Studies on transfer ribonucleic acids and related compounds. IX[1] Ribooligonucleotide synthesis using a photosensitive o-nitrobenzyl protection at the 2'-hydroxyl group

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ABSTRACT

o-Nitrobenzyl group was introduced to the 2'-hydroxyl function of uridine via 2',3'-O-(dibutylstannylene)uridine. The benzylated uridine was protected at the 5'-hydroxyl group with monomethoxytrityl chloride and condensed with 2',3'-O-dibenzoyluridine 5'-phosphate or N,N',2',3'-O-tetrabenzoyladenosine 5'-phosphate using dicyclohexylcarbodiimide (DCC). o-Nitrobenzyl ether linkage of the dinucleotides was removed by UV irradiation with wavelength longer than 320 nm. Deprotected UpU and UpA thus obtained were characterized by RNase A digestion.

INTRODUCTION

Previously we have reported syntheses of ribooligonucleotides using nucleoside 3'-phosphate derivatives.[2] Another approach involving a condensation of nucleoside 5'-phosphates with nucleosides having free 2',3'-hydroxyl groups gave a mixture of 2'-5' and 3'-5' linked dimers.[3] Although protection of the 2'-hydroxyl group via 3',5'-protected nucleosides has been investigated,[4] a selective protection of the 2'-hydroxyl function of ribonucleosides would provide a promising route for ribooligonucleotide synthesis. Recently direct 2' and 3'-O-benzylation was reported.[5,6] Catalytic hydrogenolysis to cleave the benzyl ether group, however, is not so easy to effect. It has been known that aromatic nitro compounds having a C-H bond in the ortho-position transfer their oxygen photochemically to give nitrosobenzene derivatives.[7] o-Nitrobenzyl derivatives have been used as protecting groups for amino acids[8,9] and carbohydrates.[10,11] We have also found that 6-nitroveratryl- or o-nitrobenzyl-ester of uridine 5'-phosphate was converted to uridine 5'-phosphate by UV irradiation through a pyrex filter.[12] In this paper we report a direct protection of the 2'-hydroxyl group of a nucleoside with o-nitrobenzyl ether, which can be removed by UV irradiation. The protected nucleoside (II) was proved to be a useful starting material for synthesis of 3'-5' linked ribooligonucleotides when II was converted to III and condensed with nucleoside 5'-phosphates (IV). The o-nitrobenzyl group of VI was removed under the condition in which uridine and adenosine were not altered. The structure of the products, VIIa,c were con-

Chart 1

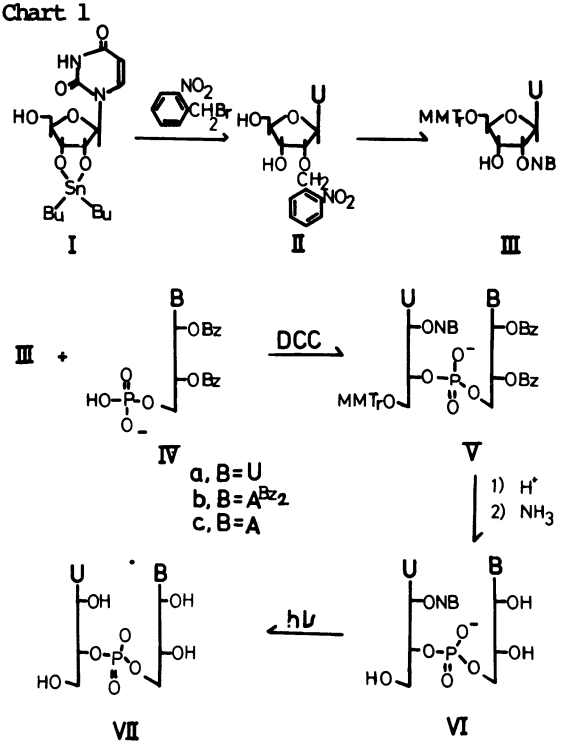

Table I Paper Chromatography and Paper Electrophoresis

	Paper Chromatography Solvent			Paper Electrophoresis pH
	A	B	C	7.5
U	0.47	0.69	0.51	0
U(ONB) (II)	0.73	0.84	0.80	
Up cyclic	0.38	0.60		0.67
pU	0.11	0.27		1.00
U(ONB)-p-U(OBz)$_2$	0.74	0.85		0.28
U(ONB)-p-U (VIa)	0.44	0.66		0.41
UpU (VIIa)	0.25	0.49		0.49
A	0.50	0.63		-0.21
Ap cyclic	0.50	0.51		0.43
pA	0.15	0.22		0.88
U(ONB)-p-A (VIc)	0.47	0.61		0.36
UpA (VIIc)	0.29	0.42		0.42

firmed by enzymatic hydrolysis with RNase A.

