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SYNTHESIS OF OLIGORIBONUCLEOTIDES USING 4-METHOXYBENZYL GROUP AS A NEW PROTECTING GROUP OF THE 2'-HYDROXYL GROUP OF ADENOSINE

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4-Methoxybenzyl group was introduced directly to the 2'-hydroxyl group from the reaction of adenosine with 4-methoxybenzyl bromide in the presence of sodium hydride. The 2'-O-(4-methoxybenzyl)-adenosine can be successfully used in the synthesis of oligoribonucleotides via phosphotriester approach. The 4-methoxybenzyl group was removed rapidly from the oligoribonucleotides by triphenylmethyl fluoroborate and the completely deblocked oligoribonucleotides were characterized by emzymatic hydrolysis.

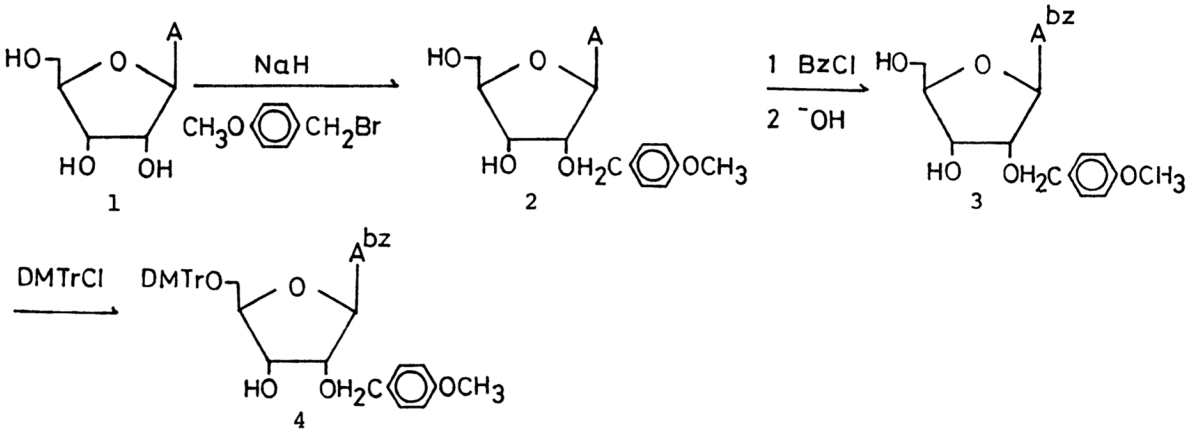
Direct protection of the 2'-hydroxyl group of ribonucleosides has been a crucial problem in the chemical synthesis of oligoribonucleotides. However, only a few examples of the direct protection of 2'-hydroxyl group of ribonucleosides have been found in literatures.[1] Consequently, the development of protecting group of 2'-hydroxyl group of ribonucleosides during the synthesis of oligoribonucleotides was required.

In this paper, we report a direct protection of the 2'-hydroxyl group of adenosine with 4-methoxybenzyl bromide, which can be removed rapidly by treatment with triphenylmethyl fluoroborate[2] from the 2'-hydroxyl group of adenosine along with the formation of 4-methoxybenzaldehyde.

We first examined the synthesis of 2'-O-(4-methoxybenzyl)adenosine 2 as a useful starting material for the synthesis of 3'-5' linked oligoribonucleotides. To a suspension of adenosine (5.34 g, 20 mmol) in dry DMF (180 ml) was added sodium hydride (50% mineral oil dispersion, 1.24 g, 26 mmol, washed with dry benzene) at -5°C. After stirring for 1 h, 4-methoxybenzyl bromide (4.83 g, 24 mmol) in dry DMF (20 ml) was added dropwise to the reaction mixture during 50 min and allowed to stand for further 1 h at -5°C. The reaction mixture was quenched with ice-water (20 ml) and the solution was concentrated to oil. The oil was dissolved in water (160 ml) and extracted with methylene chloride (2 X 20 ml). The aqueous solution was concentrated in vacuo until crystals precipitated. The precipitate was collected by filtration and recrystallization from ethanol-water gave 4.99 g (65%) of 2: mp 155-156°C; UV λmax(95% EtOH) 261 nm (ε=17,100), 227 nm, λmin(95% EtOH)

