Tetrahedron Letters,Vol.30,No.46,pp 6361-6364,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

1-(2-CHLOROETHOXY)ETHYL GROUP FOR THE PROTECTION OF 2'-HYDROXYL GROUP IN THE SYNTHESIS OF OLIGORIBONUCLEOTIDES

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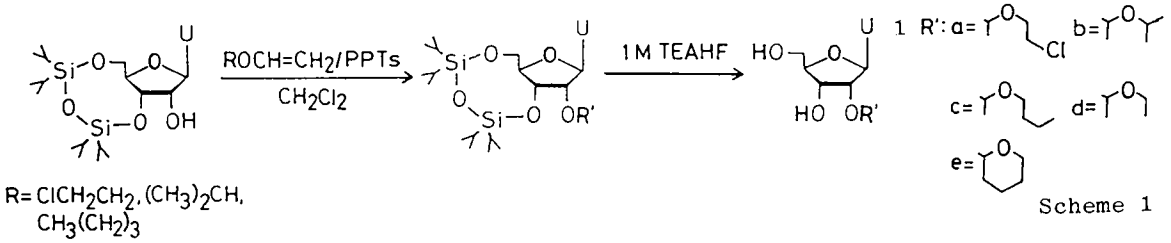
Abstract---*The 1-(2-chloroethoxy)ethyl (Cee) group is completely stable under the acidic condition required to remove the 5'-protecting groups in oligonucleotide synthesis, but can be cleaved under the similar conditions to that of the tetrahydropyranyl (Thp) in the region of pH 2-3.*

The chemical synthesis of oligoribo- and poly-ribonucleotides on a solid support is more elaborate and time-consuming than the oligo- and poly-deoxyribonucleotides primarily because of the need to protect the 2'-hydroxyl function of ribonucleosides. The choice of the protecting group for the 2'-hydroxyl function is a crucial point in polyribonucleotide synthesis and it should be completely stable under the conditions required for the removal of the 5'-protecting groups. Recently, the *tert*-butyldimethylsilyl[1] and *o*-nitrobenzyl[2] groups as the 2'-hydroxyl function have been successfully employed for the synthesis of long chain RNA fragments. On the other hand, the recent studies shown[3,4] that an acid-labile 2'-acetal protecting group was insufficiently stable under acidic conditions required for the removal of a 5'-dimethoxytrityl group to be useful for chain elongation on a solid support. Reese et al.[5] have investigated the use of a new type of acetal group such as 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl group as the 2'-protecting group and Hata et al.[6] have synthesized the oligoribonucleotides (10 and 13mers) using two different 5'-O-protecting groups [9-phenylxanthen-9-yl or (4-methoxy)phenylxanthen-9-yl] in combination with the 2'-O-tetrahydropyranyl group. More recently, Ohtsuka et al.[7] have reported the synthesis of a 21mer by the phosphoramidite approach using the base-labile levulinyl and tetrahydrofuranyl groups, respectively, for the protection of 2'-O- and 5'-O-hydroxyl functions.

In our continuing studies[8] to develop the acetal groups as the 2'-protecting groups for the synthesis of oligoribonucleotides on a solid support, we have found that 1-(2-chloroethoxy)ethyl (Cee) group is stable under the acidic conditions required for the complete removal of the DMTr group; however, under mild hydrolytic conditions (pH 2.0), the Cee group is removed.

First we examined the synthesis of uridine derivatives (**1a-c**) bearing three different 2'-acetal protecting groups. Further, we carried out investigation without separation of the diastereoisomers. The reaction between 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine and alkyl vinyl ethers and the conversion of the products obtained into **1a-c**[9] (80-83%) are indicated in outline in Scheme 1. 2'- O-Acetal uridine derivatives (**1d,e**) were prepared according to the published procedure[10,11].



