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Study on the diastereoselective synthesis of dithymidine phosphorothioates through a D-xylose derived chiral auxiliary and development of a novel catalyst

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Abstract

1,2-Di-*O*-isopropylidene-3-*C*-cyanoethyl-5-deoxy-5-isopropylamino-D-xylofuranose **16a** was synthesized. The use of **16a** as a chiral auxiliary led to the diastereoselective formation of dithymidine phosphorothioate. The chiral auxiliary was easily removed by treatment with concentrated ammonia. 2-Mesityl-4,5-dicyanoimidazole displays higher diastereoselectivity than the 2-bromo-4,5-dicyanoimidazole in the coupling reaction. © 2000 Elsevier Science Ltd. All rights reserved.

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Oligonucleotide phosphorothioates (PS-Oligos) have been one of the most advanced antisense agents.[1] The PS-Oligos currently used in clinical trials and biological studies are mixtures of diastereomers. The most useful method to address the stereoselective synthesis of PS-Oligos to date is the oxathiaphospholane approach developed by Stec and coworkers.[2] However, it suffers from the tedious and expensive chromatographic separation of the chiral precursors.

We reported[3] the use of xylose derivatives as chiral auxiliaries for the stereoselective synthesis of phosphorothioates (**A**) (Scheme 1). It had been found that the reaction proceeded with highest stereoselectivity when 2-bromo-4,5-dicyanoimidazole was used as a catalyst, and the coupling reaction was carried out at low temperature. The usual catalyst, tetrazole, gave no selectivity. The removal of the chiral auxiliary at the end of the synthesis was 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 of type **B** or **C** seemed to be good candidates. An S_N1 type displacement or β-elimination should allow for their easy removal. However, phosphite triesters have been shown to be reasonably good leaving groups in β-eliminations[4] or

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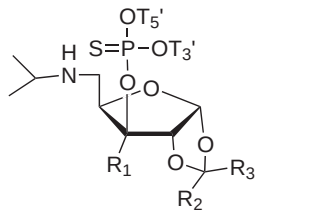
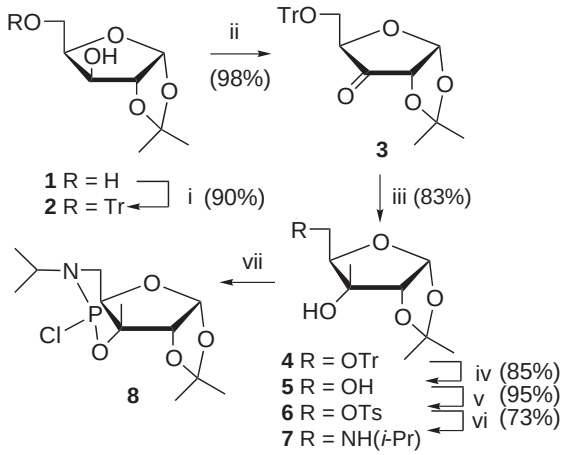


A: R_1 = H, R_2, R_3 = $CH_2CH_2CH_2CH_2$
B: R_1 = R_2 = R_3 = CH_3
C: R_1 = CH_2CN, R_2 = R_3 = CH_3
T_3'OH: 5'-O-TBDMS- thymidine
T_5'OH: 3'-O-TBDMS- thymidine

Scheme 1.

when an appropriate neighboring group can assist in their departure.[5] It was therefore not clear at the outset of this work whether intermediates such as **17**, **18** or **19** had the required stability.

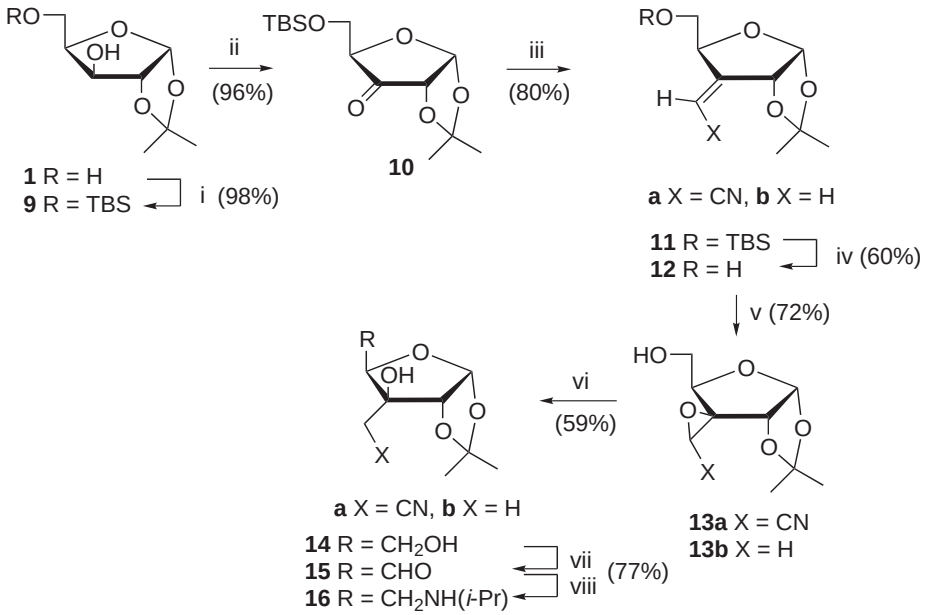
To construct chiral auxiliary type **B**, ketone **3**, obtained from 1,2-di-*O*-isopropylidene-D-xylofuranose **1** in two steps,[6] was used as a starting material. A reaction of **3** with methyl magnesium bromide gave monotrityl diol **4**. Removal of the trityl group, followed by selective tosylation and the displacement of the tosylate with isopropylamine afforded the *trans*-aminoalcohol **7** (Scheme 2).



