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Reaction mechanism for the stereoselective internucleotidic bond formation by the oxazaphospholidine approach

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ABSTRACT

This paper describes the reaction mechanisms for the stereoselective synthesis of deoxyribnucleoside 3'-cyclic phosphoramidites as well as dinucleoside phosphite derivatives by the oxazaphospholidine approach. All the key reactions in the present approach were analyzed on the basis of *ab initio* molecular orbital calculations for the model compounds at the HF/6-31G* level.

INTRODUCTION

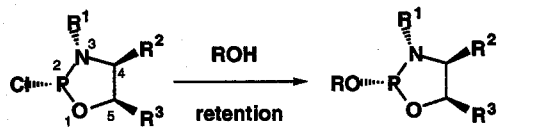
In recent years, a wide variety of backbone-modified DNA analogs have been synthesized as antisense DNAs for selective inhibition of gene expression. Among them, phosphorothioate DNA is the most widely used antisense molecule to date.[1] However, the currently used phoshorothioate antisense DNAs are random mixtures of diastereomers. Quite recently, we have reported a highly diastereoselective synthesis of dinucleoside phosphorothioates by using nucleoside 3'-oxazaphospholidine derivatives and a new class of non-nucleophilic activators, dialkyl(cyanomethyl)ammonium salts (oxazaphospholidine approach).[2] In this paper, we wish to describe unique reaction mechanisms for the diastereoselective synthesis of nucleoside 3'-oxazaphospholidine derivatives and dinucleoside phosphite intermediates by the oxazaphospholidine approach.

RESULTS AND DISCUSSION

Synthesis of nucleoside 3'-oxazaphospholidine derivatives

The phosphoramidite methods utilizing enantiopure 1,2-amino alcohols as chiral auxiliaries have been reported in recent years.[2-5] An advantageous point of these methods is that optically pure phosphoramidite units can be synthesized diastereoselectively from the appropriate enantiopure 1,2-amino alcohols. It has been reported that the reaction of an alcohol with diastereopure (2*R*,4*S*,5*R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine (*trans*-**1a**), which is derived from (1*R*,2*S*)-ephedrin, proceeds through an unusual S_N2 reaction with the retention of the *P*-configuration to give the corresponding 2-alkoxy-1,3,2-oxazaphospholidine derivatives (*trans*-**2a** and **3a**) with excellent diastereoselectivity (Scheme 1).[3,6-9] In a similar manner, we have found that the reaction of 5'-*O*-(dimethoxytrityl)thymidine with *trans*-**1b** and *trans*-**1c** gave diastereopure *trans*-**3b** and *trans*-**3c**, respectively.[2]

Scheme 1



trans-**1**

a: $R^1 = R^2 = Me$, $R^3 = Ph$
b: $R^1 = Me$, $R^2 = R^3 = Ph$
c: $R^1 = Me$, $R^2 = H$, $R^3 = Ph$
d: $R^1 = Me$, $R^2 = i\text{-}Pr$, $R^3 = H$

trans-**2** (R = Me)
trans-**3** (R = 5'-*O*-DMTr-T)

In order to elucidate the unique S_N2 reaction mechanism, *ab initio* molecular orbital calculations were carried out for *trans*-**1a** at the HF/6-31G* level. The LUMO of *trans*-**1a** on the phosphorous atom was found to be located on the backside of the N^3 atom as well as on the backside of the O^1 atom. These LUMO would be stabilized by conjugation with the large LUMO located on the phenyl group at the 5-position. The hydroxy group of an alcohol would preferentially attack at the phosphorous atom from the backside of the N^3 atom with the retention of the *P*-configuration to give the *trans* isomer as a major product. On the contrary, the nucleophilic attack of an alcohol to the phosphorous atom from the backside of the O^1 atom would give rise to the *cis* isomer with the inversion of the *P*-configuration. The latter process is, however, inherently interrupted because of the steric hindrance of the N^3-methyl group. We have successfully obtained the optimized transition state structures of these reactions. A detailed quantum chemical analysis for these reactions is now in progress.

