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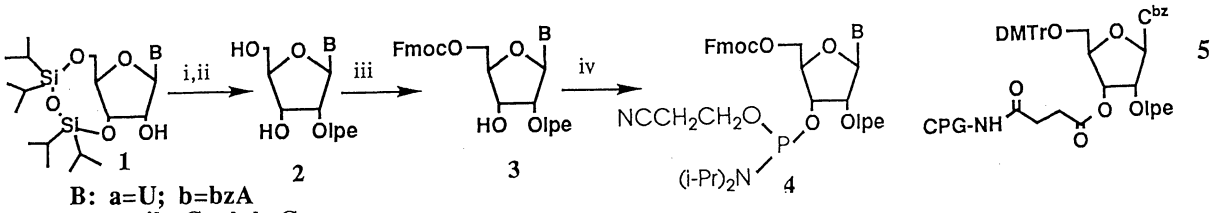
Solid-Phase Synthesis of Oligoribonucleotides Using 5'-9-Fluorenylmethoxycarbonyl and 2'-1-(Isopropoxyl)ethyl Protection

Takashi OGAWA, Hideo HOSAKA, Tatsusi MAKITA, and Hiroshi TAKAKU*
Laboratory of Bioorganic Chemistry, Department of Industrial Chemistry,
Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275

The 1-(isopropoxy)ethyl group (Ipe) has been employed for the protection of the 2'-hydroxy groups of ribonucleoside residues in the synthesis of oligoribonucleotides by the phosphoramidite approach on a solid support, using an base-labile 5'-9-fluorenylmethoxycarbonyl (Fmoc) group.

The choice of the protecting group for the 2'-hydroxy group is a crucial point in the polyribonucleotide synthesis and it should be completely stable under the conditions required for the removal of the 5'-protecting groups in the solid-phase synthesis of oligoribonucleotides. The t-butyldimethylsilyl and o-nitrobenzyl groups as the 2'-protecting group have recently been described by Ogilvie et al.[1] and Tanaka et al.[2] for use of the phosphoramidite approach of long chain synthesis. As some recent have studies shown[3] that the acid conditions required for the removal of either 5'-dimethoxytrityl or 9-phenylxanthen-9-yl groups at every cycle of oligonucleotide addition lead to appreciable concomitant removal of the acid labile 2'-O-acetal groups. In order to overcome this problem, a few workers have explored a new acetal protecting group for 2'-hydroxy groups or have searched a procedure without acid treatment during chain elongation. Reese et al.[4] have investigated the use of a new acetal protecting group designed to be stable under the conditions required for the removal of a pixyl group but cleavable under mild conditions at pH 2.0. Recently, Ohtsuka et al.[5] have reported the synthesis of a 21mer by the phosphoramidite approach using the base-labile levulinyl[6] and tetrahydrofuranyl groups, respectively, for the protection of 2'- and 5'-hydroxy groups. More recently, Gait et al.[7] have also reported that a combination of the 9-fluorenylmethoxycarbonyl group[8] for 5'-protection and 4-methoxytetrahydropyran-4yl for 2'-protection was effective for the synthesis of oligoribonucleotides.

In our continuing studies[9] to develop the acetal groups as the 2'-protecting groups for the solid phase synthesis of oligoribonucleotides



Fmoc=9-fluorenylmethoxycarbonyl; Ipe=1-(isoproxy)ethyl.
Reagents:(i) CH_2=CHOCH(CH_3)$_2$, PPTS, CH_2Cl_2; (ii) 1M TEAHF, THF; (iii) Fmoc-Cl, pyridine;
(iv) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, N,N-diisopropylethylamine, THF.

