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Synthesis of Oligoribonucleotides by Using 2'-O-(1-Methyl-1-methoxy)ethyl Nucleosides#

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3',5'-O-Tetraisopropyldisiloxanylnucleosides smoothly react with 2-methoxypropene to give 2'-O-(1-methyl-1-methoxy)ethyl nucleosides in high yields without formation of diastereoisomers. These nucleosides were used as intermediates for oligonucleotide synthesis by the phosphotriester method. The (1-methyl-1-methoxy)ethyl group was removed rapidly from oligonucleotides by acid treatment.

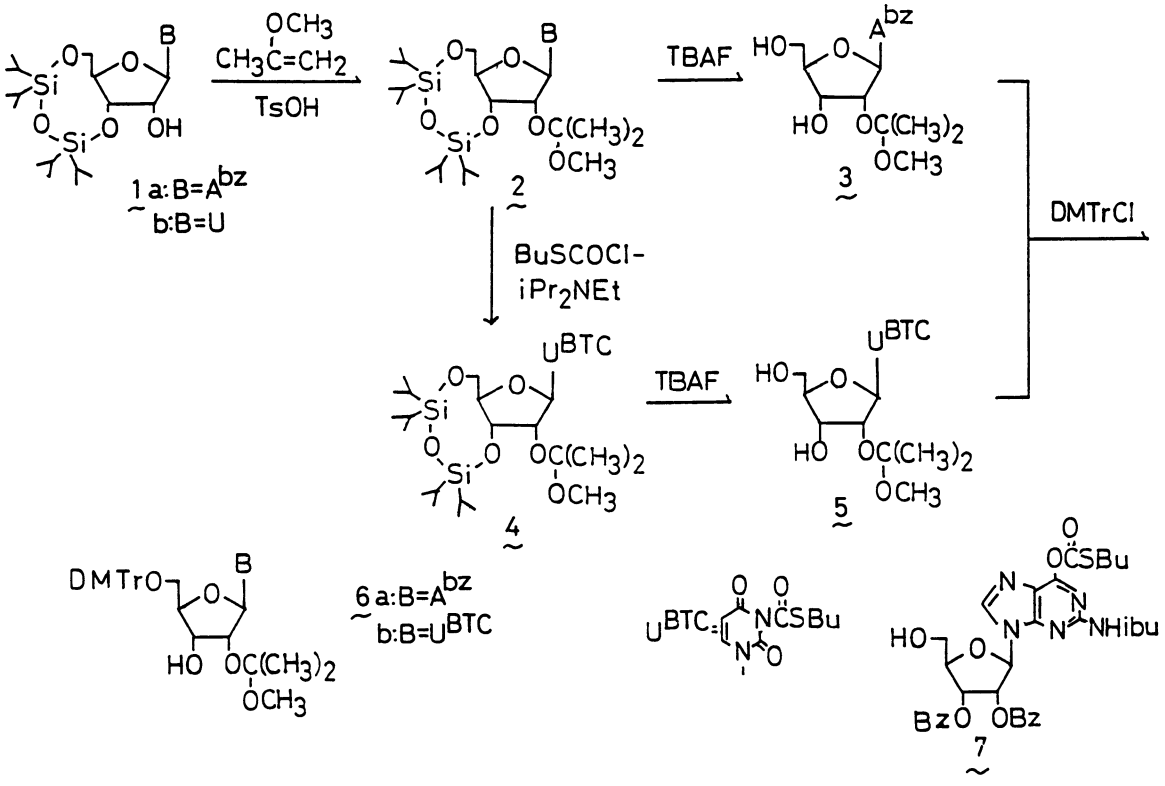
For the synthesis of oligoribonucleotides, protection of 2'-hydroxyl groups is an important procedure. In a previous paper,[1] we have described the utilization of the 3,4-dimethoxybenzyl group as a protecting group for the 2'-hydroxyl group in the synthesis of oligoribonucleotides. On the other hand, the acid labile tetrahydropyranyl,[2] methoxytetrahydropyranyl,[3] and tetrahydrofuranyl[4] groups have also been used for protection of the 2'-hydroxyl group. However, 2'-tetrahydropyranyl and tetrahydrofuranyl derivatives of ribonucleosides contained two diastereoisomers. In order to overcome this problem, we have examined the utilization of the (1-methyl-1-methoxy)ethyl (MME) group[3b,5] as a protecting group for the 2'-hydroxyl group in oligoribonucleotide synthesis. The MME group has been proposed initially by Reese and applied to ribonucleosides.[3b] We have found that this group can be removed rapidly under mild conditions which do not cause detectable isomerisation of the internucleotidic linkage.

