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A Stereoselective Synthesis of Dinucleotide Phosphorothioates, Using Chiral Indol-oxazaphosphorine Intermediates

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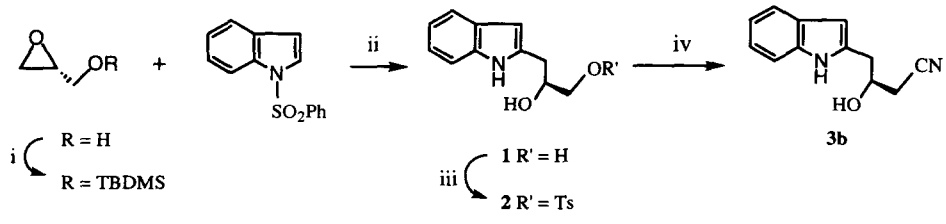
Abstract: *A stereoselective synthesis of dinucleotide phosphorothioates with a de of > 98%, using (S) and (R)-3-hydroxy-4-(2-indolyl)butyronitrile* ***3b*** *as chiral auxiliaries, is reported. The procedure may be adaptable to solid phase synthesis.* © 1997 Elsevier Science Ltd.

In our previous studies,[1] we demonstrated that indol-oxazaphosphorine **5a** is a useful intermediate for the stereoselective synthesis of a chiral phosphorothioate triester (de > 97%). However, the chiral auxiliary used did not permit its removal at the end of a sequence **3a** to **7a**. In this paper, we describe chiral auxiliaries which are easily made as both enantiomers, and can be removed under conditions adaptable to the requirements of solid phase synthesis.

The commonly used deprotection in solid phase synthesis is an ammonium hydroxide mediated β-elimination of a cyanoethylphosphate triester.[2,3] Agrawal *et al.* found that reaction of a 2-acetamido-ethylphosphate triester with ammonia also gave the corresponding diester.[4,5] We therefore synthesized chiral auxiliaries **3b** and **3c** which contain a cyano and amide group β to the potential leaving group from (R)-glycidol. The synthesis of the cyano derivative **3b** is outlined in Scheme 1.