using the phosphoramidite approach, we have found that the rate of hydrolysis of Ipe group of uridine is 37 times more rapidly than the Thp group under the acidic conditions. In this paper we describe the synthesis of oligoribonucleotides by the phosphoramidite approach using the base-labile Fmoc and acid-labile Ipe groups, respectively, for the protection of the 5'- and 2'-hydroxy groups. N-Acyl-2'-O-Ipe-nucleosides (**2a-d**) were prepared from 3',5'-O-(tetraisopropyldiloxane-1,3-diyl) nucleosides (**1a-d**) in 70-89% yields by reaction with isopropyl vinyl ether in CH_2Cl_2 in the presence of pyridinium p-toluenesulfonic acid (PPTs), followed by treatment with 1 M TEAHF as previously described for the similar 2'-O-1-(2-chloroethyl)ethoxy nucleoside derivatives.[9]

Next, we examined the synthesis of the 5'-Fmoc-nucleosides (**3a-c**) under several procedures. The yields of **3a-c** depended on the reaction temperature and the molar ratios of the 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) to the nucleosides **2**, and better results were obtained by use of a slight excess of Fmoc-Cl at a reaction temperature of 0 °C. Reaction was complete for 3 h and after work-up yields 70-78% were obtained of 5'-Fmoc-nucleoside derivatives (**3a-c**). However, in case of the acylation of **2d**, the yield of **3d** was low (55%) due to the occurrence of 3',5'-diacylation. The 5'-Fmoc-nucleosides (**2a-d**) were phosphitylated with 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite and gave the phosphoramidite units (**4a-d**) in 76-80% yields.

Table 1. Stability of the protecting groups of nucleosides (**3b,d**)

Compd.	Reaction conditions	Fmoc-group t_∞/min	bz-group $t_{1/2}$/h
Fmoc(bz)CIpe	0.1 M piperidine/CH_3CN	1	2.5
	morpholine/CH_3CN (30:70)	1.5	4
	Et_3N/CH_3CN (30:70)	12	-
Fmoc(bz)AIpe	0.1 M piperidine/CH_3CN	1	8

The stability of benzoyl group of adenine and cytosine was tested under several basic conditions (Table 1). It can be seen from Table 1 that benzoyl group of adenine and cytosine was completely stable under the basic conditions required to remove the 5'-Fmoc group at every cycle of ribo-

nucleotide addition. However, the Fmoc group was easily removed under basic conditions. Especially, in case of 0.1 M piperidine the complete removal of the Fmoc group from **3** can be detected within 1 min. On the other hand, nucleoside-functionalised glass was submitted to a 6 h treatment in the condition of 0.1 M piperidine. No cleaving of the succinate link with the support was observed.

The reaction was carried out on a small column of nucleoside-functionalised glass (**5**) (0.2 μmol) with an Applied Biosystems Model 381A DNA synthesizer. We showed the following elongation cycle to be effective: treatment with (1) 5'-unblocking [0.1 M piperidine in CH_3CN, 120 s], (2) washing [CH_3CN, 30 s], (3) coupling [0.1M phosphoramidite units (**4a-d**) and 0.3 M tetrazole in CH_3CN for 20-25 min], (4) washing [CH_3CN, 30 s], (5) oxidation [0.1 M iodine in THF/lutidine/water (40:10:1), 60 s], (6) washing [CH_3CN, 70 s], (7) capping [solution A: THF/lutidine/acetic anhydride (8:1:1, v/v), solution B: 0.27 M MeIm in THF, 60 s], (8) washing [CH_3CN, 70 s]. The utility of ribonucleoside 3'-phosphoramidites (**4**) in which the 2'- and 5'-hydroxy groups are protected with the Ipe and Fmoc groups is now demonstrated by the synthesis of 16-21 mers consisting of $rU_{20}C$, $rC_{20}C$, and a sequence similar (rCGUUUCGUACAAACAC) to C130-C145 of p2Sp1 RNA.[11] In all cases, the average coupling yields were ranging from 95 to 98%.

