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# Diastereoselective Synthesis of Phosphite Triesters

Zhili Xin and George Just*

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

***Abstract:*** Cyclic phosphoramidites have been obtained with high diastereomeric excess without chromatographic purification. They have been converted to phosphite triesters in a stereoselective manner, using the relatively bulky, acidic 2-bromo-4,5-dicyanoimidazole as a catalyst.

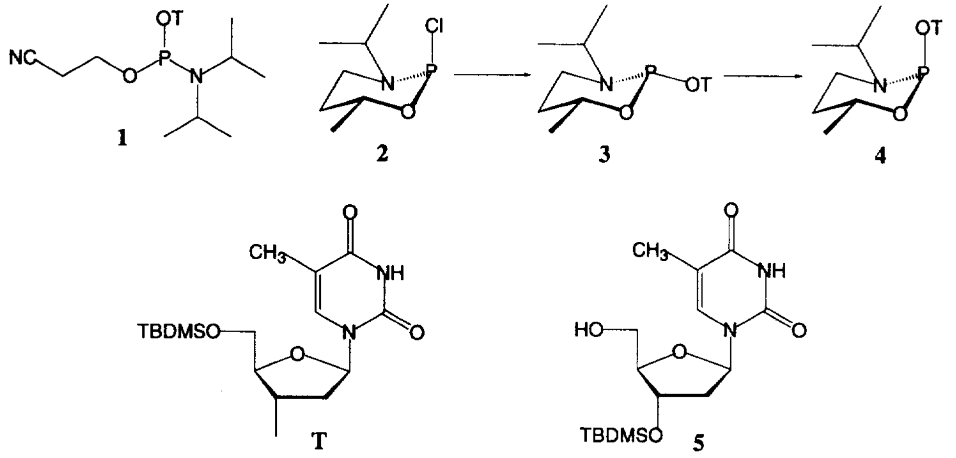
Oligonucleotides are most commonly prepared on a solid phase using the phosphoramidite method developed by McBride and Caruthers.[1] The procedure involves reacting the 5'-end of an (oligo)nucleotide attached to the solid support with an excess of 5'-protected phosphoramidite **1**. The reaction , catalyzed by tetrazole, is complete within minutes and provides a phosphite triester in nearly quantitative yield.[2] Oxidation[1] or thiation[3] then gives the desired phosphate triester or phosphorothioate. The transformation of tri- to tetracoordinate phosphorus has been shown to proceed with retention of configuration.[4] When the phosphoramidite method is applied to the synthesis of phosphorothioates or other derivatives of phosphates in which one of the non-bonding oxygen atoms has been replaced by another heteroatom, a mixture of diastereomers chiral at phosphorus is generated. This is true even when phosphoramidite **1** is separated into its Rp and Sp isomers, and these isomers coupled individually. An antisense oligonucleotide prepared by this method containing n phosphorothioate links will therefore consist of $2^n$ diastereomers, which is clearly undesirable, especially if the mixture (n usually >16) is used as a drug.[5]

Stec *et al.* [6] have found an ingenious solution to this problem by making use of oxathiaphosphospholanes as intermediates. Their method does have some disadvantages, in that "the synthesis and isolation of satisfactorily pure oligophosphorothioates longer than 12-mers ...is still difficult", in addition to requiring chromatographic separation to obtain the pure diastereomers of the phospholanes.

The phosphoramidite method is so well established and so efficient that it seemed very important to modify it to prepare chiral phosphotriesters, rather than developing a new technology. In what follows, we show that diastereomerically pure cyclic phosphoramidites can be prepared without tedious chromatographic separation, and that a certain degree of stereocontrol can be achieved by using substituted imidazoles as catalyst.

### Synthesis of a diastereomerically pure phosphoramidite (Scheme 1).

Ethyl 3R-hydroxybutanoate was transformed to its isopropyl amide by the method of Weinreb.[7] Diborane reduction[8] then gave 2R-hydroxy-4-N-isopropylaminobutane. A ~ 1M solution of the latter in methylene chloride containing 2.2 eq. NEt$_3$ was added to 1.1 eq. of 1M PCl$_3$ in CH$_2$Cl$_2$ at 0°C. After 30 min., evaporation and extraction with Et$_2$O, followed by distillation provided **2** , b.p. 105 °C / 0.5 mm Hg as a seemingly single diastereomer, $^{31}$P NMR $\delta$ 160.6 ppm, in 75% yield. To a 25 mmol CH$_2$Cl$_2$ solution of **2** was added a 50 mmol CH$_2$Cl$_2$ solution of 5'-silylated thymidine ( 0.9 eq. ) containing 1 eq. of NEt$_3$ at 0°C with stirring, and the reaction allowed to go to completion at RT. Phosphoramidites **3** ($^{31}$P NMR $\delta$ 135.0 ppm) and **4** ($\delta$ 133.6 ppm) were obtained in a ratio of 1:3. Both phosphoramidites were configurationally stable after separation by silicagel chromatography (hexanes/EtOAc/NEt$_3$ 5/3/2). When the reaction was carried out at -78°C, ratios of **3** to **4** ranging from 2:1 to 5:1 were obtained. Allowing the crude reaction mixture to stand at RT for a few days, or heating it to reflux in CDCl$_3$ changed this ratio to 1:20. Passage through a short column gave fast-eluting **4** as a single isomer in high purity. The configurations and conformations of **3** and **4** were assigned by analogy to the parent 1,3,2-dioxaphosphorinanes which have been the object of several studies.[9,10,11] They are based on the $^2$J coupling constant between the C$_{3'}$ of thymidine and phosphorus of 19.2 and 7.3 Hz for the major and the minor diastereomers, respectively, and the analogous coupling of 2.7 and 19.2 Hz for C-O-P of the oxazaphosphorinane carbon.