240 nm; ^{1}HNMR (DMSO-d_6) δ3.30 (d, 2H, H-5' and 5"), 3.60 (s, 3H, OCH_3), 4.11 (m, 1H, H-4'), 4.41-4.53 (m, 4H, H-2', H-3', $ArCH_2$), 5.35 (t, 1H, $J_{5'\text{-}OH,H}$= 6Hz, $C_{5'}$-OH), 5.54 (m, 1H, C_3'-OH), 6.08 (d, 1H, $J_{1',2'}$=7Hz, H-1'), 6.75 (d, 2H, J= 8Hz, H-3,5, Ar), 7.01 (d, 2H, J= 8Hz, H-2,6, Ar), 7.60 (s, 2H, NH_2), 8.13 (s, 1H, H-2 or H-8), 8.37 (s, 1H, H-2 or H-8), (5.35, 5.54, and 7.60 disappeared by addition of D_2O); Calcd for $C_{18}H_{21}N_5O_5.1/2H_2O$: C, 54.52; H, 5.60; N, 17.67%. Found: C, 55.09; H, 5.57; N, 17.53%. The compound 2 obtained in the above experiment was identified as the 2'-substituted compound. The ^{1}HNMR spectra showed a low field shifted H-1' signal compared with that of the 3'-isomer[3], and the H-2' signal was shifted to high field. Further evidence of the site of 4-methoxybenzyl group was obtained by determining the structure of the oligonucleotides with nuclease P1 as described later in this paper. However, the 3'-isomer could not be detected in this reaction.

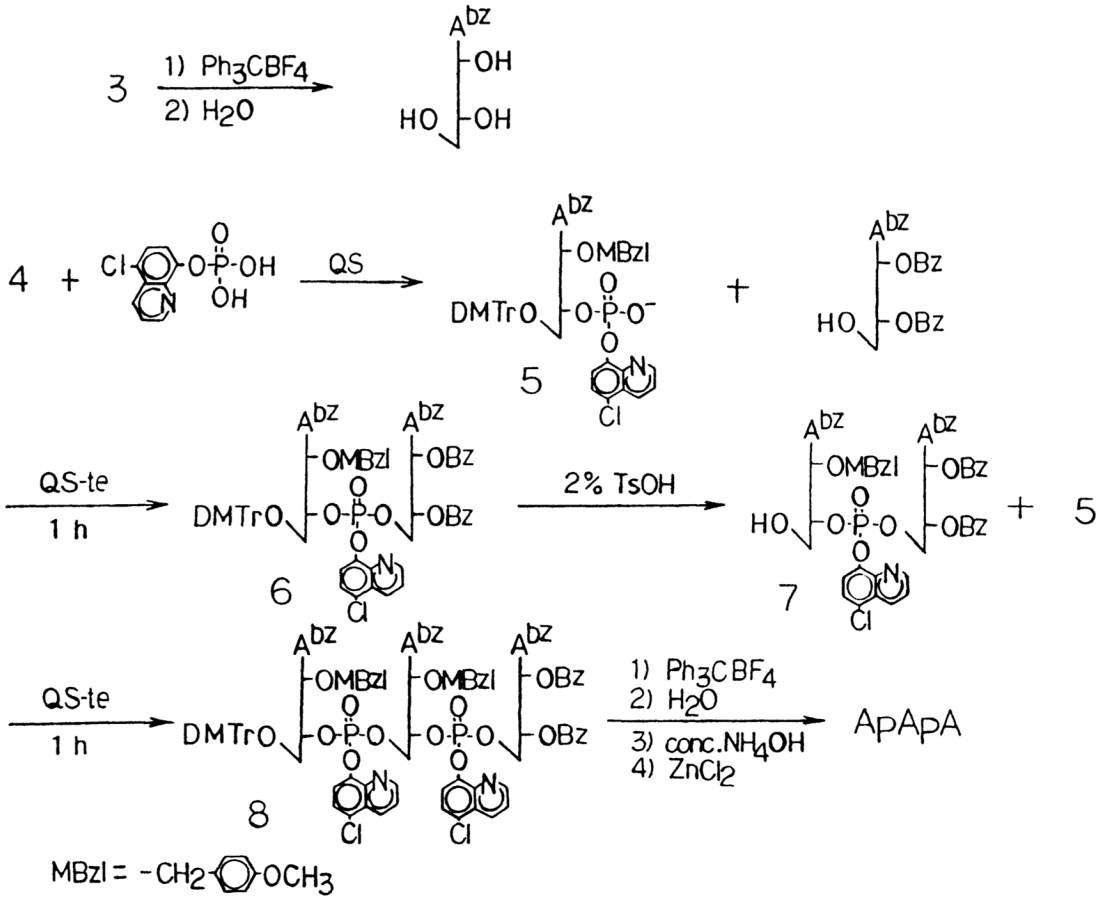
Reaction of 2 with benzoyl chloride in dry pyridine gave the corresponding benzoyl derivative which on immediate de-O-benzoylation using sodium hydroxide, gave N^6-benzoyl-2'-O-(4-methoxybenzyl)adenosine 3[4] in 91% yield. Treatment of 3 with dimethoxytrityl chloride in dry pyridine gave the expected 5'-O-dimethoxytrityl-N^6-benzoyl-2'-O-(4-methoxybenzyl)adenosine 4[5] in 99% yield.



