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# USE OF 3,4-DIMETHOXYBENZYL GROUP AS A PROTECTING GROUP FOR THE 2'-HYDROXYL GROUP IN THE SYNTHESIS OF OLIGORIBONUCLEOTIDES

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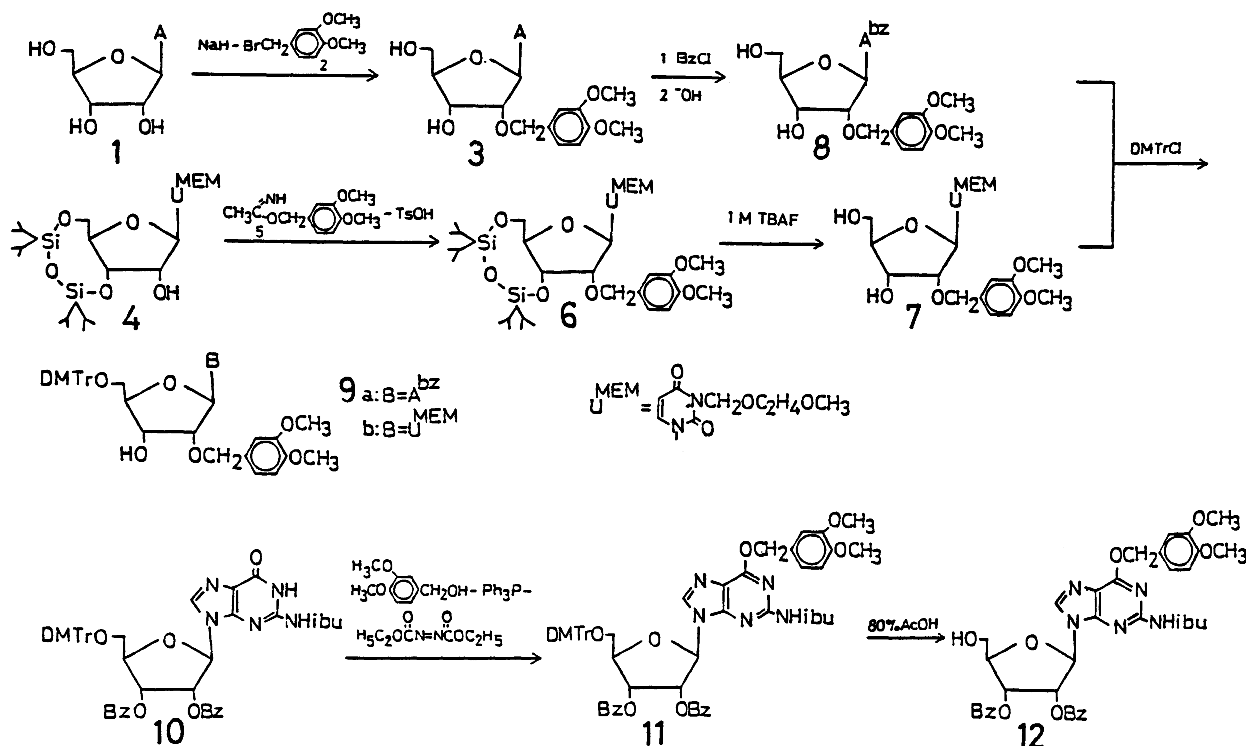
The 3,4-dimethoxybenzyl group was introduced to the 2'-hydroxyl group by the reaction of nucleosides with 3,4-dimethoxybenzyl trichloroacetimidate or 3,4-dimethoxybenzyl bromide-NaH. Further, this group can be used as a protecting group for the $O^6$-amide group of the guanine residue. The protected nucleosides are useful starting materials for the synthesis of oligoribonucleotides. The 3,4-dimethoxybenzyl group was removed rapidly from the oligoribonucleotides by treatment with oxidizing agents without any damage to the glycosidic bonds.

