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Solid-Phase Stereoselective Synthesis of Oligonucleoside Phosphorothioates: The Nucleoside Bicyclic Oxazaphospholidines as Novel Synthons

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Abstract: The nucleoside bicylic oxazaphospholidine derived from L-, or D-prolinol is a novel synthon with potential for solid-phase stereoselective synthesis of oligonucleoside phosphorothioates.

Oligonucleoside phosphorothioates (PS-oligos) (**1**) have shown promise as modulators of gene expression, and are being evaluated in clinical trials against viral diseases, cancer, and inflammatory disorders.[1] The PS-oligos are obtained by synthesis as a mixture of 2^n diastereomers (n = number of phosphorothioate linkages) using solid-phase phosphoramidite chemistry[2] in an automated synthesizer. Interestingly, preliminary biophysical, and biochemical evaluations of stereodefined phosphorothioates reveal some differences between [R_p], and [S_p] PS-oligos.[3] To gain greater insight into the antisense properties of stereodefined phosphorothioates, we were interested in devising a practical stereoselective synthesis of PS-oligos with defined *P*-stereochemistry.

Recently, enzymatic synthesis of "all [R_p]" PS-oligos was achieved using nucleoside 5'-[S_p]-α-thiotriphosphates in conjunction with *DNA polymerase*.[3a] But our enzymatic methodology is not as yet amenable to large-scale work, and in addition, does not provide [S_p] PS-oligos. Attempts to achieve stereoselective synthesis of PS-oligos using phosphoramidite chemistry in conjunction with diastereomerically pure phosphoramidites have not been successful. Thus, when pure [R_p] or [S_p] nucleoside β-cyanoethyl phosphoramidites were employed in the synthesis of PS-oligos, a mixture of [R_p] and [S_p] PS-oligos were produced.[2b] Presumably, this was because of the 1*H*-tetrazole-mediated epimerization of the phosphorous center during the coupling step of oligonucleotide synthesis. Nucleoside oxathiaphospholane is a novel synthon developed by Stec and coworkers to prepare short, but stereodefined PS-oligos.[3b] However, this approach requires: (a) prior separation of the individual *P*-diastereomers (b) longer coupling reactions compared to standard phosphoramidite chemistry, and (c) the presence of unprotected phosphorothioate functionality generated during the coupling reaction may potentially cause the formation of side products during oligonucleotide synthesis. An interesting stereoselective route towards TpsT using indoloxazaphosphorine intermediate was disclosed recently.[4] We describe here a solid-phase approach towards the stereoselective synthesis of PS-oligos using nucleoside bicyclic oxazaphospholidines as synthons.

The nucleoside bicyclic oxazaphospholidines are structurally related to oxazaphospholidines **2** and **2a** which were earlier reported as alternate synthons in oligo synthesis.[5] We also reported our attempted stereoselective synthesis of dinucleoside phosphorothioate, using the related oxazaphospholidine **2b**.[6] With the phosphoramidite **2b** as a synthon, the increased reactivity of the *P* (III) center towards nucleophilic attack and the conformational restraint imposed by the 5-membered ring in the oxazaphospholidine was expected to outcompete the tetrazole-catalyzed epimerization at the *P*-stereocenter during coupling. Additionally, the

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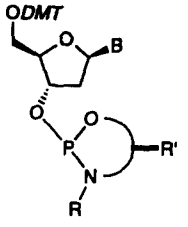
presence of the chiral auxiliary in the ring could provide the requisite facial bias in the nucleophilic attack by the support-bound nucleoside on the oxazaphospholidine **2b**. Although attempts to apply the standard



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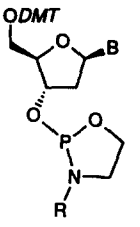
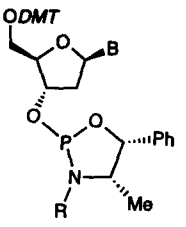