Next, we examined the synthesis of oligoriboadenylates by using 4: The nucleoside 4 (793 mg, 1.0 mmol) was treated with 5-chloro-8-quinolyl phosphate[6] (312 mg, 1.2 mmol) in the presence of 8-quinolinesulfonyl chloride (QS)[7] in dry pyridine (10 ml) for 2 h at room temperature. 8-Quinolinesulfonic acid was removed by filtration. The filtrate was then quenched with ice-water, followed by extraction with methylene chloride, and the organic layer was washed with 0.1M TEAB solution. The methylene chloride extract was concentrated in vacuo. The residue was dissolved in methylene chloride and added dropwise to hexane. The triethylammonium salt of phosphodiester 5 (1.09 g, 96%) was obtained as stable colourless solid, uncontaminated [^{31}PNMR ($CDCl_3$, 85%H_3PO_4) δ +6.42] with 5-chloro-8-quinolyl phosphate. The triethylammonium salt of 5 (715 mg, 0.63 mmol) thus obtained was treated with N^6,2',3'-O-tribenzoyladenosine (287 mg, 0.42 mmol) in the presence of 8-quinolinesulfonyltetrazolide (QS-te)[8] (412 mg, 1.57 mmol) in dry pyridine (2 ml) for 1 h at room temperature. The reaction mixture was quenched with ice-water,

followed by extraction with methylene chloride, and the organic layer was washed with water. The methylene chloride solution was concentrated in vacuo. The residue was dissolved in methylene chloride and chromatographed on a silica gel column. The fully protected dinucleotide 6 was isolated in 78% (555 mg) yield by eluting the column with a stepwise gradient of methanol (0-5%) in methylene chloride. The dinucleotide 6 thus obtained was treated with 2% p-toluenesulfonic acid in a mixture of dioxane and methanol (7:3 v/v) (12 ml) for 15 min at 0°C to give 7.[6] The 5'-hydroxyl dinucleotide 7 was isolated in 99% (453 mg) yield by precipitation with a mixture of hexane and ether (95:5 v/v) and used for the next coupling reaction without further purification. A solution of the phosphodiester 5 (268 mg, 0.23 mmol) and 7 (219 mg, 0.16 mmol) in dry pyridine (1 ml) was then condensed in the presence of QS-te (154 mg, 0.59 mmol) for 1 h. Usual work-up including separation and purification by chromatography on silica gel gave 88% (332 mg) yield of the fully protected trinucleotide 8.

