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METHOXYETHOXYMETHYL GROUP FOR THE PROTECTION OF URACIL RESIDUE IN OLIGORIBONUCLEOTIDE SYNTHESIS

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ABSTRACT: Methoxyethoxymethyl group (MEM) was useful protecting group for the protection of uracil residue. This group was selectively introduced into uracil residue in good yield and removed under mild conditions.

The useful synthetic methods for the synthesis of oligonucleotides have been recently developed and they have been applied successfully to the chemical synthesis of the important fragments of genes. However, despite the advances in synthetic methodology, there still remains the important problem of how to prevent the side reactions of the uracil residue.[1] In order to overcome this problem, a few workers have explored new protecting groups for the uracil residue for the synthesis of ribooligonucleotides.[2]

In this paper, we wish to report methoxyethoxymethyl group as a new protecting group for the uracil residue which can be introduced selectively and removed under mild conditions.

First, we examined the introduction of methoxyethoxymethyl group (MEM)[3] onto uracil residue. 2',3',5'-Tri-O-acetyluridine (1.11 g, 3.0 mmol) was treated with methoxyethoxymethyl chloride (MEMCl)[3] (1.37 ml, 12.0 mmol) in the presence of triethylamine (1.84 ml, 13.2 mmol) in dry THF (3 ml) at room temperature for 17 h. After the usual workup, chromatography on silica gel afforded the compound 2, which was further converted into the deacetylated compound 3[4] in 78% (0.78 g) yield by treatment with methanolic ammonia. The protection of uracil residue with MEM group would be expected to suppress the side reactions. To be a useful addition to the synthetic methodology a protecting group must meet criteria. These would include compatibity with other protecting groups and cleavage under mild conditions at the end of the oligonucleotide synthesis. First, 3 was treated with 80% acetic acid at room temperature, and found to be stable after 6 h. Secondly, 3 was treated with triethylamine (in acetonitrile at room temperature) or 0.1M-N^1,N^1,N^3,N^3-tetramethylguanidinium salt of 2-pyridinecarboaldoxime (PAO)[5] (in dioxane at room temperature), and found to be stable after ca. 10 and 18 h. Finally, when 3 was treated with triphenylmethyl fluoroborate[6] in CH_3CN-H_2O (4:1, v/v) at room temperature, it was quamtitatively converted to uridine after 1 h.

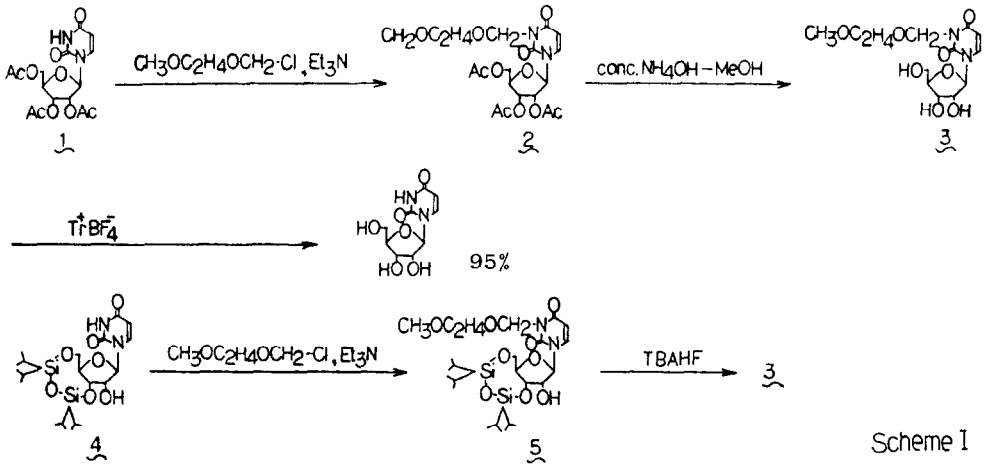
Next, we fould that MEM group can be introduced selectively onto uracil

