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NEW TYPE OF PREFABRICATED FULLY PROTECTED RIBONUCLEOTIDE MONOMER UNITS AS USEFUL SYNTHETIC INTERMEDIATES IN RAPID OLIGORIBONUCLEOTIDE SYNTHESIS

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Oligoribonucleotide synthesis has been done by employing newly constructed fully protected ribonucleotide units, S,S-diphenyl 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N-monomethoxytrityl-nucleoside 3'-phosphorodithioates 7. The units were found to be useful synthetic intermediates for rapid oligoribonucleotide synthesis.

The phosphotriester approach to the synthesis of oligonucleotides has rapidly been developed since Letsinger[1] first described the advantageous points of this method compared with the phosphodiester method. Among several improved methods via the phosphotriester approach, Cramer[2] proposed the principle where oligonucleotides could be synthesized from prefabricated fully protected mononucleotide "units". Recently, most of the improved methods via the phosphotriester approach have been designed on the basis of this principle[3].

A recent main subject of oligonucleotide synthesis lies in how to synthesize oligomers rapidly without side reactions. In order to accept such a requirement, the combination of the protecting groups of nucleotide units and the condensing agent should be reconsidered.

In our strategy, the amino groups of nucleoside bases were protected by monomethoxytrityl group (MMTr) and 2'- and 5'-hydroxyl groups were masked by tetrahydropyranyl group (Thp) and dimethoxytrityl group (DMTr), respectively. Phosphorylation of 3'-hydroxyl groups was successfully performed by use of cyclohexylammonium S,S-diphenyl phosphorodithioate[4] in the presence of 2,4-dimethoxybenzene-1,5-disulfonyl chloride (DMS), a new coupling agent, as described previously[5].

The characteristic feature of the units 7 is the use of MMTr group for the protection of amino groups of nucleoside bases[6]. Consequently, the units 7 were so lipophilic that the solubility of the units in organic solvents was much increased, and the Rf values of the two diastereoisomers resulting from introduction of the Thp group were almost the same. Therefore, we carried out further investigation without separation of the diastereoisomers.

The preparative process of 7 was described as follows: (General Procedure) Ribonucleoside 1 was treated with tetraisopropyldisiloxane dichloride (TIPDS) in

pyridine according to Markiewicz's procedure[7]. The desired 3',5'-cyclic silyl ether derivatives (2) except guanosine derivative were obtained in high yield (91-96%). In the case of guanosine, the reaction was carried out in DMF in the presence of 4 equiv. of imidazole (84% yield).

The protection of amino groups of nucleoside bases was performed by monomethoxytritylation by means of monomethoxytrityl chloride (MMTrCl) in dry pyridine. We observed that 4-(dimethylamino)pyridine (DMAP)[8] had catalytic activity for this reaction in the presence of triethylamine in CH_2Cl_2. For example, 3',5'-O-silylated guanosine (2d)(533 mg, 1.01 mmole) was dissolved in CH_2Cl_2 (10 ml) in the presence of MMTrCl (626 mg, 2.03 mmole) and triethylamine (0.28 ml, 2.01 mmole)and DMAP (5.0 mg, 0.04 mmole) and the reaction was completed in 1 h at room temperature. After the usual work-up, the desired guanosine derivative was obtained in 75% yield.

The successive tetrahydropyranylation of 2'-hydroxyl groups was performed using p-toluenesulfonic acid in dioxane, but in the case of the uridine derivative, pyridinium p-toluenesulfonate in CH_2Cl_2[9] was found to be more effective because the reaction proceeded cleanly without formation of coloured substances.

We have found that commercially available tetraethylammonium chloride and potassium fluoride (TBAF equivalent) system in acetonitrile in the presence of water[10] was very effective for the desilylation from 4. This reaction was completed in 20 h at room temperature. If the temperature was raised to 40°C, it was completed in 4 h and the desilyated product (5) was obtained in satisfactory yield.