Scheme 1

It was then interesting to explore the relative stabilities of these acetal groups in 2'-O-acetal derivatives (**1a-e**) under the acidic conditions to evaluate their possible use in the chemical

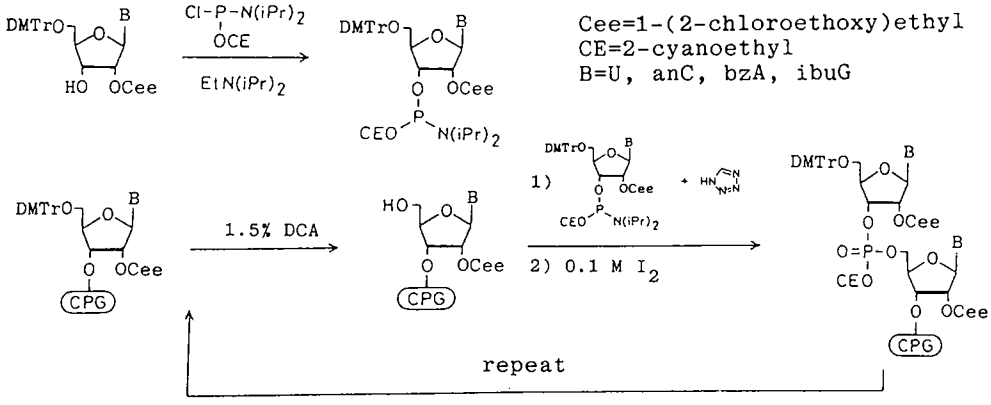
synthesis of oligoribonucleotides in conjunction with other acid-labile protecting groups on the pentose sugar. The relative rates of removal of the acetal groups from the corresponding 2'-O-acetal derivatives (**1a-e**) are shown in Table 1. It can be seen from the Table 1 that **1a** may be used as a 2'-protecting group in view of its relatively high stability even under the acidic condition (1.5% dichloroacetic acid in CH_2Cl_2) required for the complete removal of the 5'-DMTr group. Especially, in case of **1a**, no hydrolysis can be detected after 10 min.[12] However, **1a** has hydrolysis properties similar to that of the Thp or Mthp protecting groups under the relatively mild conditions, for example, by treatment with 0.01 N hydrochloric acid at room temperature.

Table 1. The relatives rates of hydrolysis of acetal groups from uridine derivatives (**1a-e**)[a].

comp.	1.5% DCA in CH_2Cl_2 $t_{1/2}$(min)	t_∞ (min)	0.01 N HCl (pH 2.0) $t_{1/2}$ (min)	t_∞ (min)
1a	420	960	96	360
1b	-	30 sec	1	4
1c	2	5	12	34
1d	20 sec	3	5	18
1e	90	273	32	150

a) These reactions were carried out with diastereomeric mixture of 2'-O-acetal uridine derivatives (**1**) at room temperature. The reactions were monitored by TLC and reversed phase TLC.

In order to study both internucleotidic cleavage and phosphoryl migration under the deprotection conditions, the dimer, UpU was treated first with ammonia and then with 0.01 N HCl (pH 2.0). The 5'-O-dimethoxytrityl derivatives (B=U, anC)[13] were phosphitylated with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite[14] and purified by chromatography on silica gel to give the corresponding phosphoramidite units (B=U, anC); ^{31}P-NMR ($CDCl_3$) 151.22, 151.10, 150.42 (B=U); 151.68, 151.25, 150.95, 150.14 (B=anC). The half-succinate of the 2',5'-protected nucleoside derivatives (B=U, anC) were attached to controlled-pore glass (CPG) in the usual method[15] to give the U- and anC-CPG



Scheme 2

(44 μmol and 39.5 μmol). The reaction was carried out on a U-CPG (0.5 μmol) in a column similar to that previously described.[16] The resin was first treated with 1.5% dichloroactetic acid in CH_2Cl_2 for 1.0 min to remove the 5'-DMTr group. To this resin, the phosphoramidite unit (B=U) (10 μmol) and 1H-tetrazole (20 μmol) dissolved in CH_3CN (150 μl) was added. After 15 min, the mixture was oxidized

with 0.1 M I_2 in THF-pyridine-H_2O (8:1:1, v/v) for 2 min. The resin was treated with conc. ammonia at room temperature for 8 h to remove the 2-cyanoethyl group and to release the dimer, UpU from resin. The 2',5'-protected dimer was dissolved in 0.01 N HCl and the pH adjusted to 2.0 by addition