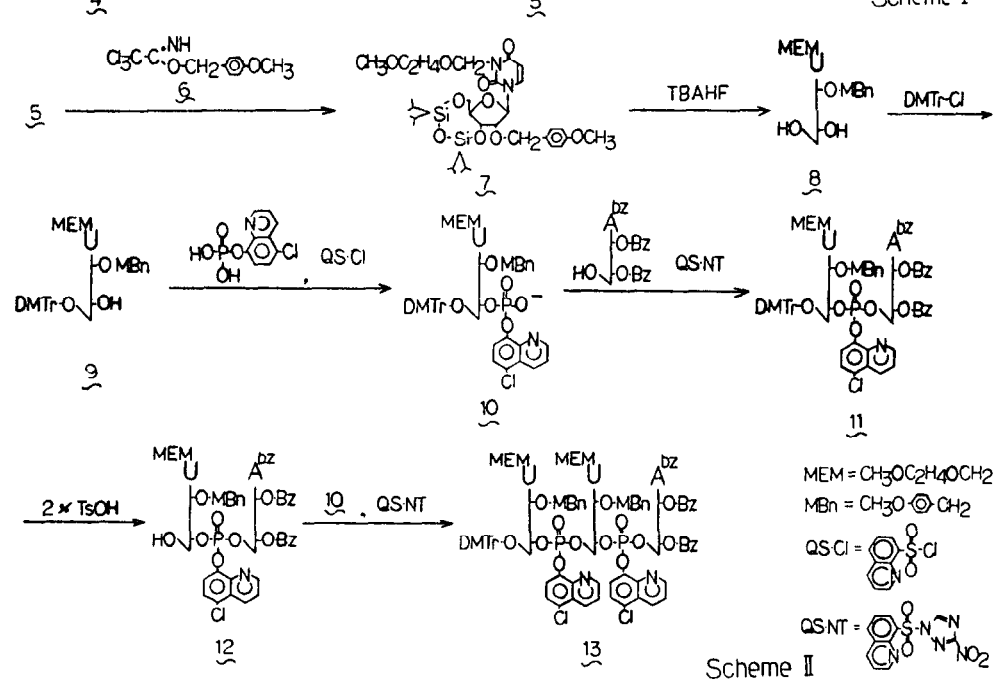
residue of 3',5'-O-(tetraisopropyldisiloxane-1,3-diol)uridine 4. Compound 4 (1.70 g, 3.5 mmol) was treated with MEMCl (1.6 ml, 14.0 mmol) in the presence of triethylamine (2.1 ml, 15.4 mmol) at room temperature for 17 h to give, after workup and chromatography of the reaction mixture, uridine derivative 5 in 71% (1.41 g) yield.[7] Desilylation from 5 (5.6 g, 9.9 mmol) was performed by use of 1M-tributylammonium fluoride (TBAHF) in THF at room temperature for 4 h to give the corresponding uridine derivative 3 in 70% (2.3 g) yield.[8] The result clearly indicates that MEM group is introduced selectively onto uracil residue.

The compound 5 thus obtained was a useful starting material for the synthesis of oligoribonucleotides containing uridine unit. To a solution of methylene chloride-cyclohexane (1:2, 2.5 ml) of 5 (1.41 g, 2.52 mmol) was added 4-methoxybenzyl trichloroacetimidate 6[9] (3.55 g, 12.6 mmol) as a new benzylating agent and trifluoromethanesulfonic acid (0.015 ml) at 0°C. The reaction mixture was gradually warmed to room temperature and stirred for 4 h. The mixture was quenched with ice-water and ectracted with methylene chloride (30 ml). The methylene chloride extract was washed with water, and concentrated in vacuo. The residue was treated with a THF solution of 1M-TBAHF at room temperature for 4 h. To the reaction mixture was added pyridine-methanol-water (3:2:2, 70 ml) and stirred for 30 min. The mixture was treated with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in methylene chloride and applied to silica gel column. The 2'-substituted uridine 8 was isolated in 3.16 g (55%) by eluting the column with methanol (5%) in methylene chloride: Rf 0.36 (CH_2Cl_2-MeOH, 9:1); UV λmax (MeOH) 266, 228 nm, λmin 241 nm. ^{1}H-NMR (DMSO-d_6) δ 3.22 (s, 3H, OCH_3), 3.32-3.64 (m, 6H, 5'-CH_2, -CH_2CH_2-), 3.78 (s, 3H, OCH_3), 4.00 (m, 1H, 4'-CH), 4.22 (m, 1H, 3'-CH), 4.45 (brs, 2H, $ArCH_2$), 4.75 (m, 1H, 2'-CH), 5.20 (brs, 4H, 5'-OH, 3'-OH, -OCH_2O-), 5.75 (d, 1H, $J_{5,6}$=8Hz, 5-H), 5.91 (d, 1H, $J_{1',2'}$=5Hz, 1'-H), 6.78 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.90 (d, 1H, $J_{5,6}$=8Hz, 6-H). Anal Calcd for $C_{21}H_{28}O_9N_2 \cdot 1/2CH_3OH$: C, 53.99; H, 6.85; N, 5.60. Found: C, 54.13; H, 7.05; N, 5.86.