The chemical synthesis of oligoribonucleotides by the phosphotriester is more elaborate and time-consuming than the chemical preparation of oligodeoxyribonucleosides. It is due to the synthetic complexity resulting from the need to protect the additional 2'-hydroxyl groups throughout the synthesis of oligoribonucleotides. In a previous paper,[1] we have described the utilization of the 4-methoxybenzyl group as the protecting group for the 2'-hydroxyl groups in the synthesis of oligoribonucleotides. However, we observed some incomplete deblocked products and cleavage of glycosidic bonds during removal of the 4-methoxybenzyl group from the oligoribonucleotides by using triphenylmethyl fluoroborate. In order to overcome this problem, we have examined the development of protecting groups for the 2'-hydroxyl groups in oligoribonucleotide synthesis and have found that the 3,4-dimethoxybenzyl group[2] is much more effective than the 4-methoxybenzyl group.

We first examined the synthesis of ribonucleotide derivatives as useful starting materials for the synthesis of the fully protected trimer (**16**). Introduction of the 3,4-dimethoxybenzyl group into the 2'-hydroxyl group of adenosine (**1**) was attemped by the reaction of **1** (1 molar equiv.) with 3,4-dimethoxybenzyl bromide (1.2 molar equiv.) in the presence of NaH (1.1 molar equiv.) in DMF as previously described,[1a] and the corresponding pure 2'-substituted compound (**3**) was isolated by crystallization from aqueous ethanol in a yield of 35%.[3] In the case of uridine, treatment of **4**[4] with a new benzylating agent, 3,4-dimethoxybenzyl trichloroacetimidate (**5**)[5] in the presence of p-toluenesulfonic acid in a $CH_2Cl_2$-cyclohexane mixture (1:2, v/v) afforded the benzylated product **6**, which was then desilylated to **7**[6] in 69% yield by 1M tri-1-butylammonium fluoride. The compound **8** was prepared in 83% yield according to a usual manner[1a] as shown in Scheme 1.

Treatment of 7 and 8 with DMTrCl in dry pyridine gave the expected 5'-tritylated products (9a,b) in good yields. On the other hand, 3'-terminal nucleoside derivative (12) was prepared as follows: the 2',3',5'-O-2-N-protected guanosine derivative (10) (4.32 g, 5.0 mmol) was treated with 3,4-dimethoxybenzyl alcohol (1.82 ml, 12.5 mmol) in the presence of diethyl azodicarboxylate (2.18 g, 12.5 mmol) and triphenylphosphine[7] (3.28 g, 12.5 mmol) in dry THF (15 ml) to afford the corresponding fully protected nucleoside (11), which was detritylated to 12[8] in 66% (2.34 g) yield by 80% AcOH treatment.

