SYMPOSIUM ON NUCLEIC ACIDS TECHNOLOGY

HELD AT
OKAYAMA, JAPAN
FEBRUARY 20th – 21st, 1988

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H.Hayatsu
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Sequence-defined dimer block synthesis from unprotected nucleoside

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ABSTRACT

Several sequence-defined dimer blocks as stable intermediates to synthesize the oligodeoxynucleotides have been synthesized from unprotected nucleoside using morpholinophosphorditetrazolide. From these dimer blocks, sequence-defined oligodeoxynucleotides were also synthesized. The general procedure is composed of following steps; a) reaction of 5'-O-protected nucleoside with morpholinophosphorditetrazolide (phosphitilation), b) reaction of the resulting mononucleoside phosphoramidite with the second nucleoside (condensation), and c) non-aqueous oxidation with t-BuOOH (oxidation).

INTRODUCTION

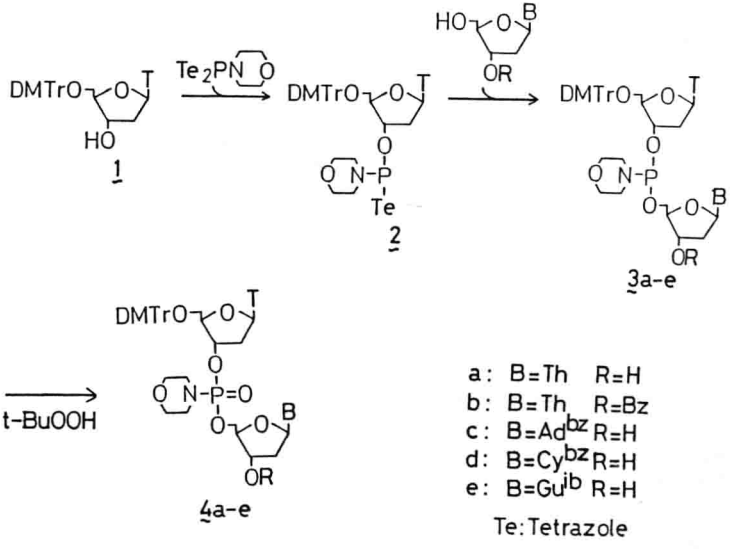
Since Letsinger[1] and Caruthers[2] introduced uses of phosphite and phosphoramidite approaches for the synthesis of oligonucleotide, extensive research has been directed toward developing synthetic method and efficient reagents.

Previously, we have developed several phosphoramidites, and synthesized homo-oligoribonucleotides[3,4] and sequence-defined oligoribonucleotides[5] by use of them as phosphitilating reagents. Further, their phosphitilating reagents were modified and applied to synthesize oligodeoxynucleotides.

Now, a development of chemical synthetic method to synthesize sequence-defined oligonucleotides in large scale is important to solve many genetic phenomena by use of physical chemical measurements and further to develop a new medicine. The purpose of the present study is a rapid and large scale synthesis of sequence-defined oligodeoxynucleotide using the phosphitilating reagent. The nucleoside phosphoramidite obtained from the phosphitilating reagent and the first nucleoside has selectivity toward hydroxyl groups of the second nucleoside.

In this paper, we wish to describe the synthesis of the sequence-defined dimer blocks, the starting materials for the synthesis of oligodeoxynucleotides, by use of the unprotected nucleosides and to refer to their chain elongation.

RESULTS AND DISCUSSION



Scheme

The synthetic route of dimer blocks is illustrated in the scheme. The procedure involves the phosphorylation of a 5'-O-protected deoxynucleoside, the subsquent condensation of the resulting nucleoside phosphoramidite with a second nucleoside, and the oxidation with t-BuOOH.

