6, 86.8 (C-2′, $^3J_{C2'-P}$=5.5 Hz), 86.3 (d, C-4′, $^3J_{C4'-P}$=3.6 Hz), 84.9, 80.9 (d, C-3′, $^2J_{C3'-P}$=8.2 Hz), 76.4, 73.6 (d, C-3′ of thymidine, $^2J_{C3'-P}$=21 Hz), 63.2, 49.8 (d, NCH(CH_3)$_2$, $^2J_{C-P}$=37.7 Hz), 40.3 (d, C-2′ of thymidine, $^3J_{C2'-P}$=5.5 Hz), 34.5 (d, C-5′ of thymidine, $^4J_{C5'-P}$=14.5 Hz), 27.1, 25.9, 21.9 (d, NCH(CH_3)$_2$, $^3J_{C-P}$=10.1 Hz), 21.6 (d, NCH(CH_3)$_2$, $^3J_{C-P}$=6.4 Hz), 19.5, 12.5, 10.1, −5.4, −5.3;

HRMS (FAB) *m/e* calcd for $C_{29}H_{51}N_3O_9PSi$ $[M+H]^+$ 644.3132, found: 644.3133.

4.1.23. Dithymidine phosphorothioate 26b. Phosphoramidite **24b** (18 mg, 0.028 mmol), $T_5'OH$ (15 mg, 0.03 mmol) and 2-bromo-4,5-dicyanoimidazole (13.7 mg, 0.07 mmol) were dried under vacuum overnight. Dry dichloromethane (0.45 ml) was added slowly to the mixture at 0°C and the mixture was kept at 0°C for 4 h. Beaucage's sulfurizing (6.7 mg, 0.034 mmol) reagent was then added to the mixture. After 10 min, tetrabutylammonium fluoride (TBAF) was introduced and the reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with dichloromethane, washed with water and brine, and dried over $MgSO_4$. Purification by flash chromatography (eluting solvent: acetone) afforded the **26b** as a white solid (8.8 mg, 40%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (s, 1H), 7.53 (3, 1H), 6.26–6.30 (m, 2H), 5.98 (d, 1H, J=3.5 Hz), 5.21 (m, 1H), 5.16 (d, 1H, J=3.5 Hz), 4.29–4.38 (m, 3H), 4.20 (m, 1H), 4.07 (m, 2H), 3.79 (m, 3H), 3.03–3.12 (m, 2H), 2.95 (m, 1H), 2.48–2.51 (m, 1H), 2.15–2.40 (m, 3H), 1.92 (s, 3H), 1.88 (s, 3H), 1.72 (s, 3H), 1.48 (s, 3H), 1.31 (s, 3H), 1.17 (m, 6H); ^{31}P NMR (202.3 MHz, CD_3OD) δ 61.5, 61.3 (1/7); MS (FAB) 790 ($(M+1)^+$).

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