After dimethoxytritylation of 5'-hydroxyl groups of 5 was performed in the usual manner, phosphorylation of 3'-hydroxyl groups was carried out as follows: Compound 6a (3.15 g, 5.0 mmol) was dissolved in dry pyridine (30 ml) and this solution was added to a mixture of cyclohexylammonium S,S-diphenyl phosphorodithioate (2.86 g, 7.5 mmol) and DMS (3.02 g, 9.0 mmol) in dry pyridine (20 ml). The starting material 6 disappeared on tlc after 2 h, then ice-water (30 ml) was added to the reaction mixture. The desired product 7a was extracted with CH_2Cl_2 and obtained by silica-gel column chromatography. (Table 1)

Table 1. Isolated Yields of the Protected Nucleotide Derivatives (2-7).

nucleoside	compound (%)						Rf value of 7 (Solvent A*)
	2	3	4	5	6	7	
a) Ur	91	—	***	81(from 3)	91	89	0.58
b) Ad	96	96	***	84(from 3)	89	86	0.39**
c) Cy	96	92	97	93	97	77	0.55
d) Gu	84	75	80	75	83	70	0.50

* Solvent A: CH_2Cl_2/MeOH, 9:1 (v/v) ** Solvent B: CH_2Cl_2/MeOH, 20:1 (v/v)
*** It was not isolated and applied to the successive reaction.

One of the two phenylthio groups was able to be selectively removed from 7 by treatment with alkaline solution or pyridinium hypophosphonate. Both phenylthio groups could be removed simultaneously by treatment with silver acetate[11].

All the other protecting groups of 7, DMTr, MMTr, and Thp groups were removed under acidic conditions (0.01 M HCl, pH 2), whereas the DMTr group from 7 was selectively removed by treatment of 2% TsOH (CH_2Cl_2/MeOH-7:3) at 0°[12] without any loss of MMTr and Thp groups.

According to the above facts, the synthesis of the fully protected dinucleotide units was performed as follows.