Finally, removal of 4-methoxybenzyl group from 3 and 8 by using triphenylmethyl fluoroborate was examined. The nucleoside 3 was treated with triphenylmethyl fluoroborate (10 equiv.) in methylene chloride for 30 min at room temperature. After addition of water, the mixture was left to stand for 30 min. N^6-Benzoyladenosine was obtained by paper chromatography in 99% yield. The nucleoside 2 was stable in concentrated ammonia and 80% acetic acid for 24 h at 22°C. The trimer 8 was completely deblocked by treatment with (i) triphenylmethyl fluoroborate



in methylene chloride for 30 min at room temperature; (ii) water for 30 min; (iii) concentrated ammonia for 5 h at 50°C; and finally, zinc chloride in aqueous pyridine for 24 h. The deblocked trinucleotide, ApApA was obtained in 85% yield after purification by chromatography on DEAE-Sephadex A-25. The purity of ApApA was checked by PE and HPLC on Finepak C_{18}[9] as well as hydrolysis with nucleoase P1[10] to A and pA in the ratio 1.00:1.98. This hydrolysis indicated that ApApA had a 3'-5' internucleotidic bonds. Further studies on the synthesis of other 2'-O-(4-methoxybenzyl)nucleosides and their use in oligoribonucleotide synthesis are now in progress.

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3) When 4-methoxyphenyldiazomethane [prepared by a modification of the procedure of Cross; G.L.Cross and R.A.Moss, J.Amer.Chem.Soc., 86,4042(1968)] was allowed to react with adenosine in dry DMF in the presence of stannous chloride for 2 h at 45°C, 3'-O-(4-methoxybenzyl)adenosine was obtained along with the 2'-isomer 2; ^{1}HNMR (DMSO-d_6) δ 3.35 (m, 2H, H-5' and H-5"), 3.70 (s, 3H, OCH_3), 4.10 (m, 2H, H-4', H-3'), 5.81 (d, 2H, $ArCH_2$), 4.90 (m, 1H, H-2'), 5.18 (t, 1H, C_5'-OH), 5.60 (d, 1H, C_2'-OH), 5.98 (d, 1H, $J_{1',2'}$= 6Hz, H-1'), 6.79 (d, 2H, H-3,5, Ar), 7.29 (d, 2H, H-2,6, Ar), 7.65 (s, 2H, NH_2), 8.31 (s, 1H, H-2 or H-8), 8.49 (s, 1H, H-2 or H-8), (5.18, 5.60, and 7.65 disappeared by addition of D_2O).
4) mp 186-187°C; UV λmax(95% EtOH) 278 nm, 225 nm, λmin(95% EtOH) 245 nm; ^{1}HNMR (DMSO-d_6) δ 3.58 (m, 2H, H-5'), 3.74 (s, 3H, OCH_3), 4.12 (m, 2H, H-4', H-3'), 4.59 (d, 2H, $ArCH_2$), 4.80 (m, 1H, H-2'), 6.22 (d, 1H, $J_{1',2'}$= 5Hz, H-1'), 6.85 (d, 2H, H-3,5, Ar), 7.10 (d, 2H, H-2,6, Ar), 7.65-8.20 (m, 5H, Ar), 8.59 (s, 1H, H-2 or H-8), 8.62 (s, 1H, H-2 or H-8), 11.15 (brs, 1H, NH, disappeared with D_2O); Calcd for $C_{25}H_{25}N_5O_6$: C, 61.09; H, 5.13; N, 14.25%. Found: C, 61.22; H, 5.10; N, 14.25%.
5) UV λmax(95% EtOH) 277 nm, 228 nm, λmin(95% EtOH) 255 nm; ^{1}HNMR (DMSO-d_6) δ 3.60 (m, 2H, H-5'), 3.68 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 4.22 (m, 1H, H-4'), 4.41 (m, 1H, H-3'), 4.78 (m, 1H, H-2'), 5.01 (d, 2H, $ArCH_2$), 5.49 (d, 1H, C_3'-OH, disappeared with D_2O), 6.27 (d, 1H, $J_{1',2'}$= 4Hz, H-1'), 6.60-8.10 (m, 22H, Ar), 8.54 (s, 1H, H-2 or H-8), 8.67 (s, 1H, H-2 or H-8), 11.07 (brs, 1H, NH, disappeared with D_2O).
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