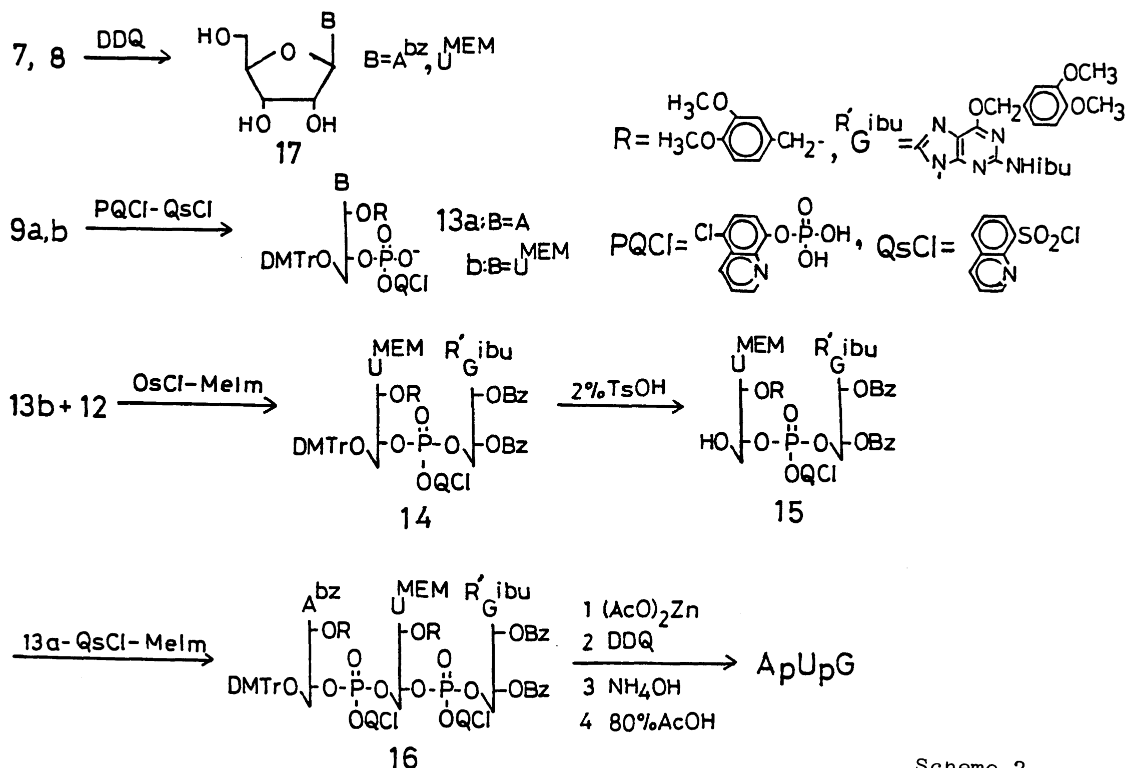


Scheme 1.

Next, we examined the synthesis of the fully protected trimer (16) by using 9 and 12. The tritylated compounds (9a,b) (1.0 mmol) were phosphorylated by 5-chloro-8-quinolyl phosphate (PQCl)[9] (1.1 mmol) and 8-quinolinesulfonyl chloride (QsCl)[10] in dry pyridine for 2 h. The mixture was quenched with ice-water and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ extract was washed with 0.1 M tetraethylammonium hydrogencarbonate (TEAB) and evaporated in vacuo. The residue was dissolved in $CH_2Cl_2$ and added dropwise to a hexane-ether mixture (95:5, v/v). The phosphodiester derivatives (13a,b) were isolated in 90-92% yields, which were used in the next coupling reaction without further purification. The triethylammonium salt of 13b (1.69 g, 1.5 mmol) thus obtained was treated with 12 (0.72 g, 1.0 mmol) in the presence of QsCl (0.69 g, 3.0 mmol) and N-methylimidazole (MeIm) (0.24 ml, 3.0 mmol) in dry pyridine (6.7 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 (0-5%) in $CH_2Cl_2$ to give the fully protected dimer (14) (1.43 g, 87%). Removal of the DMTr group from 14 (1.43 g, 0.86 mmol) was performed by treatment with 2% TsOH in a mixture of dioxane and methanol (1:1, v/v, 20 ml) for 15 min at 0 °C. After

the usual workup, chromatography afforded the detritylated dimer (15) (1.20 g, 95%). A solution of both compounds 13a (1.45 g, 1.2 mmol) and 15 (1.19 g, 0.82 mmol) in dry pyridine was treated with QsCl (0.56 g, 2.46 mmol) and MeIm (0.18 ml, 2.46 mmol) for 1 h. The fully protected AUG trimer (16) was obtained in 91% (1.70 g) yield after separation by silica gel column chromatography. The above results indicate that the protection of the guanine and uracil residues was very effective for the the synthesis of oligoribonucleotides containing guanosine and uridine units.

Finally, removal of the 3,4-dimethoxybenzyl group from 7, 8, and 16 by using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) was examined. When the nucleoside derivatives (7 and 8) in a $CH_2Cl_2$-$H_2O$ mixture (18:1, v/v) were treated with DDQ (5 molar equiv.) at room temperature for 1 h, the oxidative cleavage of the 3,4-dimethoxybenzyl group readily occurred and the corresponding debenzylated products (17) were obtained by TLC in 91-93% yields. Under these neutral conditions, other protecting groups (N-benzoyl and N-methoxyethoxymethyl groups) and the glycosidic bonds remained unchanged. However, we could not obtain satisfactory results when DDQ was used for removal of the 4-methoxybenzyl group from the 2'-O-(4-methoxybenzyl)nucleosides. Further, we observed that the 3,4-dimethoxybenzyl group could be selectively removed from the nucleoside derivative (7) by treatment with triphenylmethyl fluoroborate in a $CH_3CN$-$H_2O$ mixture (4:1, v/v) for only 5 min. Deprotection of 16 was performed as follows: 1) zinc acetate in pyridine-$H_2O$ (9:1, v/v) at room temperature for 24 h to remove the 5-chloro-8-quinolyl group; 2) DDQ in $CH_2Cl_2$-$H_2O$ (18:1, v/v) at room temperature for 1 h to remove the 3,4-dimethoxybenzyl group; 3) concentrated ammonia-pyridine (9:1, v/v) at 50 °C for 6 h to remove the acyl and MEM groups; 4) 80% AcOH at room temperature for 1 h to remove the DMTr group. The deblocked trimer, AUG, could be isolated in 51% yield by paper chromatography. The trimer was degradated by nucleoase P1 to give A, pU,




