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The Utility of 2'-Thp Group in the Synthesis of the Relatively Long RNA Fragments on the Solid Phase

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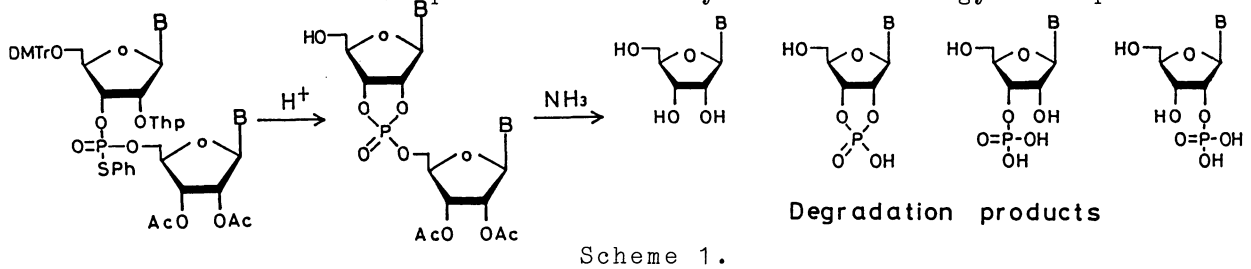
The stability of 2'-O-tetrahydropyranyl (Thp) group during several acidic treatments used for removal of 5'-O-9-(4-methoxy)phenyl xanthenyl (Mox) group in synthesis of 30 nucleotide long uridine molecules on the solid supports via cyanoethylphosphoramidite method is discussed.

We have obtained the medium sized RNA fragments by solid phase synthesis via cyanoethylphosphoramidite approach.[1] We chose the combination use of 2'-Thp group and 5'-Pix and Mox groups for protection of each hydroxyl group and protic solvent was employed during 5'-detritylation stage in a chain elongation cycle. It has been previously reported that even if a part of Thp group was eliminated during acidic treatment required for 5'-deprotection stage, synthetic oligoribonucleotides were isolated as 2'-5' linkage free products[1a] (Scheme 1). However, Gait et al. have examined the stability of 2'-Thp group at the uridine residue attached to a solid support against treatment with 3% dichloroacetic acid (DCA) in 1, 2-dichloroethane.[2] And Hirao et al. have also evaluated the extent of removing 2'-Thp group from the protected uridine derivative under the several acidic conditions, in which DMTr group was introduced into 5'-hydroxyl group.[3] Both two papers have suggested that the combined protection of 2'-acetal blocking groups and 5'-trityl type protecting groups was inappropriate for the long RNA sequence assembly on the solid supports, as far as protic acids were employed for the 5'-detritylation step. This point is the most serious problem in our synthetic strategy. Despite their



Scheme 1.

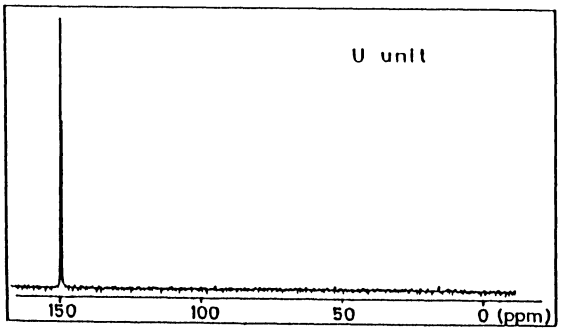


Fig. 1. ^{31}P-NMR spectra of uridine phosphoramidite unit.

Table 1. manipulation for the automatic synthesizer

Step	Reagent or manipulation	Time/s
1	CH_3CN	30
2	Argon gas flow	20
3	0.1 M amidite/0.4 M tetrazole	6 x 3
4	coupling reaction	300
5	0.25 M Ac_2O/1-MeIm/lutidine in THF	30
6	0.1 M I_2 in THF/pyridine/H_2O	30
7	CH_3CN	20 x 5
	Argon gas flow	10 x 5
8	detritylation step	a)
9	CH_3CN	90

a)These conditions are described in the text.

observations, we considered that it would be possible to synthesize the relatively long oligoribonucleotides using Thp group for blocking of 2'-hydroxyl group, if the appropriate protic condition was chosen as 5'-detritylation reagents, and Pix[4a] and Mox[4b] groups were employed as more labile 5'-blockers in order to minimize the simultaneous cleavage of 2'-Thp group. In this study, the stability of 2'-Thp group was tested under the several acidic conditions in preparation of 30-ribonucleotides long uridine on the solid phase by use of an automatic synthesizer (ABI DNA synthesizer Model 381 A) via the phosphoramidite approach.