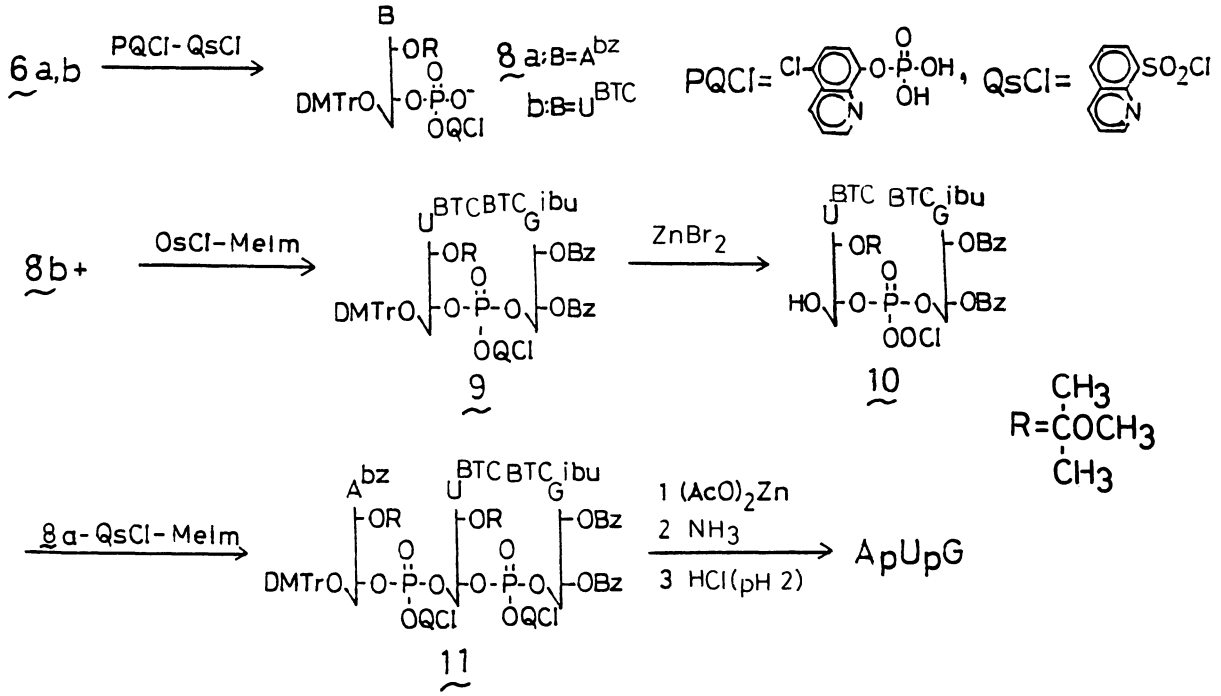
We first examined the synthesis of ribonucleoside derivatives (3 and 4) as key intermediates for the fully protected trimer (11) as shown in scheme 1. The 3',5'-subsituted adenosine (1a)[6] was treated with 2-methoxypropene (20 molar equiv.) in the presence of p-toluenesulfonic acid (0.05 molar equiv.) in dry THF at room temperature for 2 h to give the intermediate (2a) which was then treated with tetra-n-butylammonium fluoride (TBAF) to remove the silyl groups. 2'-O-(1-Methyl-1-methoxy)ethyl-N^6-benzoyladenosine (3)[7] was obtained in 87% yield after separation by silica gel chromatography. In the case of uridine, the intermediate 2b was treated with butyl chlorothioformate (BuSCOCl)[8] in the presence of diisopropylethylamine in pyridine for 24 h to yield 4. Desilylation from 4 was performed by use of TBAF in THF to give the corresponding 2'-O,N^3-protected uridine (5)[9] in 75% yield.

Dedicated to Professor Teruaki Mukaiyama on the occasion of his 60th birthday.

In a similar manner, the guanosine and cytidine derivatives were synthesized in good yields.[10] Tritylation of the nucleoside derivatives (3 and 5) with DMTrCl in pyridine gave the expected 5'-tritylated products (6a,b) in good yields. The 3'-terminal guanosine derivative (7) was prepared by the previously described procedure.[8]

Next, we examined the synthesis of the fully protected trimer (11) by using 7 and 8. Phosphorylation of the tritylated compounds (6a,b) (1.5 mmol) with 5-chloro-8-quinolyl phosphate (PQCl) (1.65 mmol) in the presence of 8-quinolinesulfonyl chloride (QSCl)[11] in pyridine (15 ml) for 2 h gave the phosphorylated products (8a,b) in 85-87% yields. The triethylammonium salt of 8b (974 mg, 0.88 mmol) thus obtained was treated with 7 (474 mg, 0.59 mmol) in the presence of QSCl (404 mg, 1.76 mmol) and methylimidazole (MeIm) (1.4 ml, 1.76 mmol) in pyridine (3 ml) for 1 h. After the usual workup, the resulting residue was applied to a column of silica gel and eluted with a stepwise gradient of MeOH in (0-5%) in CH_2Cl_2 to give the fully protected dimer (9) (808 mg, 88%). Removal of the 5'-DMTr group in 9 (303 mg, 0.2 mmol) was performed by treatment with 1 M-zinc bromide in CH_2Cl_2-iPrOH (85:15, v/v, 10 ml) for 15 min at room temperature to give 10 (234 mg, 88%). It was found that treatment of 1 M zinc bromide did not affect the acid labile 2-O-(1-methyl-1-methoxy)ethyl function.[12] The 5'-hydroxyl dimer (10) (220 mg, 0.2 mmol) was treated with 6a (330 mg, 0.3 mmol) in the presence of QSCl (140 mg, 0.6 mmol) and MeIm (0.5 ml, 0.6 mmol) in dry pyridine for 1 h. The fully protected trimer (11) was isolated in 91% (420 mg) yield after separation by silica gel column chromatography.