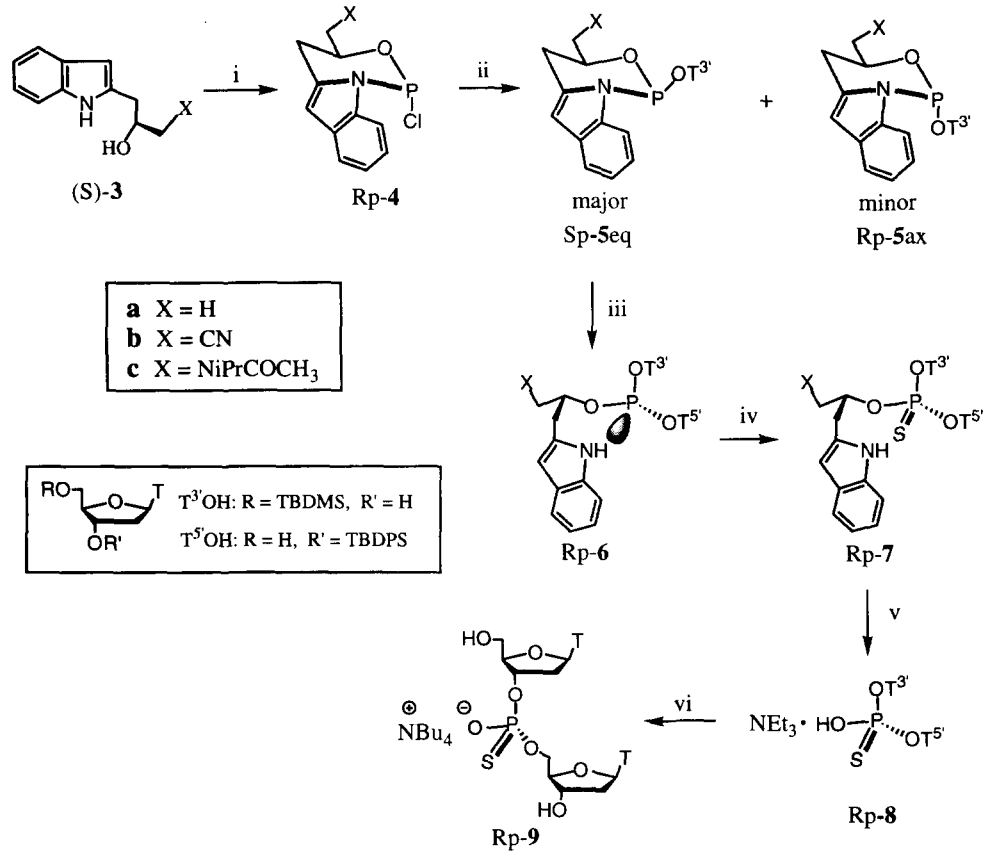
Scheme 1



i) TBDMSCl, NEt_3, DMAP, CH_2Cl_2, 81.2%. ii) a. n-BuLi, -78 °C - 25 °C, overnight, 48.4%. b. KOH, CH_3OH/H_2O (3:1), reflux, 87%. iii) TsCl (1.1 eq.), pyridine, 0 °C, overnight, 100%. iv) NaCN, DMF, 100 °C, 3 hours, 64%.

In THF, the reaction of **3b** with PCl_3 was complete in several minutes to give phosphorochloridite **4b** with a major ^{31}P NMR peak at 144 ppm. 5'-O-TBDMS-thymidine ($T^{3'}OH$) was then added at 0 °C to provide the indol-oxazaphosphorines as a mixture of a major **5beq** (^{31}P NMR in $CDCl_3$, 120.53 ppm) and a minor **5bax** (^{31}P NMR in $CDCl_3$, 120.72 ppm) stereoisomer in a ratio of 12:1. The coupling reaction of **5b** with 3'-O-TBDPS-thymidine ($T^{5'}OH$) was carried out on the unpurified mixture in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU).

Scheme 2



i) PCl_3, THF, Et_3N (3.3 eq.), 0 °C, ii) $T^{3'}OH$, 0 °C iii) $T^{5'}OH$, DBU. iv). Beaucage's reagent. v) 28% NH_4OH, 50 °C, 0.5 hour, flashed chromatography on silica gel (acetone:triethylamine 10:1). vi) 1 M TBAF in THF.

As reported,[1] equatorial indol-oxazaphosphorine **5eq** reacted much faster than the axial one **5ax**. When 3 eq. of **5b** was treated with 1 eq. of $T^{5'}OH$, only one isomer **6b** (^{31}P NMR in THF, 141.61 ppm) was

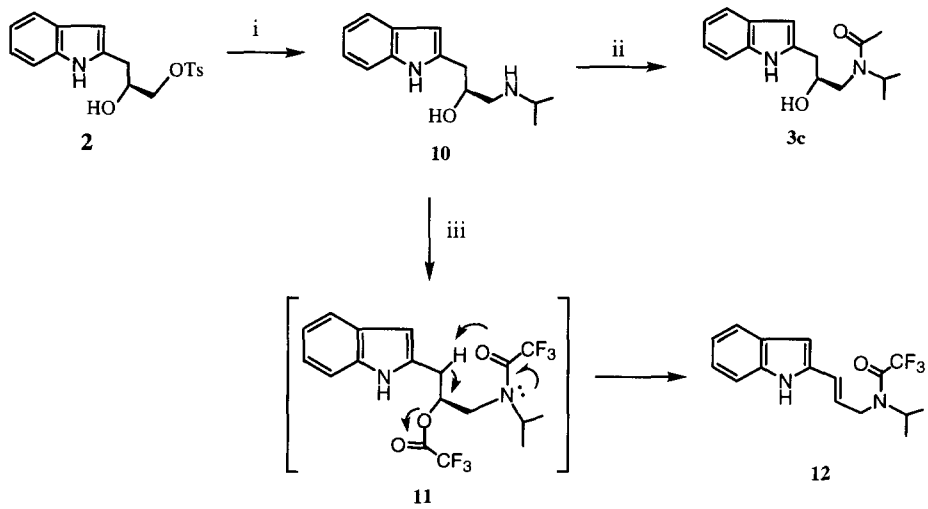
obtained. With 2 eq. or 5eq of DBU at room temperature, the coupling was complete in less than 10 or 5 minutes, respectively. Interestingly, the corresponding reaction of **3a** carrying no cyano group with T[5']OH required several hours. Phosphite triester **6b** was stable to DBU under the conditions used. After filtering off DBU on a short silica gel column, the phosphite triester **6b** was sulphurized with Beaucage's reagent to give phosphorothioate triester **7b** (^{31}P NMR in $CDCl_3$, 66.31 ppm).