An uridine phosphoramidite unit (2'-O-tetrahydropyranyl-5'-O-9-(4-methoxy)phenylxanthenyl-N^3-anisoyl uridine 3'-O-cyanoethyl-N, N-diisopropylaminophosphoramidite) was prepared in 90% yield by a method similar to that previously reported.[1d] This unit was obtained as excellent pure form determined by ^{31}P-NMR (500 MHz) (Fig. 1). The CPG support loaded with the uridine derivative (0.2 μmol, 10 mg) was packed in a column which is part of an automatic synthesizer. The condition for the manipulation of the machine are summarized in Table 1. To investigate the stability of 2'-Thp groups of uridine fragments on the solid support, we selected following acidic conditions as the detritylation stage. 1) 0.5% trifluoroacetic acid (TFA) in CH_2Cl_2 for 60 s; 2) 0.5% TFA in CH_2Cl_2 for 180 s; 3) 2.0% DCA in CH_2Cl_2 for 60 s; 4) 2.0% DCA in CH_2Cl_2 for 180 s. Among these conditions entries 1) and 3) were employed for our standard conditions of 0.2 μmol scale synthesis when Pix and Mox groups were introduced into the 5'-hydroxyl group protection. Each coupling reaction in a series of uridine 30 mer was estimated by measurement of the absorbance at 370 nm (Mox cation). For each fragment the average coupling efficiency was ca. 99%.