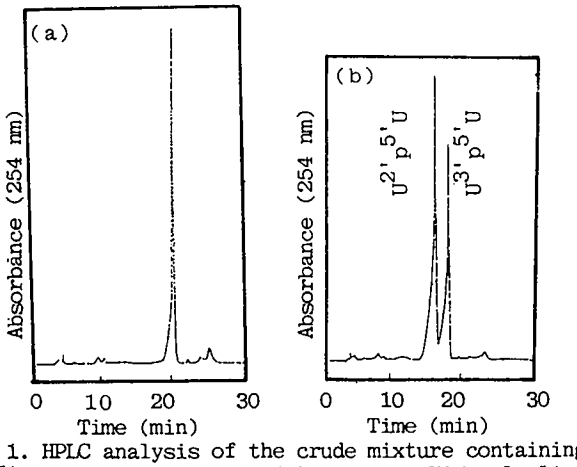


Fig. 1. HPLC analysis of the crude mixture containing U3'p5'U after deprotection (a), using a TSK gel oligo-DNA RP column with a linear gradient of 50% aqueous methanol (from 0 to 50% during 30 min) in 0.05 M ammonium phosphate (pH 7.0), and after addition of $U^{2'}p^{5'}U$ (b), using the same gradient.

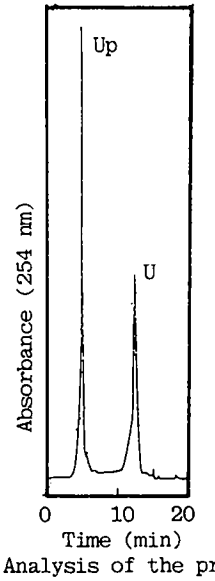


Fig. 2. Analysis of the products obtained after hydrolysis of UpU with ribonuclease T_2 on a TSK gel oligo-DNA RP column. Elution was performed with a linear gradient of CH_3CN (1-25%) in 0.05 M ammonium phosphate (pH 7.0). The flow rate was 1.0 ml/min.

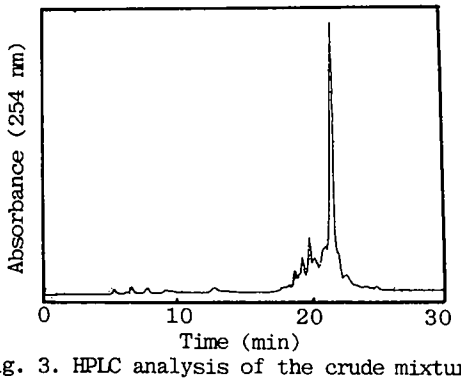


Fig. 3. HPLC analysis of the crude mixture containing C_{12} after deprotection using a TSK gel oligo-DNA RP column with a linear gradient of CH_3CN (from 5 to 25% during 30 min) in 0.1 M TEAA (pH 7.0).

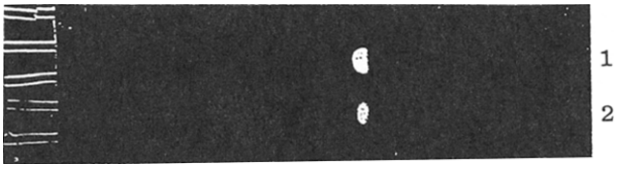


Fig. 4. 20% Polyacrylamide gel electrophoresis in 7 M urea of 12-mer. Lane 1: U_{12} chain length standard; Lane 2: C_{12}.

of 0.1 N HCl. The mixture was stirred at room temperature for 12 h and neutralized with dil ammonia, followed by reversed phase HPLC analysis (Fig. 1). Integration of the main peak in Fig. 1a reveals that the dimer, UpU account for 98% of the total absorbance at 254 nm. Further, no isomerization to uridylyl-(2'-5')-uridine can be detected under the deprotection conditions (Fig. 1b). The ratio of

Up and U were estimated by the reversed phase HPLC after digestion of UpU with ribonuclease T_2 and was found to be Up:U=1.00:1.04 (theoretical, 1.00:1.00) (Fig. 2). Under the condition of digestion, ribonuclease T_2 did not cleave a uridylyl-(2'-5')-uridine. No peak corresponding to UpU was observed in the chromatogram of the digests, indicating the absence of any 2'-5' internucleotidic bonds.