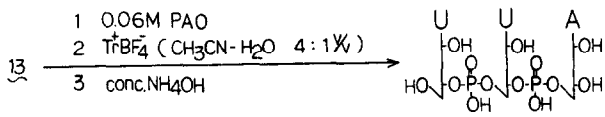
Next, we examined the synthesis of trinucleotide, UUA 14 by using 8 as shown in scheme II. The nucleoside 8 was tritylated with dimethoxytrityl chloride in dry pyridine for 2.5 h to give the tritylated product 9 in 83% yield. The nucleoside 9 (754 mg, 1.0 mmol) was treated with 5-chloro-8-quinolyl phosphate[10] (312 mg, 1.2 mmol) in the presence of 8-quinolinesulfonyl chloride (QS)[11] (544 mg, 2.4 mmol) in dry pyridine (10 ml) for 2 h. The reaction mixture was quenched with ice-water, followed by extraction with methylene chloride, and the organic layer was washed with 0.1M-tetraethylammonium bicarbonate (TEAB) solution. The methylene chloride extract was concentrated in vacuo. The residue was dissolved in methylene chloride and added dropwise to hexane-ether (95:5, v/v). The triethylammonium salt of phosphodiester 10 was isolated in 1.00 g (93%), which was used in the next coupling reaction without further purification. The triethylammonium salt of 10 (536 mg, 0.49 mmol) thus obtained was treated with N^6,2',3'-O-tribenzoyladenosine 11

(121 mg, 0.33 mmol) in the presence of 8-quinolinesulfonyl-3-nitro-1H-1,2,3-triazole (QSNT)[12] (383 mg, 1.26 mmol) in dry pyridine (3 ml) for 2 h. After the usual workup, chromatography on silica gel afforded the dinucleotide derivative 11 (418 mg, 91%). The fully protected dinucleotide 11 thus obtained was treated with 2% p-toluenesulfonic acid in methylene chloride-methanol (7:3, v/v) (24 ml) at 0°C for 15 min to give 12.[13] The 5'-hydroxyl dinucleotide 12 was isolated in 82% (268 mg) yield by precipitation with hexane-ether (95:5, v/v) and used for the next coupling reaction without further purification. A solution of phosphodiester 10 (443 mg, 0.41 mmol) and 12 (278 mg, 0.26 mmol) in dry pyridine (2 ml) was then condensed in the presence of QSNT (291 mg, 0.96 mmol) for 1 h. Usual workup including separation and purification by chromatography on silica gel gave the fully protected trinucleotide 13 (461 mg, 88%).





Removal of all the protecting groups from 13 was performed as follows: i) The trimer 13 (20.5 mg, 10 μmol) was treated with 0.06M PAO in dioxane-water (2:1, v/v) (1.0 ml) at room temperature for 20 h.[14] The mixture was treated with Dowex 50W-X2 (pyridinium form) and the resin was removed by filtration. The flitrate was concentrated in vacuo. ii) The residue was treated with triphenylmethyl fluoroborate (99 mg, 300 μmol) in CH_3CN-H_2O (4:1, v/v) (0.5 ml) for 1.5 h. The mixture was quenched with pyridine and the solution was cencentrated. At this stage MEM, de-dimethoxytrityl, and 4-methoxybenzyl groups were removed. iii) To the residue was added conc.ammonia and the mixture was kept at 50°C for 6 h. The solution was concentrated and the residue was dissolved in water. The solution was washed with ether.



Thus, UpUpA was isolated in 78% yield after chromatographic separation using Whatmann 3 MM paper with n-PrOH-conc.NH_4OH-H_2O (55:35:10, v/v). The purity of UpUpA was checked with PE and HPLC as well as hydrolysis with nuclease P1 to U, pU, and pA in the ratio 1.00:1.09:0.99.

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4) mp 90-92°C; UV λmax(MeOH) 262 nm, λmin 231 nm. ^{1}H-NMR (DMSO-d_6) δ 3.23 (s, 3H, OCH_3), 3.43 (m, 2H, $-CH_2-$), 3.65 (m, 4H, $-CH_2-$, 5'-H), 3.79-4.21 (m, 3H, 4'-H, 3'-H, 2'-H), 5.12 (m, 2H, 5'-OH, 3'-OH), 5.30 (brs, 3H, $-OCH_2O-$, 2'-OH), 5.69 (d, 1H, $J_{5,6}$=8Hz, 5-H), 5.90 (d, 1H, $J_{1',2'}$=4Hz, 1'-H), 8.01 (d, 1H, $J_{5,6}$=8Hz, 6-H). Anal Calcd for $C_{13}H_{20}N_2O_8$: C, 46.98; H, 8.01; N, 8.43. Found: C, 47.05; H, 6.07; N, 8.25.
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7) ^{1}H-NMR (Cl_3CD) δ 1.08, 1.12 (28H, Si-CH(CH_3)$_2$), 3.38 (d, 4H, OCH_3, 2'-OH), 4.21-4.79 (m, 6H, $-CH_2CH_2-$, 5'-H), 5.01-5.35 (m, 3H, 4'-H, 3'-H, 2'-H), 5.40 (brs, 2H, $-OCH_2O-$), 5.62 (d, 1H, $J_{5,6}$=8Hz, 5-H), 5.78 (d, 1H, $J_{1',2'}$=8Hz, 1'-H), 7.68 (d, 1H, $J_{5,6}$=8Hz, 6-H).
8) The structure was confirmed by ^{1}H-NMR spectra compared with standard compound.
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