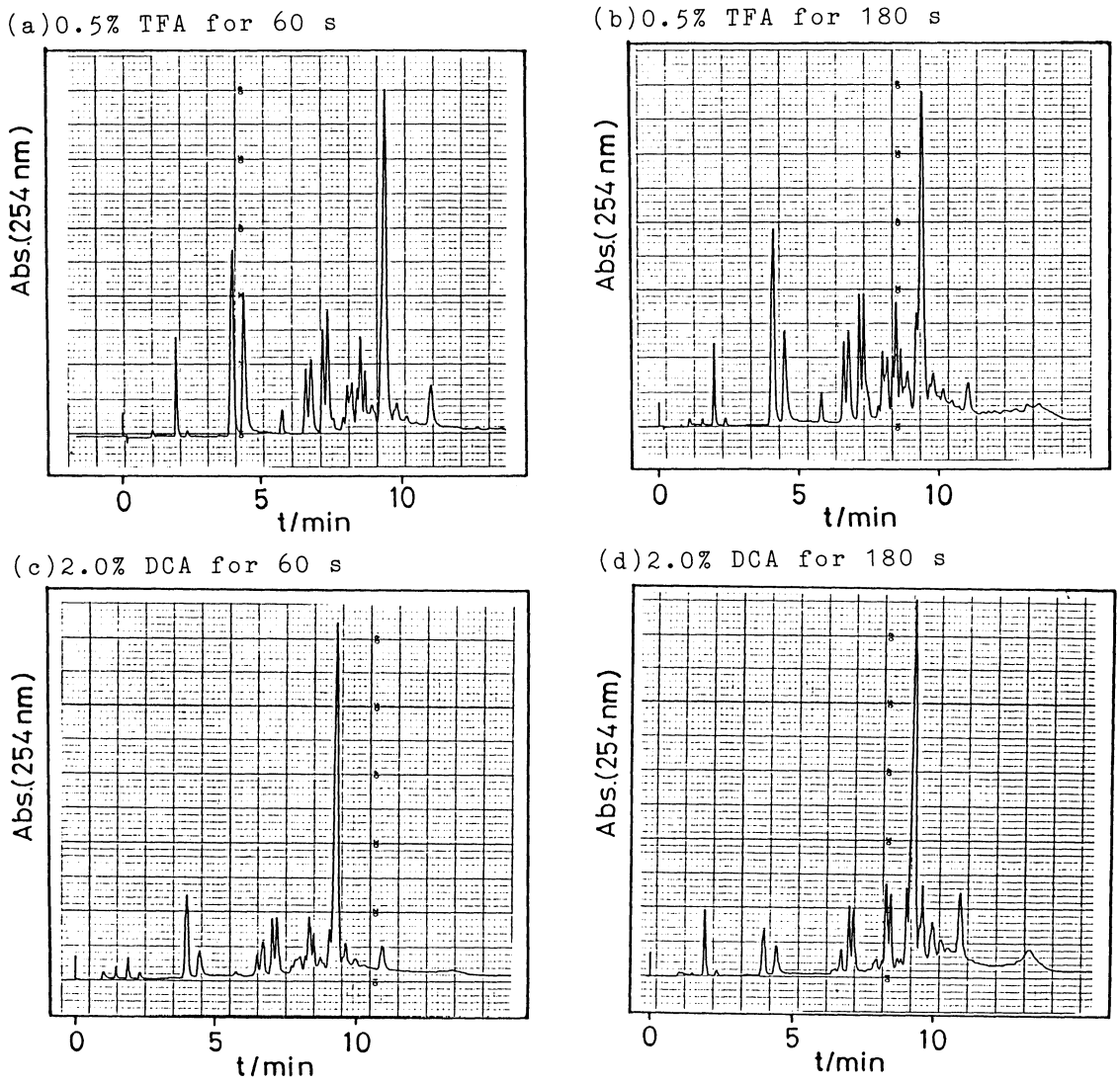


Fig. 2. Reversed phase HPLC (μBondasphere C_{18}) profiles of 30 long uridine molecules after full deprotection.

Fully protected RNA fragments on the support was first treated with concentrated ammonia-pyridine solution (at r.t for 12 h and at 55 °C for 3 h). The reaction mixture was filtered, then the filtrate was evaporated under reduced pressure. The residue was dissolved in sterilized water and then the aqueous solution was washed with ether and concentrated under reduced pressure. The aqueous residue was further dissolved in a 0.01 M solution of HCl in sterilized water and the solution was adjusted to pH 2.0. Then the reactant was kept at room temperature for 24 h. A small amount of diluted ammonia was added to neutralize the solution. The aqueous solution was concentrated under reduced pressure, and the final products were analyzed by the reversed phase HPLC (μBondasphere C_{18} column) using a gradient of acetonitrile in 0.1 M triethylammonium acetate buffer.

As shown in Figs. 2 (a) and (c), using the standard acid treatment (entries 1 and 3), the desired product (uridine 30 mer) was obtained as main peak on the HPLC profile, and the chain length was analyzed by ion exchange HPLC (TSKgel DEAE-2SW). (Data not shown.) Comparing entry 1 (0.5% TFA for 60 s) with entry 3 (2.0% DCA for 60 s), the side products increased in the latter condition, and we considered that the minor products seemed to be mainly chain cleavage compounds.[1a] This phenomenon means that the release of 2'-Thp groups bearing fully protected oligoribonucleotides was relatively minimized during 2.0% DCA treatment. In the case of entries 2 and 4, the manipulation time for each protic acid treatment was 180 s, which is 3 times longer than that of standard condition. As shown in Figs. 2 (b) and (d), the desired uridine 30 mers were also synthesized by using both acidic conditions, although HPLC profiles were slightly complicated. These results were suggested that Thp groups were sufficiently protected for 2'-hydroxyl groups on the relatively long RNA fragments against our acidic conditions required for removal of 5'-Mox group.

In summary, Thp group as well as t-butyldimethylsilyl group,[5] was also employed for 2'-hydroxyl protection in the practical and rapid synthesis of oligoribonucleotides, when the appropriate 5'-deprotection condition was chosen. And as shown in Fig. 1, it is necessary to prepare excellent pure starting compounds in order to obtain the relatively long RNA sequence assembly on the solid supports.

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