A dodecaribonucleotide, CCCCCCCCCCCC was synthesized by similar procedure on anC-CPG (0.3 mol). The oligomer was cleaved from CPG, deprotected first with ammonia at 55°C for 5 h, and then 0.01 N-HCl (pH 2.0) at room temperature for 20 h, and analyzed by the reversed phase HPLC (Fig. 3). A sample purified by the reversed phase HPLC was completely digested by ribonuclease T_2 and the ratios of Cp and C were judged from the reversed phase HPLC analysis. Furthermore, a sample thus obtained was characterized by polyacrylamide gel electrophoresis (Fig. 4). The overall yield from anC-CPG was 15% (3.8 A_{260}).

It is reasonable to conclude from the work described in this paper that the 1-(2-chloroethoxy)-ethyl group is likely to be suitable for the synthesis of olgoribonucleotides on solid supports.

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9. **1a:** UV λmax (MeOH) 263 nm, λmin (MeOH) 232 nm; ^{1}H-NMR (DMSO-d_6) δ 7.90 (dd, 1H, $J_{5,6}$=9 Hz, C-6), 5,72 (d, 1H, $J_{1',2'}$=6 Hz, H-1_1', 5.59 (d, 1H, $J_{5,6}$=9 Hz, C-5), 5.35-5.00 (m, 1H, HO-3'), 4.82 (d, 1H, CH), 4.20-3.68 (m, 3H, HO-5', H-2', H-3'), 3.75 (m, 1H, H-4'), 3.55 (br s, 6H, H-5', CH_2CH_2), 1.23 (dd, 3H, CH_3). Anal. Calcd for $C_{13}H_{19}N_2O_7Cl.1/2CH_3OH$: C, 44.22; H, 5.77; N, 7.64. Found: C, 43.99; H, 5.82; N, 7.62.
   1b: UV λmax (MeOH) 261 nm, λmax (MeOH) 230 nm, ^{1}H-NMR (DMSO-d_6)δ 8.10 (dd, 1H, $J_{5,6}$=9 Hz, C-6), 6.05 (d, 1H, $J_{1',2'}$=6 Hz, H-1'), 5.80 (d, 1H, $J_{5,6}$=9 Hz, C-5), 5.50-4.90 (m, 3H, OH-3', H-2', CH), 4.30 (m, 3H, OH-5', H-3', H-4'), 4.00 (m, 3H, $CH(CH_3)_2$, H-5'), 1.75 (d, 3H, CH_3), 1.15 (d, 6H, CH_3). Anal. Calcd for $C_{14}H_{22}N_2O_7.1/4CH_3OH$: C, 50.60; H, 6.98; N, 7.61. Found: C, 50.68; H, 7.20; N, 7.69.
   1c: UV λmax (MeOH) 262 nm, λmin (MeOH) 239 nm, ^{1}H-NMR (DMSO-d_6) δ7.95 (dd, 1H, $J_{5,6}$=9 Hz, C-6), 5.94 (d, 1H, $J_{1',2'}$=6 Hz, H-1'), 5.65 (d, 1H, $J_{5,6}$=9 Hz, C-5), 5.20-4.72 (m, 2H, OH-3', CH), 4.20-3.82 (m, 4H, OH-5', H-2', H-3', 4'-H), 3.65 (m, 2H, H-5'), 3.30 (m, 2H, CH_2), 1.30 (m, 7H, CH_2CH_2, $CHCH_3$), 0.81 (t, 3H, CH_3). Anal. Calcd for $C_{15}H_{24}N_2O_7.CH_3CH_2OH$: C, 52.97; H, 7.74; N, 7.17. Found: C, 52.70; H, 7.74; N, 7.41.
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12. The loss of the 2'-O-acetal groups of compounds **1b-e** were often observed under acidic condition required for the complete removal of the 5'-O-DMTr group.
13. The other 2'-O-Cee-ribonucleosides (anC, bzA, ibuG) can be prepared in 78-83% yields by similar procedure.
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(Received in Japan 16 June 1989)