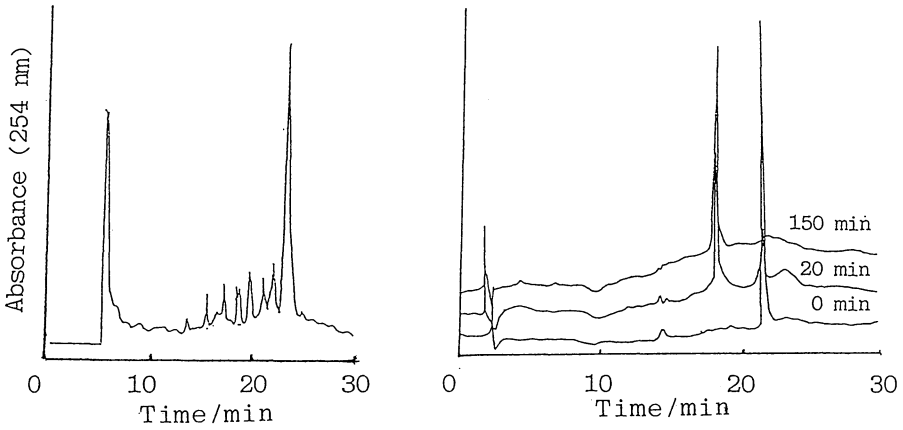


Fig. 1. HPLC analysis of $rC_{20}C$ using a TSKgel ODS-80TM column with a linear gradient of CH_3CN (from 0-25% during 30 min) in 0.1 M TEAA (pH 7.0). The flow rate was 2 ml/min. (a) Chromatography of crude 2'-Ipe protected oligomer, $rC_{20}C$; (b) Chromatography of time course of 2'-deprotection of the oligomer, $rC_{20}C$.

All of the sequences synthesized above were treated with concentrated ammonia solution at room temperature for 1 h to cleave of the reversible bond and then ammonia solution heated at 55 °C for 6-12 h to the complete removal of the exocyclic amino acyl protecting groups. The protocol for deprotection by the authors makes it rather difficult to evaluate which process is the main source of truncated fragments. However, the oligoribonucleotide with their 2'-hydroxy groups protected could be used to advantage and almost separation of desired oligomers from truncated fragments

could be obtained by reversed phase chromatography ($rC_{20}C$, Fig. 1a). In final deprotecting step, as labile protecting group was removed from the partially protected oligomer ($rC_{20}C$) by treatment with dilute HCl (pH 2.0) at room temperature. The rate of removal of 2'-protecting groups was monitored by reverse phase HPLC (Fig. 1b) and the deprotection was performed completely with 3 h to give the essentially a single product. The main peak was ^{32}P-labeled and analyzed by gel electrophoresis (Fig. 2). Further, no isomerization to 2'-5' oligomer can be detected under deprotection conditions. The complete deprotected oligomer, $rC_{20}C$ was complete digested with RNase T_2. HPLC analysis showed conversion into rCp and rC in the expected proportions. The over all yield was ca. 7%.

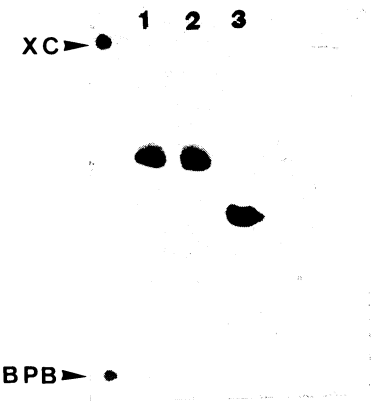


Fig. 2. Electrophoresis on a 20% polyacrylamide gel of the 5'-^{32}P-labeled oligomers. Lane 1: $rC_{20}C$; lane 2: $rU_{20}C$; lane 3: 16mer.

We thus conclude that the base-labile 9-fluorenylmethoxycarbonyl and acid-labile 1-(isopropoxy)ethyl groups are a promising candidate for the protection of the 5'- and 2'-hydroxy groups of the building blocks engaged in the synthesis of oligoribonucleotides by the phosphoramidite approach on solid supports.

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10) ^{31}P-NMR ($CDCl_3$, 85% H_3PO_4): **4a**=δ 149.70, 143.85, 138.76, 138.19 ppm; **4b**=δ 151.13, 150.25, 149.50, 149.00 ppm; **4c**=δ 151.65, 150.54, 149.60, 148.75 ppm; **4d**=δ 151.67, 150.90, 149.60, 149.05 ppm.
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