Scheme 2. (i) TrCl, Et_3N, CH_2Cl_2; (ii) DMSO, Ac_2O, rt; (iii) CH_3MgBr, THF, 0°C to rt; (iv) AcOH, MeOH, reflux; (v) TsCl, pyridine, 0°C; (vi) *i*-$PrNH_2$, 55°C; (vii) PCl_3, Et_3N, CH_2Cl_2

We then proceeded to see whether cyclic phosphoramidite could be formed efficiently. It should be noted that similar *trans*-fused cyclic sulfides have been obtained efficiently and in high yield.[7] *Trans*-aminoalcohol **7** in dichloromethane was treated with phosphorus trichloride in the presence of triethylamine. ^{31}P NMR spectroscopy revealed several peaks ranging from 10 to 220 ppm after the addition. The equilibration of the mixture at 50°C for 2 days failed to produce any major product. We concluded from this experiment that it is necessary to have hydroxy and amino groups *cis* to each other in the chiral auxiliary in order to have an efficient six-membered ring formation.

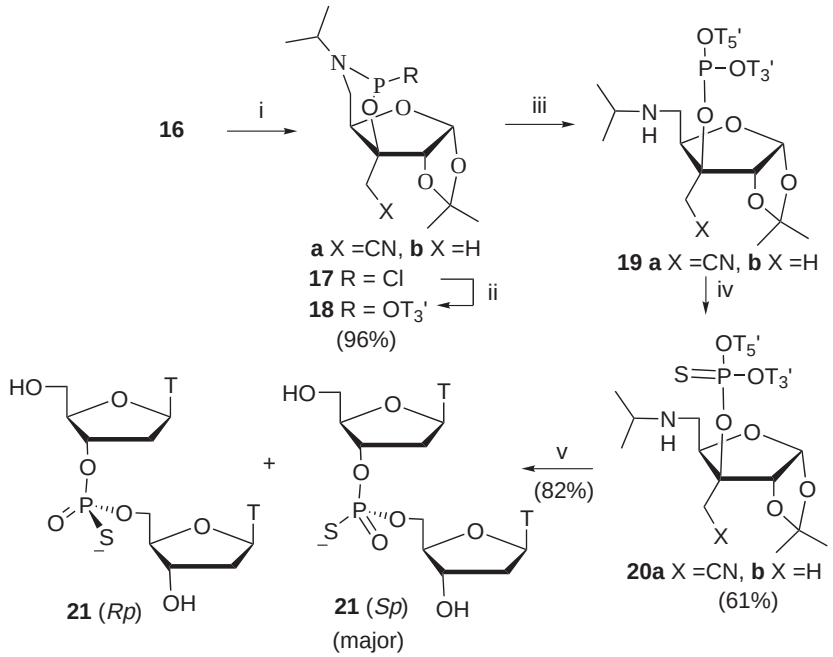
The chiral auxiliary of type **C** was synthesized as illustrated in Scheme 3. D-Xylose derivative **1** was first protected regioselectively at the 5-position as its silyloxy ether **9**, and then oxidized to ketone **10**. Wittig reaction of **10** with diethyl cyanomethylphosphonate afforded two isomers of alkenes in a 5:1 ratio, and these were separated by flash chromatography. The major isomer **11a** was deprotected to give **12a**, which was treated with *N*-benzyltrimethylammonium hydroxide (triton-B) and *tert*-butyl hydroperoxide (TBHP) to furnish epoxide **13a** in which the epoxide oxygen had been formed by reaction at the β-face of the molecule. Samarium diiodide-mediated[8] reduction of the α,β-epoxy nitrile was unsuccessful, and only starting material was recovered. The selective reduction of the epoxide with lithium borohydride in diglyme gave *cis*-diol **14a**, whose configuration was confirmed by 2D-NOESY. Swern oxidation of diol **14a** followed by reductive amination with isopropylamine afforded the desired γ-aminoalcohol **16a**.