Scheme 2.

and pG in the ratio of 1.00:1.04:0.97. This hydrolysis indicated that AUG had a 3'-5' internucleotidic bonds.

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3) Mp 146-148 °C; UV $\lambda$max (MeOH) 261, 234 nm, $\lambda$min (MeOH) 242 nm; $^1$H-NMR (DMSO-$d_6$) $\delta$ 3.34 (s, 2H, H-5'), 3.56 (s, 3H, $OCH_3$), 3.70 (s, 3H, $OCH_3$) 4.10 (m, 1H, H-4'), 4.25-4.70 (m, 4H, H-2', H-3', and $ArCH_2$), 5.30 (d, 1H, HO-5'), 5.49 (m, 1H, HO-3'), 6.03 (d, 1H, $J_{1',2'}$=6 Hz, H-1'), 6.65 (s, 4H, Ar), 7.20 (br s, 2H, $NH_2$), 8.05 (s, 1H, H-8), 8.25 (s, 1H, H-8). Anal. Calcd for $C_{19}H_{23}N_5O_6 \cdot 1/2H_2O$: C, 53.52; H, 5.67; N, 16.42. Found: C, 53.69; H, 5.47; N, 16.24.

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6) UV $\lambda$max (MeOH) 266, 232 nm, $\lambda$min (MeOH) 241 nm. $^1$H-NMR ($CDCl_3$) $\delta$ 3.32 (s, 3H, $OCH_3$), 3.45-3.70 (m, 6H, H-5', $-CH_2CH_2-$), 3.81 (s, 3H, $OCH_3$), 3.92 (s, 3H, $OCH_3$), 4.10 (m, 4H, H-2', H-3', H-4', HO-5'), 4.70 (br s, 2H, $ArCH_2$), 5.39 (br s, 3H, $-OCH_2O-$, HO-3'), 5.63 (d, 1H, $J_{5,6}$=6 Hz, H-5), 5.78 (d, 1H, $J_{1',2'}$=4 Hz, H-1'), 6.61 (br s, 1H, Ar), 6.83 (br s, 2H, Ar), 7.79 (d, 1H, $J_{5,6}$=6 Hz, H-6). Anal. Calcd for $C_{22}H_{30}N_2O_{10}$: C, 53.61; H, 6.43 ; N, 5.95. Found: C, 53.76, H, 6.64; N, 5.90.

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8) Mp 115-118 °C; UV $\lambda$max (MeOH) 273, 255 (sh), 227 nm, $\lambda$min (MeOH) 267 nm. $^1$H-NMR ($CDCl_3$) $\delta$ 1.21 (d, 6H, $-CH(C\underline{H}_3)_2$), 2.70 (m, 1H, $-C\underline{H}(CH_3)_2$), 3.89 (br s, 8H, H-5', $OCH_3$), 4.00 (m, 1H, H-4'), 4.21-4.50 (m, 2H, H-2', H-3'), 5.42 (br s, 3H, HO-5', $ArCH_2$), 6.05 (t, 1H, $J_{1',2'}$=5.5 Hz, H-1'), 6.86 (m, 3H, Ar), 7.55 (m, 6H, Ar), 7.92 (m, 4H, Ar), 8.15 (s, 1H, H-8), 12.40 (br s, 1H, NH). Anal. Calcd. for $C_{37}H_{36}N_5O_{10}$: C, 62.50; H, 5.11; N, 9.86. Found: C, 62.67; H, 5.31; N, 9.57.

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