Synthesis of 2'-O-(o-nitrobenzyl)uridine (II).

Moffatt and his coworkers reported that dibutylstannylene function of 2', 3'-O-(dibutylstannylene)nucleosides served as an activating group for the 2' and 3'-oxygen groups and synthesized 2',(3')-O-benzyluridine.[6] When 2',3'-O-(dibutylstannylene)uridine (I) was allowed to react with o-nitrobenzyl bromide in DMF, only 2'-O-(o-nitrobenzyl)uridine (II) was obtained by crystallization from ethanol in a yield of 24%. The 3'-alkylated isomer was not detected in the mother liquor. From the elemental analysis and mass spectrum it was evident that only one nitrobenzyl group was present. UV spectra of II showed appreciable hypochromicity in water compared with ε value in ethanol. The hypochromic effect was assumed to be characteristic for the 2'-benzyl nucleosides.[5a] The absolute location of the o-nitrobenzyl group in II was confirmed by spin-decoupling studies of the diacetate of II. Since protons of $C_{2'}$ and $C_{3'}$ of II were not resolved, II was acetylated with acetic anhydride in pyridine to give the diacetate, in which one proton showed low-field shift and did not couple with $C_{1'}$-H. Further evidence of the location of the benzyl group was obtained by determining the structure of the condensed product with RNase A as described later in this paper. II was stable in concentrated ammonia and 80% acetic acid at room temperature for 24 hr. Removal of the o-nitrobenzyl group of II was performed by UV irradiation in 40% ethanol for 2 hr through a pyrex filter which was assumed to cut UV light shorter than 320 nm. Conversion to uridine examined by tlc and ppc (C) was 93% in both cases.

Synthesis of dinucleoside monophosphates (VIIa,c) using II as the starting material.

For condensation of II with nucleoside 5'-phosphates, II was converted to III by treatment with monomethoxytrityl chloride in pyridine at room temperature. III was condensed with 2',3'-O-dibenzoyluridine 5'-phosphate(IVa) or N,N',2',3'-O-tetrabenzoyladenosine 5'-phosphate(IVb) using DCC as a condensing reagent. After 22 hr the reaction was nearly completed as judged by ppc and pep of the partially deprotected reaction mixture. The Rf values and relative mobilities of compounds are listed in Table I. The products (Va,b) were precipitated in ether-pentane mixture. Aliquots of Va,b were treated with 80% acetic acid and then with concentrated ammonia. The 2'-protected compounds(VIa,c) were isolated by pep and the recovery of absorbance from IV at this stage was 61%. VIa,c were subjected to UV irradiation to remove the 2'-O-benzyl group. The extent of the reaction was checked by ppc and found to be 94-95% after 1 hr in both cases. The recovery of the UV absorbance of UpU was confirmed by irrad-

iating an authentic sample of UpU under the same condition used. VIIa,c were digested with RNase A to give the expected products in correct ratio. These hydrolyses indicated that VIIa,c had a 3'-5' phosphodiester linkage and the starting material(III) was alkylated at the 2'-position.

Further use of o-nitrobenzyl ether derivatives of nucleosides and o-nitrobenzyl ester of nucleotides in ribooligonucleotide syntheses is in progress.

EXPERIMENTAL

General Methods. Paper chromatography (ppc) was performed using the following solvent systems: solvent A, isopropanol-concentrated ammonia-water(7:1:2,v/v), solvent B, ethanol-1 M ammonium acetate, pH 7.5(7:3,v/v); solvent C, n-butanol-acetic acid-water (5:2:3.v/v). Paper electrophoresis (pep) was performed at 900 V/40 cm using 0.05 M triethylammonium bicarbonate,pH 7.5.