2a

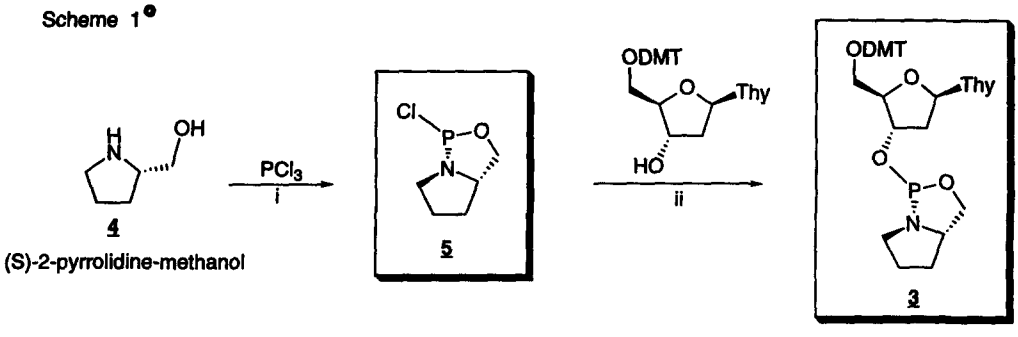


2b

phosphoramidite chemistry to effect the solid-phase stereoselective synthesis were not successful, the corresponding *P*(V) compound of **2b** could be used as synthons for a potential stereoselective approach.

In continuing studies on the synthons of the general structure **2**, we report here the use of the bicyclic oxazaphospholidine **3**, derived from the β-aminoalcohol (*S*) (+)-2-pyrrolidine methanol (L-prolinol) (**4**), for the stereoselective synthesis. The bicyclic system was accessed as shown in Scheme 1. Thus, the reaction of **4**

Scheme 1[•]



4
(S)-2-pyrrolidine-methanol

5

3

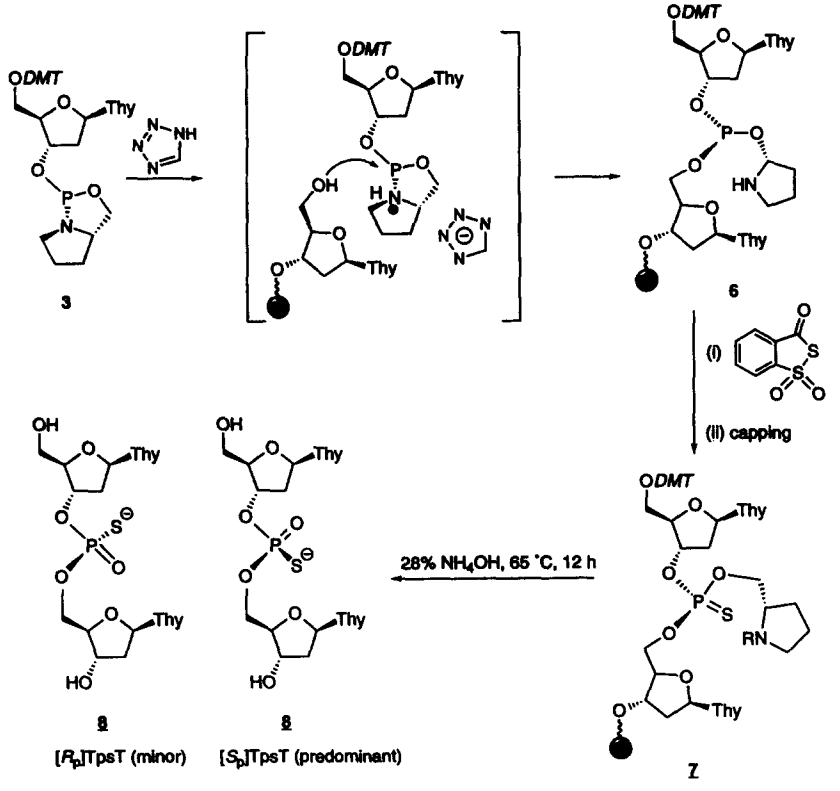
[•] Key: (i) $N(Et)_3$, 12 h (ii) Diisopropylethylamine, 6 h

with PCl_3 at -78 °C in the presence of triethylamine gave the *P*-chloro-oxazaphospholidine **5** as a colorless liquid following vacuum distillation (77.5 °C, at 0.2 mm Hg, 55% yield). ^{31}P NMR of **5** revealed a singlet at δ 178 ppm representing a single isomer. From considerations of steric, and electronic effects, the *P*-stereochemistry of **5** could be tentatively assigned as indicated in which the chlorine atom was pseudoaxially disposed at the face opposite to the prolinol ring.

The reaction of **5** with 5'-*O*-dimethoxytritylthymidine in the presence of *N,N*-diisopropylethylamine at -78 °C gave the nucleoside **3** as a single diastereomer (90% yield). Following work up, examination of the crude product by ^{31}P NMR revealed the presence of a single isomer as revealed by TLC and ^{31}P NMR (δ 151.8 ppm). In analogy with substitution reactions of *P*-chloro-oxazaphospholidines involving carbon-, oxygen-, and nitrogen-based nucleophiles[7] which gave substitution products with overall retention of configuration, **3** could be formulated as having the structure with S_p configuration (experimental determination of absolute stereochemistry of **3** is in progress). The oxazaphospholidine **3** was obtained as a white solid after work up, and stored at 0 to -5 °C until ready to use.