Scheme 3. (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

The synthesis of phosphoramidites and phosphorothioates is depicted in Scheme 4. γ-Aminoalcohol **16a** was treated with phosphorus trichloride in the presence of triethylamine. ^{31}P NMR spectroscopy indicated that a major peak at 147.6 ppm was formed immediately, which was assigned to **17a**. Without isolation, the reaction mixture was treated with 5′-*O*-TBDMS-thymidine ($T_{3'}OH$)[9] in dichloromethane. A single isomer of **18a** with ^{31}P NMR signal at 133.1 ppm appeared. Purification furnished pure phosphoramidite **18a**. The coupling reaction between **18a** and 3′-*O*-TBDMS-thymidine ($T_{5'}OH$) was then carried out, using 2-bromo-4,5-dicyanoimidazole as the catalyst. Phosphite triesters **19a** were obtained, as established by ^{31}P NMR spectroscopy (138.5:137.5 ppm = 1:6). Without purification, phosphite triesters were sulfurized[10] to give phosphorothioates **20a** with ^{31}P NMR signals at 59.05 and 59.13 ppm in the same ratio. After treatment with concentrated aqueous ammonia at room temperature for 5 min, the ^{31}P NMR resonances shifted to 57.3 and 56.8 ppm (1:6). The silyl protecting groups were removed

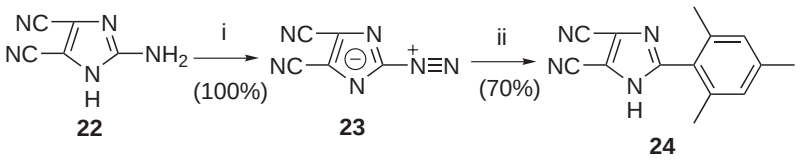
to give dithymidine phosphorothioates **21** (56.8:56.6 ppm = 1:6). The minor and major isomers of **21** were assigned as *Rp* and *Sp*, by comparison of ^{31}P NMR and HPLC data.[11]



Scheme 4. (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) concd ammonia, rt, TBAF

Chiral auxiliary **16b** was synthesized in a similar manner, and transformed to **B**, using the procedure described to transform cyanoethyl derivative **16a** to **20a** (**C**). However, we were not able to remove the chiral auxiliary to form free phosphorothioate **21**.

The stereoselectivity of the coupling reaction is lower than that of the similar system.[3] Different stereochemical outcomes might suggest different coupling mechanisms. When a precursor of type **A** was used in the coupling reaction,[3] the coupling mechanism we suggested was the single protonation mechanism in which the catalyst only served as a proton donor. This is in agreement with the fact that the different sized catalysts give similar selectivities. To verify whether reactions reported here follow the same mechanism, we synthesized the more sterically hindered catalyst **24** (Scheme 5). Commercially available **22** was first diazotized to give **23**, which was then refluxed in mesitylene to give **24**.[12] When **24** was used as the activator in the coupling reaction of **20b**, the same phosphite triesters were obtained in a 15:1 ratio, as compared to a 6:1 ratio when 2-bromo-4,5-dicyanoimidazole was used as a catalyst under the same conditions.



Scheme 5. (i) $NaNO_2$, HCl; (ii) mesitylene

This preliminary result suggests that catalysts not only serve as proton donors, but may also participate in the coupling reactions in our system. Catalyst **24** has a pK_a value similar to tetrazole, and will therefore probably be compatible with the dimethoxytrityl group used in conventional DNA synthesis.

In summary, cyclic phosphoramidite **18a** incorporating a tertiary alcohol was stable enough to be isolated, and allows for the stereoselective synthesis of *Sp* dithymidine phosphorothioate **21**. The chiral auxiliary was removed rapidly by reaction with concentrated ammonia at room temperature. With the removable chiral auxiliary and less acidic catalyst in hand, we are currently optimizing our system and adapting the methodology for routine oligonucleotide synthesis. It is not clear whether the substituted imidazole-based catalyst functions as an acid catalyst only, or as both an acid catalyst and a nucleophile.[3b]

Acknowledgements

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11. ^{31}P NMR resonances of *Sp* and *Rp* isomers in CD_3OD are at 58.86 and 58.81 ppm, respectively. HPLC conditions: Phenomenex C8 column; solvent A: water; solvent B: acetonitrile; flow rate: 1.5 ml min^{-1}; 3% B increases linearly to 7% for the first 30 minutes, then increases to 40% during the next 20 minutes. Retention times of *Sp* and *Rp* isomers are 14.62 and 10.04 minutes, respectively.
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