A solution of tetrazole(1.1 mmol) in CH_2Cl_2-pyridine (1:1, v/v)(1.0 ml) was added to morpholinophosphordichloridite(77.6 µl) in CH_2Cl_2(1.0 ml) containing N,N-diisopropylethylamine(0.2 ml). After the reaction mixture was stirred at 0°C for 20 min, a solution of 5'-O-protected nucleoside 1(0.5 mmol) in CH_2Cl_2(1.5 ml) was added dropwise over a period of 10 min to the reaction mixture at 0°C. The mixture was stirred for additional 30 min, and then it was added dropwise to second nucleoside(0.5-1.0 mmol) in CH_2Cl_2-pyridine(3.0-5.0 ml) containing N,N-diisopropylethylamine(0.2 ml) and kept for 30 min. A 3M t-BuOOH

Table Yields and Physical Chemical Data for Compounds

Compound	Yield[a] (%)	δ-^{31}P[b] (ppm)	Isomer I/II	λ_{max}(nm)	m.p. (°C)	Rf[c]
4a	67	7.08	36/64	266, 235	137-139 135-138	0.39
4b	62	6.96 7.03	33/67	266, 231	137-139 140-141	0.57
4c	62	7.01 7.08	33/67	275, 234	144-148 142-144	0.42
4d	55	6.98 7.22	31/69	304, 261, 236	146-149 146-147	0.44 0.43
4e	70	6.04	30/70	261, 237	>159 >155	0.28 0.27

a) Based on HPLC except for compound 4a (isolated yield).

b) Relative to external 85% H_3PO_4

c) TLC on silica using CH_3OH : $CHCl_3$ (1:8, v/v)

in toluene(0.2 ml) was added to the mixture and then the solution was stirred for 10 min at 0°C.

Yields and physical chemical data for compounds obtained are shown in Table.

The advantages of the present approach are as follows.

i) Nucleoside phosphoramidite 2 has selectivity toward 5'-OH of the second nucleoside and yields 3'-5' linked phosphite compound predominantly. Hence a protection of hydroxyl groups of the second nucleoside is not required. This feature leads to a simple and rapid synthesis of oligodeoxynucleotides.

ii) The P-N bond is so chemically stable that nucleotide chain can be elongated in similar manner.

iii) A large scale synthesis is feasible, since this synthesis proceeds in homogeneous system.

This procedure will provide an attractive synthetic route for a large amount of sequence-defined oligodeoxynucleotide.

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Efficient oligoadenylate synthesis catalyzed by uranyl ion complex in aqueous solution

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ABSTRACT

Polymerization of adenosine-5'-phosphorimidazolide in an aqueous solution was conducted with uranyl ion as a catalyst. Oligoadenylate formation took place efficiently with high regio-selectivity (2'-5' linkage). The oligoadenylates up to hexadecamer were obtained in a high total yield. The chain length and the regio-selectivity of the resulting oligoadenylates varied greatly depending on the concentration of the uranyl ion catalyst. The oligonucleotide formation is likely to be mediated by uranyl-nucleotide complex.

INTRODUCTION

Nucleoside-5'-phosphorimidazolide is an activated nucleotide and easily hydrolyzed to nucleoside-5'-phosphate in an aqueous solution. It is proposed as a precursor for prebiotic synthesis of nucleic acids.[1,2] Previously we reported that divalent metal ions such as Pb^{2+}, Zn^{2+} and Co^{2+} catalyze the polymerization of nucleoside-5'-phosphorimidazolide to give oligonucleotides.[3] The resulting oligonucleotides contain mainly 2'-5' internucleotide linkages. Pb^{2+} ion-catalyzed oligonucleotide formation was applied to the synthesis of 2'-5' oligoadenylate,[4] which is implicated in the interferon's antiviral action.[5] We have further studied the mechanism of the metal-ion catalyzed oligonucleotide formation and explored other efficient catalysts. We have found that uranyl ion is a highly effective catalyst for the oligonucleotide synthesis. Now we wish to present the synthesis of oligoadenylates from adenosine-5'-phosphorimidazolide catalyzed by uranyl ion.

EXPERIMENTAL

Adenosine-5'-phosphorimidazolide (ImpA) was prepared from adenosine 5'-phosphate and imidazole as described previously.[4] The polymerization of ImpA was carried out in an aqueous solution. A typical reaction mixture (200 μl) contained 50 mM ImpA, 1 mM uranyl nitrate and 0.2 M N-ethylmorpholine buffer (pH 7.3). After incubation for 1 day at 20°C, the reaction mixture was treated with EDTA solution to form EDTA-uranyl complex. The reaction mixture was analyzed by HPLC using an RPC-5 column. The elution was carried out with a linear gradient of 0 - 0.05 M $NaClO_4$ containing 2.5 mM Tris-acetate buffer (pH 7.5) and 0.1 mM EDTA in 60 min at a flow rate of 1.0 ml/min.