Photolysis apparatus had a 300 W high pressure mercury lamp (Eikosha Co. Model PIH 300) with a quartz water-circulating jacket. Compounds were irradiated through a pyrex filter (2 mm thick) inserted in the water jacket.

Molecular extinction values used were: uridine, 10,000; U(ONB)(II), 13,700; U(ONB)-p-U(VIa), 23,700; UpU(VIIa), 20,000; adenosine, 15,400; U(ONB)-p-A(VIc), 29,100; UpA(VIIa), 25,400 at 260 nm in water.

RNase A digestion was carried out using 50μg of the enzyme in 0.1 M ammonium acetate, pH 7.5 (0.1 ml) at 37^{o} for 6 hr.

Other general methods were as described previously.[2a,f]

2'-O-(o-Nitrobenzyl)uridine (II).

2',3'-O-(Dibutylstannylene)uridine(I) (4.75 g, 10 mmoles) was treated with o-nitrobenzyl bromide (4.32 g, 20 mmoles) in DMF(60 ml) at 110^{o}until tlc did not show increase of the product. After 4 hr DMF was evaporated and the residue was stirred with water-ether mixture(5:3) (80 ml). The ether layer was removed and the rest was washed with ether(20 ml). The aqueous phase and the oil were heated with water(100 ml) and the solution was left at room temperature for 4 hr. Further cooling in refrigerator for overnight gave a precipitate (923 mg) and the second crop (234 mg) was obtained from the mother liquor. The precipitate was contaminated with a trace of a faster travelling side product as detected by tlc ($CHCl_3$-EtOH, 5:1). Recrystallization from ethanol gave prisms (892 mg, 24%):mp 204-205; λmax (H_2O) 263 nm (ε13,700); λmax (0.1 N HCl) 263 nm (ε 13,500); λmax (0.1 N NaOH) 263 nm (ε 11,300); ε_{263}(EtOH) 15,100; ε_{320}(H_2O)1,470; mass spectrum (70 eV) m/e 379 (M^+); nmr (DMSO-d_6) 3.65 (m, 2, C_5,-H_2), 3.85-4.30 (m,3, C_2,-H, C_3,-H, C_4,-H), 5.0 (d, 2, J_{gem}= 2 Hz Ar-CH_2), 5.08 (t, 1, C_5,-OH), 5.27 (d, 1, $J_{3'OH,H}$ = 6 Hz, C_3,-OH), 5.58 (q, 1, $J_{C5,6}$ = 8 Hz C_5-H, $J_{N3H,C5}$

= 1.5 Hz), 5.93 (d, 1, $J_{1',2'}$ = 5 Hz $C_{1'}$-H), 7.4-8.1 (m, 5, Ar 4, C_6-H 1).

Anal. Calcd for $C_{16}H_{17}O_8N_3$ (379.32): C, 50.66; H, 4.52; N, 11.08. Found; C, 50.51; H, 4.77; N, 11.04.

Nmr spectrum of 2'-O-(o-nitrobenzyl)-3',5'-O-diacetyluridine.

II (100mg, 0.26 mmole) was treated with acetic anhydride (0.2 ml) in pyridine (0.4 ml) overnight at room temperature. The reaction was completed as checked by tlc ($CHCl_3$-EtOH, 10:1). The mixture was poured on ice and extracted with chloroform. The chloroform layer was washed with dil.sodium bicarbonate and water. The solvent was removed after drying with sodium sulfate and the residue was coevaporated with toluene. Recrystallization with benzene gave an amorphous compound (76 mg); nmr ($CDCl_3$) 4.25-4.5 (m, 4, $C_{2'}$-H, $C_{4'}$-H, $C_{5'}$-H), 4.98-5.2 (m, 3, $C_{3'}$-H, Ar-CH_2), 5.73 (q, 1, $C_{5,6}$ = 8 Hz, C_5-H), 5.98 (d, 1, $J_{1',2'}$ = 4 Hz, $C_{1'}$-H, becoming singlet by irradiation at 4.38 but not at 5.09).

5'-O-Monomethoxytrityl-2'-O-(o-nitrobenzyl)uridine (III).

