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Convenient Syntheses of Oligoribonucleotides Having 2'-5' and/or 3'-5' Phosphodiester Linkage

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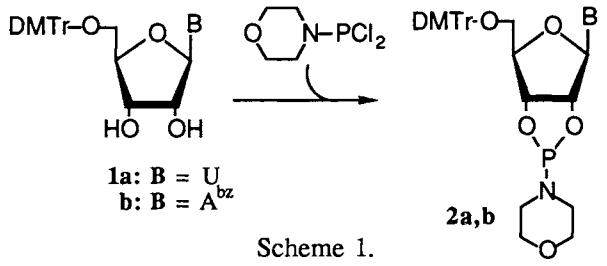
Sequence-defined oligoribonucleotides having 2'-5' and/or 3'-5' phosphodiester linkage have been synthesized by use of 5'-protected ribonucleoside 2',3'-cyclic phosphoramidite on solid support. In coupling reaction, pKa of azole as a catalyst governs the activation. The resulting 2'-5' and/or 3'-5' linked oligoribonucleotides were easily separated by reversed-phase chromatography.

Recently, an oligodeoxyribonucleotide was easily obtained by polymer-supported phosphite approach which was developed and improved by Letsinger[1] and Caruthers[2]. However, the synthesis of oligoribonucleotides is not easy because the protection of hydroxyl group of ribonucleosides is a piecework job. In order to overcome this job, several highly reactive and selective phosphitylating reagents such as tris(azolyl)phosphine were developed in our laboratory.[3] Their phosphitylating reagents reacted with 2',3' cis-glycol of ribonucleoside to give ribonucleoside 2',3'-cyclic phosphoramidite derivatives which have highly selective reactivity with 5'-OH of ribonucleoside. They were useful for syntheses of homooligoribonucleotides by polymerizations of unprotected ribonucleosides.[3,4] Also, syntheses of sequence-defined diribonucleotides have been studied preliminary with (dialkylamino)-dichlorophosphines as a phosphitylating reagent.[5] Here, for the purpose of synthesis of sequence-defined oligoribonucleotides having 2'-5' and/or 3'-5' phosphodiester linkage, we have investigated an effective method for activation of 2',3'-cyclic phosphoramidite and a chain elongation through coupling with nucleoside derivatives by solid-phase method.

In the coupling reaction of nucleoside phosphoramidite with nucleosides, tetrazole is normally used as a catalyst. It was reported that azoles behave as an acid catalyst and a nucleophilic catalyst for the tetrazole-activated coupling step.[6] In order to achieve an effective coupling reaction, the effect of azoles was investigated by ^{31}P NMR. 5'-O-Dimethoxytrityluridine 2',3'-cyclic phosphoromorpholidite (DMTr-U>p(mor)) was synthesized by the phosphitylation of 5'-O-dimethoxytrityluridine (0.28 mmol) with dichloromorpholinophosphine (0.28 mmol) in acetonitrile containing ethyldiisopropylamine. To the reaction solution, a solution of azole (2.8 mmol) in DMF

was added and the reaction mixture was stirred at 0 °C for 60 min. The reaction mixture was analyzed by ^{31}P NMR. A signal of DMTr-U>p(mor) (146 ppm) decreased and a new signal appeared at 133 ppm by the addition of azoles. Although this new signal has not been identified, it is presumably an active intermediate. This is because dinucleotide derivative was produced by the addition of ribonucleoside to the reaction mixture containing the active intermediate. Fig. 1 shows the relation between the pKa of azoles and the peak areas of the signal (133 ppm). The smaller became pKa of azole, the larger amount of the intermediate was produced. This result indicates that the more acidic azole is the effective for the activation of the phosphoramidate derivatives. Hence 5-(*p*-nitrophenyl)tetrazole, which is most acidic azole in this study, was used.

In order to perform a solid phase synthesis effectively, isolations of 5'-O-protected ribonucleoside 2',3'-cyclic phosphoromorpholidites were tried (Scheme 1). Dichloromorpholinophosphine (1.0 mmol) was added to a solution of 5'-O-dimethoxytrityluridine or N^6-benzoyl-5'-O-dimethoxytrityladenosine (1.0 mmol) in THF (5 mL) containing 2,6-lutidine (2.0 mmol) at 0 °C and then the mixture was



Scheme 1.