The stage was now set for the use of **3** in solid-phase synthesis using CPG-bound nucleoside. Initially, the manual coupling mode was employed in which an acetonitrile solution of **3** was contacted with CPG-T for 5 to 10 min at room temperature in the presence of 1*H*-tetrazole (Scheme 2). After washing to remove the excess

Scheme 2



reagents, the dinucleoside phosphite **6** was sulfurized with 3*H*-1,2-benzodithiole-3-one-1,1-dioxide[8] (2% in CH_3CN). Following capping, washing, and removal of the DMT group, the CPG-bound product **7** was heated with aqueous ammonium hydroxide (28%, 65 °C, 12 h).[9] Examination of the crude product by HPLC, and ^{31}P NMR (Figure 1) revealed the predominant formation of the S_p isomer of TpsT (**8**) in greater than 90% selectivity[10] (S_p:R_p, 9:1) and coupling efficiency greater than 97%. Encouraged by the results of the manual coupling, we prepared **8** using the standard 1 and 10 μmol coupling program. Again, both ^{31}P NMR and HPLC revealed the predominant formation of the S_p isomer (Figure 1). In a parallel run, the oxazaphospholidine derived from (*R*) (-)-2-pyrrolidine methanol (D-prolinol) gave the predominant formation of [R_p] TpsT (S_p:R_p, 1:9). Preliminary studies with oxazaphospholidines derived from A, C, and G nucleosides also revealed similar stereoselectivity in the coupling reactions. Further efforts are under way to enhance the stereoselectivity by systematic variation in the mixing modes, activating reagents, concentration of reactants, and temperature.

In summary, the bicyclic oxazaphospholidine **3** represents a novel class of nucleoside synthons amenable towards solid-phase stereoselective synthesis of oligonucleoside phosphorothioates.

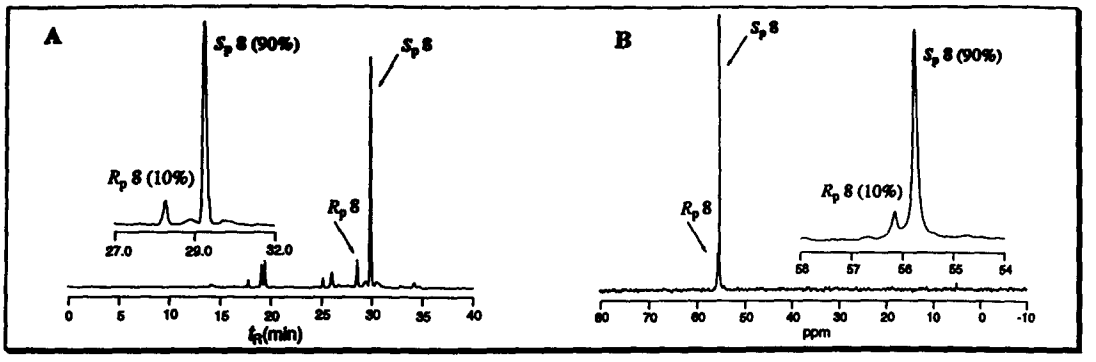


Figure 1. Panel A. HPLC profile of crude TPsT (8) prepared using 3; Panel B. ^{31}P NMR spectrum of TPsT (8) prepared as 10 μmol scale following HPLC purification.

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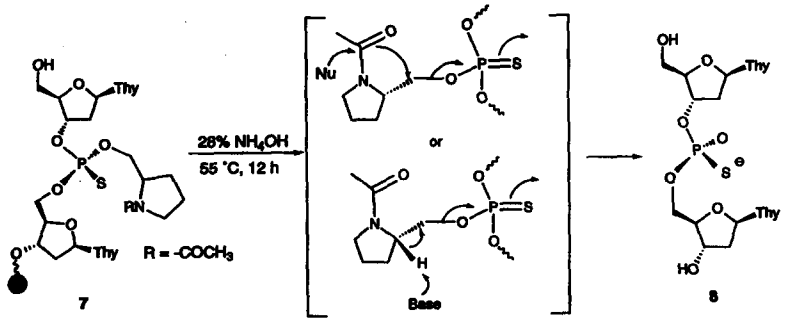
9. The deprotection of the phosphate appendage could follow the sequence shown below:



Efforts are under way to identify the fate of the chiral auxilliary following deprotection.

10. Configurations were assigned as described previously. see: Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2471.