The coupling reactions proceeded smoothly and gave a high yield of the oligoribonucleotides containing guanosine and uridine units.

In order to determine the life time of the MME group under deprotective conditions, 2'-O-protected adenosine derivatives were treated with various acidic conditions. It can be seen from the Table 1 that, as expected, the rate of hydrolysis of 3 is very faster than 2'-O-(4-methoxytetrahydropran-4-yl)-N^4-benzoyladenosine (12).[3]

Table 1. Removal of the 2'-O-protecting groups by acidic hydrolysis at room temperature[a]

Entry No.	Substrate	Reagent	$t_{1/2}$/min	t_∞/min
1	12	0.01 M HCl (pH 2)	36	280
2	3	0.01 M HCl (pH 2)	0.45	5
3	3	5% AcOH	20	100

a) Aliquots of solution were taken after suitable interval time, neutralized with triethylammonium bicarbonate and analyzed by HPLC (Finepak C_{18}).

Deprotection of 11 was performed as follows: 1) zinc acetate (35 equiv. per one phosphate) in pyridine-H_2O (9:1, v/v) at room temperature for 24 h to remove the 5-chloro-8-quinolyl groups, 2) concd ammonia at 60 °C for 6 h, and 0.01 M HCl (pH 2.0) at room temperature for 2 h. The deblocked trimer, AUG was obtained in 75% yield by paper chromatography. The trimer was degradated by spleen phosphodiesterase to give Ap, Up, and G in the ratio of 1.02:1.01:1.00.

In conclusion, the MME group described in this paper could be used for the 2'-hydroxyl group of ribonucleosides in the synthesis of oligoribonucleotides.

Especially, the MME group can be introduced to protect the 2'-hydroxyl group of ribonucleosides without formation of diastereoisomers. The MME group was removed rapidly from the oligoribonucleotides by acid treatment.

References

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6) W. T. Markiewicz, J. Chem. Res., (S), 1979, 24.
7) Mp 135-136 °C; UV λmax (MeOH) 278, 227 nm, λmin (MeOH) 246 nm, ^{1}H-NMR ($CDCl_3$) 1.20 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 2.67 (s, 3H, OCH_3), 3.95 (m, 3H, HO-3', H-5'), 4.45 (m, 2H, H-2', H-4'), 5.30 (m, 2H, HO-5', H-3'), 5.95 (d, 1H, $J_{1',2'}$=8 Hz, H-1'), 7.60 (m, 3H, Ar), 8.10 (m, 3H, H-2), 8.80 (s, 1H, H-8). Found: C, 56.35; H, 5.97; N, 15.10%. Calcd for $C_{21}H_{24}N_5O_6.1/2CH_3OH$: C, 56.20; H, 5.70; N, 15.24%.
8) M. Fujii, Y. Horinouchi, and H. Takaku, Chem. Pharm. Bull., in press.
9) UV λmax (MeOH) 261, 238 nm, λmin (MeOH) 249 nm. ^{1}H-NMR ($CDCl_3$) 0.94 (br s, 9H, CH_3), 1.38 (s, 6H, CH_2), 3.15 (s, 3H, OCH_3), 3,63 (br s, 2H, H-5'), 4.17 (m, 3H, H-2', H-3', H-4'), 4.60 (m, 2H, HO-2', HO-5'), 5.80 (d, 1H, $J_{5,6}$=8 Hz, H-5), 5.95 (d, 1H, $J_{1',2'}$=6 Hz, H-1'), 7.85 (d, 1H, $J_{5,6}$= 8 Hz, H-6), Found: C, 50.31; H, 6.65; N, 6.36%. Calcd for $C_{18}H_{28}N_2O_8$: C, 50.53; H, 6.69; N, 6.37%.
10) The manuscript is in preparation and will be fully reported elsewhere.
11) H. Takaku, M. Yoshida, M. Kato, and T. Hata, Chem. Lett., 1979, 811.
12) The MME group of C and G was found to be stable under the deprotective conditions of the DMTr group (1 M zinc bromide in CH_2Cl_2-iPrOH (85:15, v/v)).

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