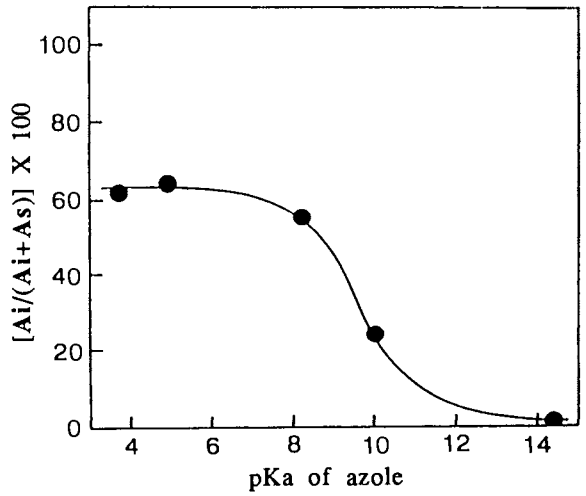


Fig. 1. The effect of azole for the activation of DMTr-U>p(mor). As and Ai are the areas of the signals of 146 ppm and 133 ppm in ^{31}P NMR spectra, respectively. Acidic pKa of azoles used are as follows: 5-(*p*-nitrophenyl)tetrazole, 3.7; tetrazole, 4.9; benzotriazole, 8.2; 1,2,4-triazole, 10.0; imidazole, 14.4.

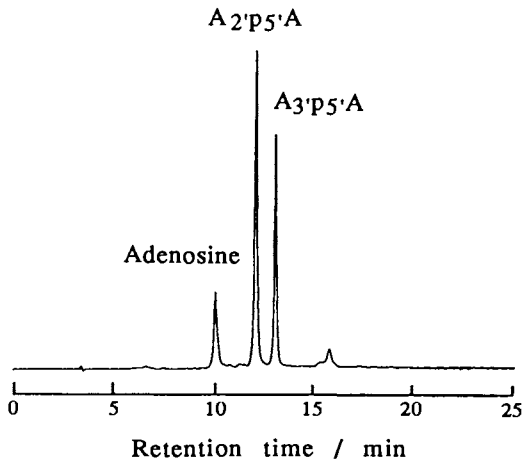
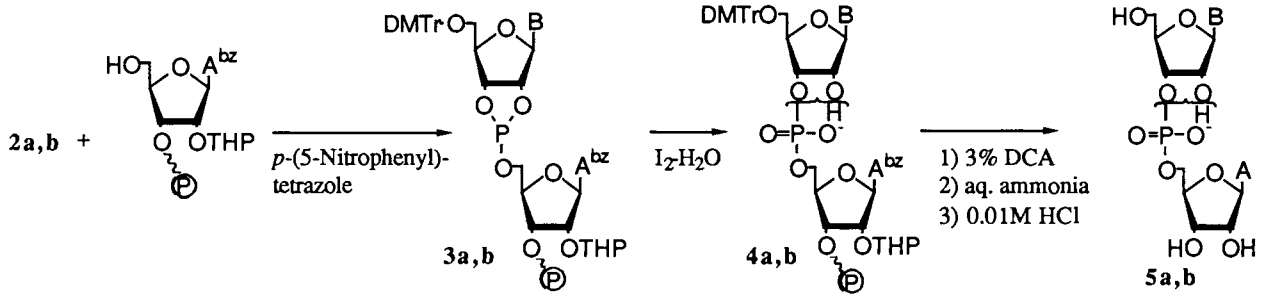


Fig. 2. HPLC profile of adenylyl-adenosine synthesized on solid support. A2'p5'A and A3'p5'A were adenylyl-adenosine having 2'-5' or 3'-5' phosphodiester linkages, respectively. HPLC condition: column, DAISO ST-120-5-C_{18}; eluent, 3-27% acetonitrile gradient (1%/min) in 0.1 M triethylammonium acetate; flow rate, 1.0 mL/min.

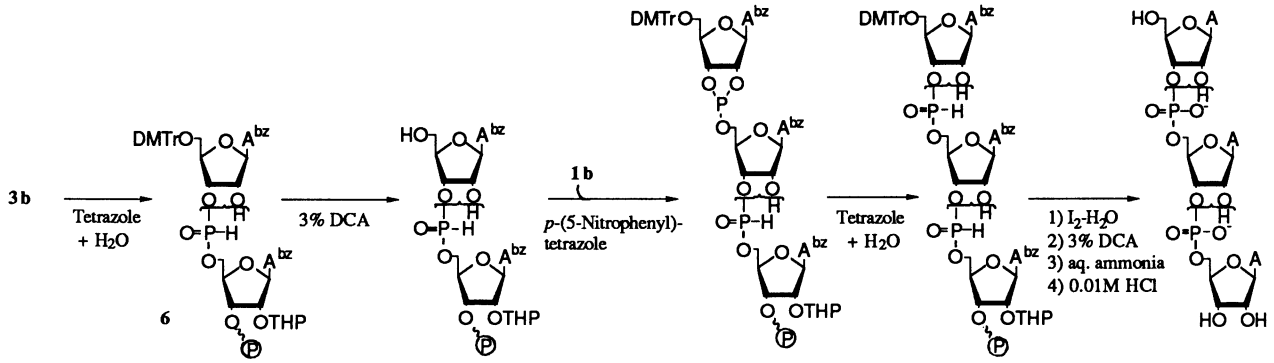
stirred for 20 min under Ar atmosphere. The resulting suspension was centrifuged at 6000 rpm for 20 min. The supernatant was applied on silica-gel column and eluted by dichloromethane/ethyl acetate/triethylamine (4/5/1, v/v/v). Appropriate fractions were collected and evaporated. The residue was dissolved in a small amount of dichloromethane and the solution was added dropwise to hexane at 0°C. The resulting white precipitate was collected by filtration and dried over phosphorus pentoxide *in vacuo*. Yields of DMTr-U>p(mor) and N^6-benzoyl-5'-O-dimethoxytrityl-adenosine 2',3'-cyclic phosphoromorpholidite (DMTr-bzA>p(mor)) were 31% (205 mg) and 31% (245 mg), respectively. Structures of these compounds were identified by elemental analyses[7] and NMR studies. ^{31}P NMR spectrum of DMTr-U>p(mor) exhibited singlet at 146.3 ppm and that of DMTr-^{bz}A>p(mor) exhibited two singlets at 146.9 and 147.0 ppm. Their chemical shifts indicate that phosphoramidite moiety, such as (-O-)$_2$P-N-, exists in the compounds. In ^1H NMR analysis, the signals of $H_{2'}$ and $H_{3'}$ shifted downfield and became the complicated signals by phosphorus-proton couplings. Isolated 5'-O-protected ribonucleoside 2',3'-cyclic phosphoromorpholidite could be stored at -20°C under Ar atmosphere.