RESULTS AND DISCUSSION

ImpA hydrolyzed to pA easily in an aqueous solution in the absence of metal ion catalyst. Addition of catalytic amount of uranyl ion promoted the polymerization of ImpA. The HPLC profile of the reaction mixture, in which 1 mM of uranyl ion (molar ratio, $[ImpA]/[UO_2^{2+}]=50$) was used as a catalyst, is shown in Fig. 1. The formation of the hydrolyzed product, pA was very small. High total yields of oligoadenylates from dimer to hexa-decamer were obtained. The 2'-5' internucleotide linkage was formed selectively, especially in the short-chained oligomers.

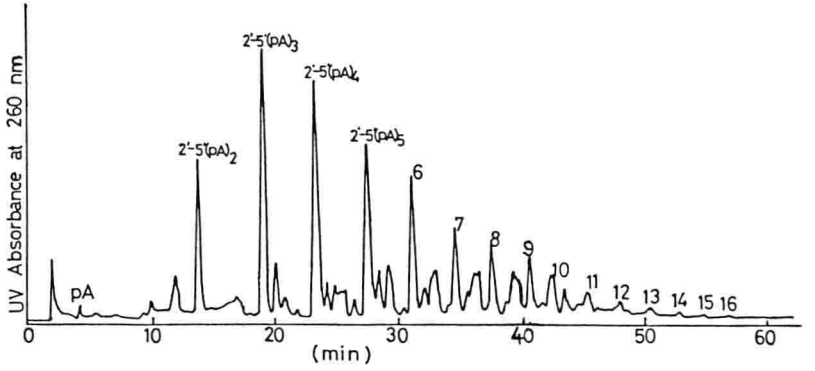

Fig. 1. HPLC Profiles of Oligoadenylic Acids Obtained from ImpA Catalyzed by Uranyl Ion
The reaction was run at 20°C and pH 7.3 for 1 day. 50 mM ImpA and 1 mM uranyl nitrate were used for the reaction.

The yield of 2'-5' linked dimer, trimer, tetramer, pentamer, hexamer, heptamer and octamer was 5.6%, 14.8%, 14.2%, 12.0%, 8.9%, 5.2% and 3.6%, respectively. On the other hand, linkage isomers of oligoadenylic acids which contain at least one 3'-5' internucleotide linkage were formed in small amounts. The uranyl ion catalyzes the oligoadenylates formation far more efficiently than Pb^{2+} ion which has been known to be the effective catalyst[3,4] When the same amount of Pb^{2+} ion was used as a catalyst, 2'-5' linked dimer, trimer, tetramer and pentamer were formed in 20.4%, 2.8%, 0.6% and 0.2% yield, respectively, after 5 days.

The concentration of the uranyl ion had a large effect on the chain length and the internucleotide linkage of the resulting oligoadenylates. At low concentrations of uranyl ion, hydrolysis of ImpA competed with the condensation of ImpA and decreased the chain length of the oligomers. When 10 μM of the uranyl ion was used as a catalyst ($[ImpA]/[UO_2^{2+}] = 5,000$), 2'-5' and 3'-5' linked dimers were formed in 10.2% and 5.0% yield, respectively. Hydrolysis of ImpA was predominant and a very small amount of long-chained oligomers was formed. 50 μM of the uranyl ion gave the highest yield of 2'-5' linked diadenylate. The 2'-5' linked trimer, tetramer and pentamer were formed in significant yields when 100-500 μM of the uranyl ion was used as a catalyst.

The oligoadenylate formation is likely to be mediated by uranyl-ImpA complex.

The uranyl ion-catalyzed oligoadenylates synthesis provides the most effective and convenient method for the synthesis of 2'-5' oigoadenylates with desired chain length from dimer to octamer

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(Butylthio)carbonyl group: a new protecting group for the guanine and uracil residues in oligoribonucleotide synthesis

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ABSTRACT

The protection of the O^6-amide and N^2-amino groups of guanosine and the N^3-imide group of uridine with the (butylthio)-carbonyl group is described. This group could be rapidly introduced in good yields and removed very easily under the conventional deprotective conditions of the exo-amino acyl groups of other nucleoside bases.