The chiral auxiliary on **7b** was easily removed with 28% ammonium hydroxide at 50 ^{0}C for 30 minutes. Chromatographic purification on silica gel (acetone:triethylamine 10:1) and deprotection of silyl groups with TBAF afforded dithymidine phosphorothioate Rp-**9** (^{31}P NMR in D_2O, 55.87 ppm).

In a parallel run, (R)-**3b** was transformed *via* indol-oxazaphosphorine Rp-**5** (^{31}P NMR in $CDCl_3$, 120.78 ppm) and phosphorothioate triester Sp-**7b** (^{31}P NMR in $CDCl_3$, 66.43 ppm) to Sp-**9** (^{31}P NMR in D_2O, 55.55 ppm). The absolute stereochemistry of dimers **9** were confirmed by comparing ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR with the standard Rp and Sp-**9** sample's.[6]

Acetamide derivative **3c** was synthesized by the reaction of acetic anhydride with amine compound **10** obtained from **2** by reaction with isopropylamine. Reaction of **10** with trifloroacetic anhydride gave olefin **12** as the only product.

Scheme 3



i) a. Isopropylamine, 110 ^{0}C, overnight, 81%. ii) Acetic anhydride, CH_2Cl_2, 5 hours, then washed with saturated $NaHCO_3$ solution, 92%. iii) $(CF_3CO)_2O$ (1.2 eq.), CH_2Cl_2.

The reaction of acetamide derivative **3c** with PCl_3 and T[3']OH afforded **5c**eq and **5c**ax in a ratio of 6:1. It was transformed to phosphorothioate triester **7c** (^{31}P NMR in THF, 68 ppm) as described for the corresponding cyano derivative **7b**. In contrast to the cyano derivative, its tetrahydrofuran solution hydrolyzed spontaneously upon sulphurization within several hours to provide **8** (^{31}P NMR in THF, 58 ppm). By adding a base such as

triethylamine, the reaction was complete in several minutes. The mechanism of the reaction has not been elucidated yet and is reasonably complex, since it involves intermediates having ^{31}P NMR signals at 22 and 55 ppm. It may involve either direct neighboring group participation or an elimination analogous as that depicted for **11** to **12**. The development of using an amide derivative as a leaving group is still in progress.

In conclusion, we reported here a methodology for stereoselective synthesis of dinucleotide phosphorothioates by chiral indol-oxazaphosphorine **5b**. Currently, we are adapting this methodology to the solid phase synthesis of oligonucleotide phosphorothioates.

Acknowledgments

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References and notes

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6. The stereochemistry of standard Rp and Sp-**9** samples, made by E. Marsault and Y. Jin in our lab, was confirmed by snake venom phosphodiesterase digestion and HPLC analysis carried out at ISIS Pharmaceuticals (Carlsbad, CA) by Ms. Alice Symons. See Y. Jin, G. Biancotto, and G. Just, *Tetrahedron Lett.* **1996**, *37*, 973, and E. Marsault, Ph.D. Thesis, McGill University, 1996.

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