Scheme 2.

For the synthesis of diribonucleotide on the solid support, isolated DMTr-U>p(mor) and DMTr-^{bz}A>p(mor) were used (Scheme 2). The synthesis was carried out on N^6-benzoyl-5'-O-dimethoxytrityl-2'-O-tetrahydropyranyladenosine loaded silica-gel support (0.049 mmol/g, 20 mg) by syringe technique[8] as follows; 1) washing with dichloromethane, 2) detritylation with 3 % dichloroacetic acid in dichloromethane (1 min), 3) washing with dry acetonitrile, 4) coupling with 5'-O-protected ribonucleoside 2',3'-cyclic phosphoromorpholidite (0.06 mmol) using 5-(*p*-nitrophenyl)tetrazole (0.06 mmol) in dry acetonitrile (10 min), 5) oxidation with iodine-water, 6) washing with acetonitrile, and further step 1) to 3). The product was removed from the resin and deprotected by treatment with conc. ammonia/pyridine/water (2/1/1, v/v/v) at r.t. for 24 h followed by 0.01 M HCl aq. solution at r.t. for 1h. The couping yields were 82 and 78 % for UpA and ApA, respectively, based on dimethoxytrityl cation assay. The products were analyzed by reversed-phase HPLC, indicating the ratio of 2'-5' and 3'-5' linkage isomers, 68 : 32 for UpA and 54 : 46 for ApA. For example, HPLC profile of crude ApA is shown in Fig. 2. The yield based on the HPLC analysis was 73%. Since the relatively pure products were obtained without further purifications, the purification was easy as compared with a solution-phase synthesis.

Additionally, for the purpose of chain elongation, the oxidation of phosphite triester into phosphodiester linkage must be performed in the final step. Therefore, the phosphite triester linkage was converted into a H-phosphonate linkage by using 0.5 M tetrazole in acetonitrile/water (4/1, v/v) prior to the oxidation, and the oxidation of the H-phosphonate linkages was carried out with 0.1 M iodine in THF/2,6-lutidine/water (2/2/1, v/v/v). The synthetic route of trimer is illustrated in Scheme 3. The average coupling yield of ApApA synthesis was 83 %, based on dimethoxytrityl cation assay. The ratio of linkage isomers was $A_{3'}p_{5'}A_{3'}p_{5'}A$: $A_{3'}p_{5'}A_{2'}p_{5'}A$: $A_{2'}p_{5'}A_{3'}p_{5'}A$: $A_{2'}p_{5'}A_{2'}p_{5'}A$ = 21:33:21:25. The products were identified by treatment with snake venom phosphodiesterase and spleen phosphodiesterase.



Scheme 3.

The phosphitylating reagent having selective reactivity brought a simple method for the syntheses of sequence-defined oligoribonucleotides having 2'-5' or 3'-5' phosphodiester linkage on solid support. Recently, oligoribonucleotides having 2'-5' phosphodiester linkage have become of interest for the synthesis of branched oligoribonucleotides[9] and 2'-5' linked oligoadenylates[10].

References

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7) Anal. Found of (DMTr-U>p(mor)): C, 61.46; H, 5.58; N, 6.26; P, 4.55. Calcd. for $C_{34}H_{36}N_3O_9P$: C, 61.72; H, 5.48; N, 6.35; P, 4.68. Anal. Found of (DMTr-^{bz}A>p(mor)): C, 63.35; H, 5.23; N, 10.38; P, 3.73. Calcd. for $C_{42}H_{41}O_8N_6P$: C, 63.95; H, 5.24; N, 10.65; P, 3.93.
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