In contrast to the above facts, when diastereopure (2*R*,4*S*)-2-chloro-3-methyl-4-isopropyl-1,3,2-oxazaphospholidine (*trans*-**1d**) was allowed to react with 5'-*O*-(dimethoxytrityl)thymidine, *cis*-**3d** was obtained as a major product with inversion of the *P*-configuration along with the formation of the *trans* isomer. *Ab initio* molecular orbital calculations for *trans*-**1d** revealed that the LUMO on the phosphorous atom is located on the backside of the chlorine atom. In this case, the

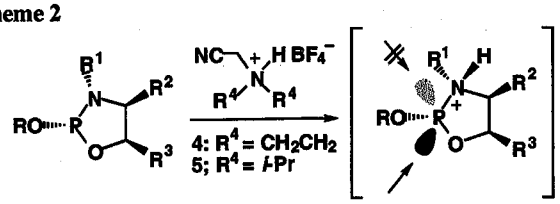
nucleophilic attack of an alcohol at the phosphorous atom from the backside of the chlorine atom would give rise to the product with the inversion of the *P*-configuration as a usual S_N2 reaction.

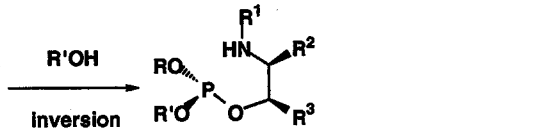
Stereospecific internucleotidic bond formation

In the previous paper, we have demonstrated that the diastereoselectivity of the internucleotidic bond formation depends on both the substituent groups of the oxazaphospholidine ring and the structure of dialkyl-(cyanomethyl)ammonium salts.[2] We have ultimately found that a monomer *trans*-**3c** (R^1 = Me, R^2 = H, R^3 = Ph) and an activator *N*-cyanomethylpyrrolidinium tetrafluoroborate **4** were highly effective for the diastereoselective internucleotidic bond formation. For instance, the reaction of *trans*-**3c** with 3'-*O*-(TBDMS)-thymidine in the presence of **4** in MeCN gave the corresponding phosphite triester **9** with higher than 99% of the diastereoselectivity.[2] On the other hand, a highly bulky **5** was employed as an activator in place of **4**, a significant loss of the diastereoselectivity was observed in the condensation reaction.[2]

Next, *ab initio* MO calculations for conpound **6c**, which is a model of *N*-protonated nucleoside 3'-cyclic phosphoramidite (**7c**), were carried out. The LUMO of **6c**, calculated at the HF/6-31G* level, suggested that there exist two possible directions for the nucleophilic attack of an alcohol to the phosphorous atom (Scheme 2).

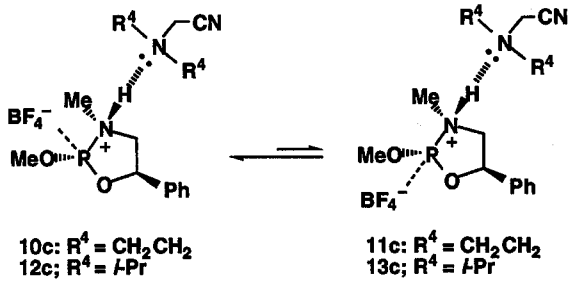
Scheme 2





The hydroxy group of an alcohol would preferentially attack at the phosphorous atom from the backside of the protonated N^3 atom to give the corresponding phosphite triester **8c** with the inversion of the *P*-configuration. It is apparently disadvantageous that the nucleophilic attack of an alcohol to the phosphorous atom from the backside of the O^1 atom because of the steric hindrance of the N^1-methyl group. In the case of using a highly bulky activator **5**, however, the result of low diastereoslective condensation cannot be explained only in terms of the steric effect of the *N*-protonated species **7** as described above. We have also carried out geometry optimization for various ternary complexes, which consist of *N*-protonated oxazaphospholidine, dialkyl(cyanomethyl)-amine, and tetrafluoroborate anion, in MeCN using the Onsager reaction field model.[10] It was found that a ternary complex **10c**, in which BF_4^- is coordinated to the phosphorous atom from the backside of the O^1 atom and a *N*-cyanomethylpyrrolidine is located on the protonated N^1 atom by the hydrogen bond, is a highly plausible intermediate for the diastereoselective condensation reaction (Scheme 3). When a bulky activator **5** is employed, BF_4^- is hardly coordinated to the phosphorous atom from the front side of the protonated N^1 atom because of the steric hindrance of the neighboring bulky cyanomethylamine. As a result, relative stability of the ternary complex **13c**, in which BF_4^- is coordinated to the phosphorous atom from the backside of the protonated N^1 atom, would be increased. In this complex, the nucleophilic attack of an alcohol to the phosphorous atom from the backside of the protonated N^1 atom would be blocked by the coordinated BF_4^-. Geometry ptimization of the transition state structures involving these ternary complexes is now in progress.

Scheme 3



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