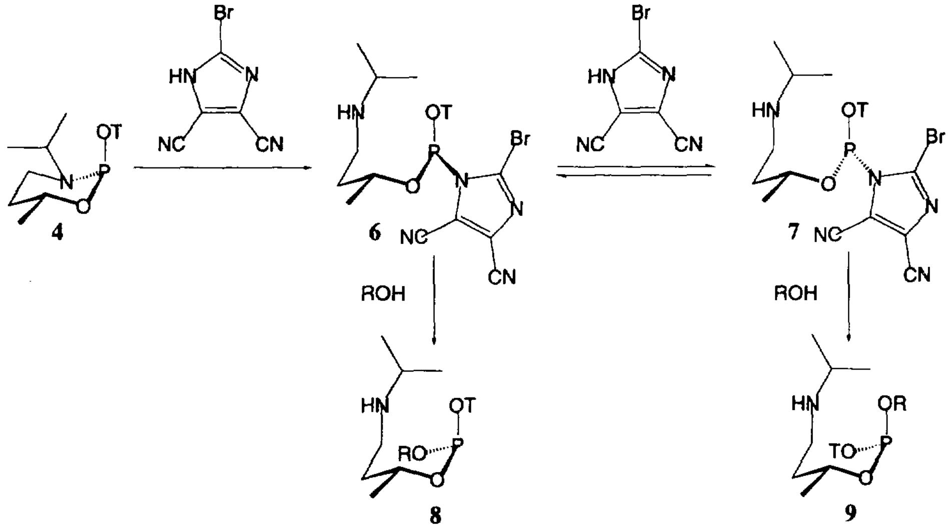
Scheme 1

### Triazoles and imidazoles as catalysts.

In order to assess the role of the catalyst in phosphite triester formation, a variety of commercially available triazoles and imidazoles were investigated. All reactions were carried out with **4** as the phosphoramidite and methanol as the nucleophile in an NMR tube, using CDCl$_3$ as a solvent and monitoring the reaction by $^{31}$P NMR ($\delta$ 139.4 and 138.8 ppm for the major and minor phosphite triester), with the following results: tetrazole (ratio of triesters: 1:1), 3-nitroimidazole, benzotriazole and 5-chlorobenzotriazole (2:1), 4,5-dichloroimidazole (3:1), 4,5-dicyanoimidazole (12:1), 2-nitroimidazole (17:1) and tribromoimidazole (20:1). By far the most rapid reaction took place with dicyanoimidazole ($\tau/2$ <1min), followed by nitro- and tribromoimidazole ($\tau/2$ ~ 10-20 min). Since acidity of the imidazoles seemed to have some relation with the rate of triester formation, and their size with the stereoselectivity of the reaction, we synthesized 2-bromo-4,5-dicyanoimidazole[12] (pKa 2.7). It gave a phosphite triester ratio of 50:1 at a faster rate than with tetrazole (pKa 4.8). Unfortuately, this selectivity dropped to 3:1 when silylated thymidine **5** was used as nucleophile.

In another set of experiments, in which dichloroimidazole was used as catalyst, reaction of phosphoramidite **3**, containing 10 % of **4**, with methanol gave a 1:2 ratio of phosphite triesters as compared to a 2:1 ratio for **4**. Whatmore, **3** reacted considerably more rapidly than **4**, as judged from the rate of disappearance of the two isomers. We also observed that a lowering of the catalyst concentration improved the selectivity of the reaction.

All these data are consistent with a modification of the tetrazole catalyzed reaction of phosphoramidites, as proposed by Stec[13] and evidenced by Berner *et al*.[14,15] In this mechanism, tetrazole displaces the (protonated) amine function in **1** to form a tetrazolide, which undergoes further rapid reaction with tetrazole to give a mixture of epimeric tetrazolides. In a slower reaction, a nucleophilic alcohol displaces tetrazole. In the case of substituted imidazoles, increase of the size of the catalyst allows for successful stereoselective completion of the reaction (**6** to **8**) rather than equilibration (**6** to **7**), provided the nucleophilic alcohol is small (Scheme 2).



Scheme 2

In conclusion, we have shown that cyclic phosphoramidites can be prepared predominantly as one diastereomer without cumbersome chromatographic separations. Using bulky, fairly acidic imidazoles as catalyst, the phosphoramidites can be transformed to phosphotriesters with some degree of stereoselectivity, which seems to depend on the size of the nucleophile used to carry out the displacement reaction. In a companion paper, we describe a similar reaction in which the chiral auxiliary can be removed to form phosphorothioates.

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## References and Notes

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