II (379 mg, 1 mmole) was made anhydrous azeotropically with pyridine and allowed to react with monomethoxytrityl chloride (370 mg, 1.2 mmoles) in pyridine at 25° for 20 hr. Tlc ($CHCl_3$:EtOH, 10:1) showed that the reaction was completed. Pyridine was evaporated and the residue was dissolved in chloroform, washed with water, dried with sodium sulfate and precipitated in pentane. The precipitate was contaminated with a trace of monomethoxytritanol but used for the next reaction without further purification.

Condensation of III with 2',3'-O-dibenzoyluridine 5'-phosphate (IVa).

Pyridinium salt of IVa[1] (0.1 mmole) and III (78 mg, 0.12 mmole) were co-evaporated with pyridine and treated with DCC (124 mg, 0.6 mmole) in pyridine (0.8 ml) at room temperature. After 22 hr an aliquot was deprotected with 80% acetic acid for 1 hr and then with concentrated ammonia for 1 hr at 50°in a sealed tube. Ppc and pep (Table 1) at this stage showed VIa as the major spot. Besides II and benzamide , faint spot of pU and an unidentified compound (Rf 0.42 in solvent B) were detected. After 5 days the rest of the reaction mixture was treated with aqueous pyridine overnight and extracted with pentane. The urea was removed by filtration and the product (Va) was precipitated with ether-pentane (3:2, 100 ml) from its solution in anhydrous pyridine. The precipitate was washed with ether 3 times and dried over P_2O_5 in vacuo to give 100 mg. An aliquot (1.221 mg) was deprotected as above and subjected to pep. VIa thus isolated was 16.8 A_{260} units. The total product was estimated as 1379 A_{260}units (0.058 mmole, yield 61%).

Photolysis of VIa and characterization of UpU (VIIa).

VIa obtained from the above aliquot sample was isolated from a paper electrophoretogram. The aqueous solution of VIa (6.2 A_{260}/ml) was irradiated in a pyrex tube through the pyrex filter. One ml of the solution was taken at time intervals and subjected to ppc in solvent A and B. The chromatogram in solvent A showed fluorescent spots at Rf 0.66 and 0.79 together with a faint spot of nitrosobenzaldehyde (Rf 0.86). Spots corresponding to VIa and VIIa were eluted with water (3 ml). The percentages of the product (VIIa) were 89, 94 and 94 after 30 min, 1hr and 2 hr, respectively. Incubation of the irradiated product with ammonium bicarbonate (0.05 M, pH 9.2, 0.4 ml) for 2hr at 37° to affect 5-hydro-6-hydroxyuridine[13] did not give any change of the product. The unprotected product UpU (4.6 A_{260}) eluted from the paper chromatogram was digested with RNase A to confirm complete cleavage of the phosphodiester linkage. Samples irradiated at different time periods were digested with the RNase found to give the ratio of Up : U between 1.00 : 1.00 to 1.00 : 1.05. The product VIIa was also analyzed by Nucleic Acid Analyzer (Varian LCS 1000) to give the same retention time as an authentic sample synthesized from 2'-O-benzoyluridine 3'-phosphate.

Synthesis of uridylyl-(3'-5')-adenosine (VIIc).

III (50 mg, 0.076 mmole) and N,N',2',3'-O-tetrabenzoyladenosine 5'-phosphate (IVb)[3] (872 A_{260}, 0.048 mmole) were condensed using DCC (78 mg) as described for the synthesis of Va. After 2 days an aliquot was partially deprotected and subjected to ppc and pep. The mononucleotide was almost disappeared and VIc was the major product. The reaction mixture was worked up as described for Va. The yield was 65 mg, 61%, as estimated by the same way as in the case of Va. Photolysis of VIc was performed in aqueous solution (5.2 A_{260}/ml) using the same condition used for VIa. Conversion to VIIc was 94% after 2 hr and no phosphorus containing spot was detected besides VIc and VIIc on paper chromatogram in solvent A. VIIc was completely hydrolyzed with RNase A to give Up (1.04 A_{260}) and A (1.60 A_{260}) in pep. Ratio of Up to A was 1.00 : 1.00.

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