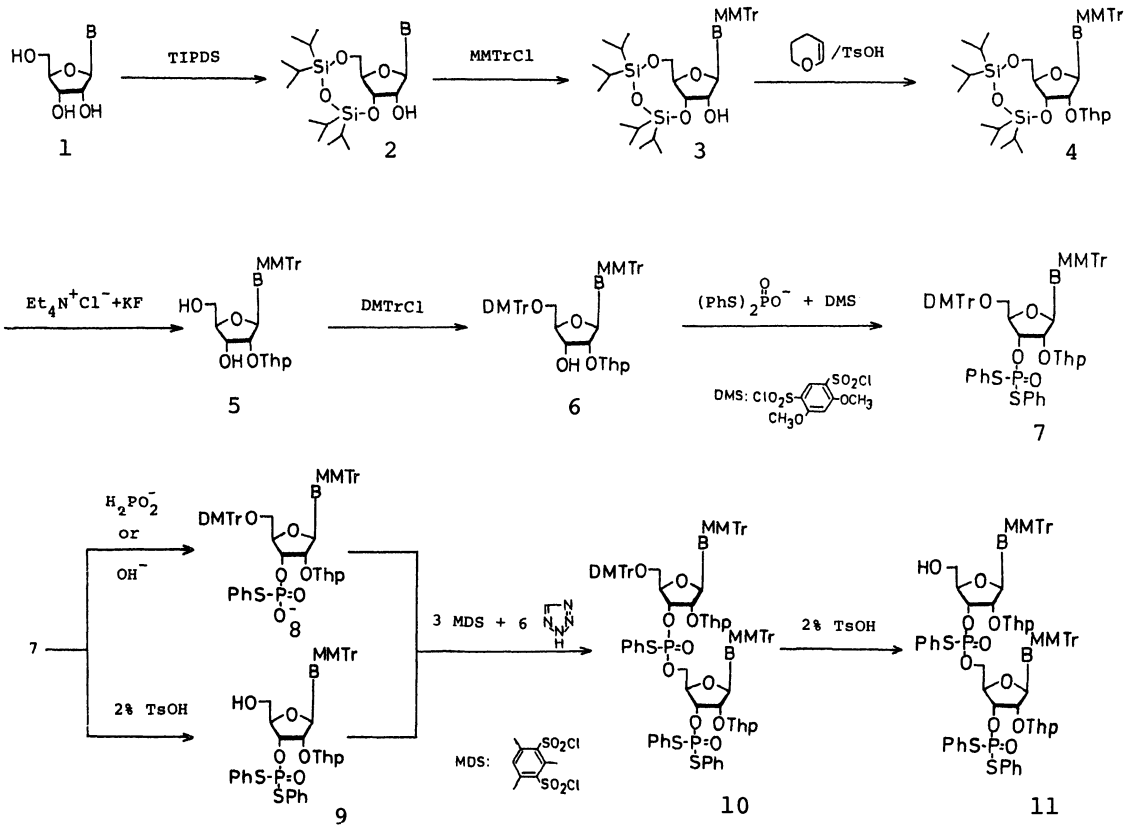


Table 2. Synthesis of the Fully Protected Dinucleotides (10).[13]

phosphate 8 component (B) (mmol)	hydroxyl 9 component (B) (mmol)	condensing agent (mmol)	pyridine (ml)	time (h)	product yield (%)	Rf value (Solvent A)
Ur (0.11)	Ur (0.12)	DMSTe * (0.33)	1.1	3.0	10a 72	0.66
Ad (0.50)	Ur (0.52)	MDS + tetrazole (0.75) (1.50)	5.0	1.5	10b 74	0.72
Ur (0.19)	Ad (0.15)	MDS + tetrazole (0.46) (0.91)	1.5	1.5	10c 68	0.58

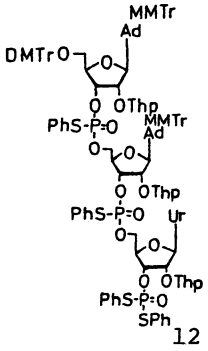
*DMSTe=2,4-Dimethoxybenzene-1,5-disulfonyltetrazole.

Dinucleotide synthesis: The phosphate component 8 (triethylammonium salt) prepared from 7 by means of pyridinium hypophosphonate in pyridine and the 5'-hydroxyl component 9 in the presence of tetrazole[14] (6 equiv.) were coevaporated

with dry pyridine, then mesitylenedisulfonyl chloride (MDS, 3 equiv.) and dry pyridine were added.

After 1 h the starting material 8 disappeared on tlc, then ice-water (15 ml) was added. The aqueous solution was extracted with CH_2Cl_2 and the desired fully protected dinucleotide unit 10 was purified by silica-gel column chromatography.(Table 2)

Trinucleotide synthesis: Fully protected dinucleotide 10b (933 mg, 0.56 mmole) was treated with 2% TsOH solution, then the desired product 11b was obtained after purification by silica-gel column chromatography (633 mg, 83% yield). The phosphate component 8b (180 mg, 0.15 mmol) and the hydroxyl component 11b (138 mg, 0.10 mmol) were condensed in the presence of MDS (85 mg, 0.30 mmol) and tetrazole (43 mg, 0.61 mmol) at room temperature for 2 h to afford the desired fully protected DMTr $Ad^{MMTr}p(SPh)Ad^{MMTr}p(SPh)Up(SPh)_2$ (12) by silica-gel column chromatography (168 mg, 70% yield). [Rf. 0.64 (Solvent A)]



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Complete deprotection of the fully protected oligonucleotides was performed as follows; 1) 0.2 M NaOH-dioxane (1:1, v/v) for 2 h for removal of internucleotidic PhS groups and one of the two PhS groups at the 3'-terminal phsophate; 2) 15 equiv. of silver acetate in pyridine-water (2:1) for 16 h for removal of the remaining PhS group at the 3'-phosphate; 3) 0.01 M HCl (pH 2) for simultaneous removal of all of DMTr, MMTr and Thp groups. After the above work-up unprotected desired nucleotides were obtained (75-90% yields) by paper chromatography using Whatman 3MM paper developed with 2-propanol-conc.ammonia-water (7:1:2 v/v).

The trimer, ApApUp (29 OD unit), was treated with spleen phosphodiesterase. It was completely degraded to Ap and Up (Ap/Up=1.7).

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