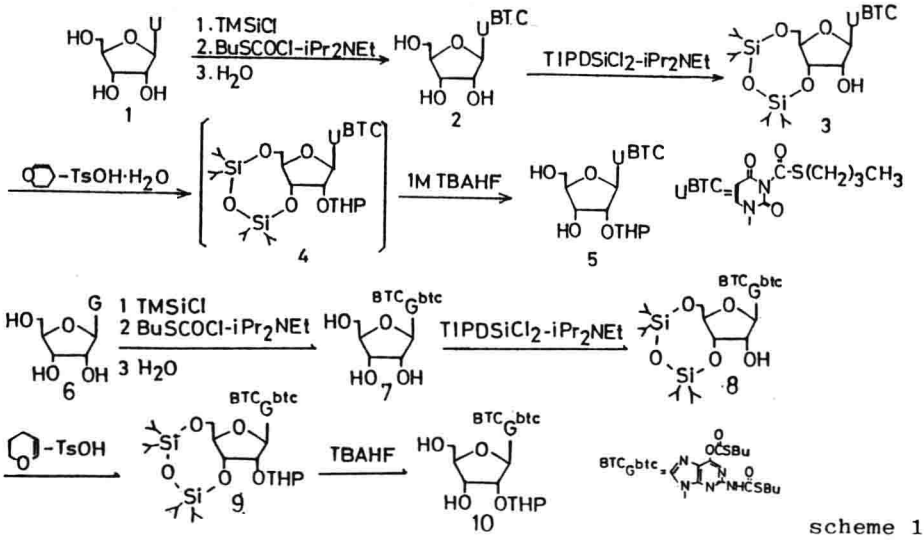
The synthesis of oligoribonucleotides containing guanosine and uridine has long been associated with the low yields and obvious side reactions. The source of the side reactions have been shown recently to be the reactively of the O^6-position of guanosine and the N^3-position of uridine toward the condensing and phosphorylating agents commonly employed in oligoribonucleotide synthesis.[1] Several protecting groups have recently been proposed to prevent the side reactions.[2]

Here we wish to report the (butylthio)carbonyl group as a more efficient protecting group to the O^6- and N^2-positions of guanosine and the N^3-position of uridine. We describe its application to the synthesis of octaribonucleotide, GACCGUCA, i.e., box 9R sequence of r-RNA precurson of Tetrahymena.[3]

The (butylthio)acrbonyl group could be introduced to the N^3-imide group of uridine and the O^6-amide and N^2-amino groups of guanosine in good yields by a one pot synthesis as outlined in scheme 1.

It was then interesting to explore the relative stabilities of the (butylthio)carbonyl group on guanosine and uridine under a variety of deprotective conditions to evaluate their possible use in the oligoribonucleotide synthesis in conjunction with

other sugar and phosphate protecting groups in our approach to the oligoribonucleotide synthesis. The results indicate that the (butylthio)carbonyl group had the suitable properties for oligoribonucleotide synthesis. The (butylthio)carbonyl group on the guanine and uracil residues was removed very easily under the conventional deprotective conditions for the exo-amino acyl groups of other nucleoside bases. In particular, compounds 2 and 7 were treated with NH_4OH-MeOH (9:1, v/v) at room temperature for 3 h to afford uridine and guanosine without the formation of cytidine, 2,6-diamino purine, and 6-thiobutylguanosine.



scheme 1.

To demonstrate the utility of the (butylthio)carbonyl group, octaribonucleotide, GACCGUCA was synthesized. Compounds 5 and 10 were treated with DMTrCl in pyridine to give the corresponding tritylated nucleoside derivatives. The fully protected mononucleotide units (14) were prepared in good yields by treatment of the tritylated nucleoside derivatives with the phosphorylating agent 13 prepared simply from 2,6-dichlorophenyl phosphorodichloridate and 5-chloro-8-hydroxyqunoline in the one flask reaction