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NUCLEOSIDES AND NUCLEOTIDES. 126. INCORPORATION OF A MUTAGENIC NUCLEOSIDE, 5-FORMYL-2'-DEOXYURIDINE, INTO AN OLIGODEOXYRIBONUCLEOTIDE[1]

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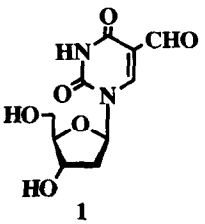
Abstract: An oligodeoxyribonucleotide (TT1TTT) containing 5-formyl-2'-deoxyuridine (**1**) has been synthesized. Protection of the formyl group of 3',5'-di-*O*-acetyl-5-formyl-2'-deoxyuridine (**3**) with *N,N*-di-(*m*-chlorophenyl)-ethylenediamine (**4d**) afforded the imidazolidine derivative **5d**, which was converted into the nucleoside 3'-phosphoramidite **7**. Incorporation of **7** into an oligonucleotide and then acid-hydrolysis gave the oligomer containing **1**. The nucleoside composition was confirmed after conversion of **1** in the oligomer into 5-hydroxymethyl-2'-deoxyuridine.

γ-Irradiation therapy has been widely used for the treatment of cancer patients. Strand breaks caused by the radiation are major lesions causing tumor cell death. Hydroxyl radicals appear to be responsible for a variety of sugar and base lesions in DNA and induction DNA-protein cross-links.[2] Kasai *et al.* reported that 5-formyl-2'-deoxyuridine (**1**) was newly isolated from γ-irradiated DNA molecules.[3] Among a number of modified nucleosides isolated from damaged DNA by γ-radiation, **1** also had potent mutagenic properties on *Salmonella* strain TA102.[3] They also reported the possibility of formation of DNA-protein cross-links in DNA molecules containing **1**.[3] Since **1** is known to form Schiff bases with amines,[4] the formation of **1** in DNA by the radiation is plausible to induce DNA-protein cross-links with histone. It seems to be possible that such oxidative damage of DNA molecules would cause mutagenesis and even carcinogenesis. Therefore, it is of importance to synthesize DNA oligomers containing **1** at a distinct position and examine properties of the oligomers.



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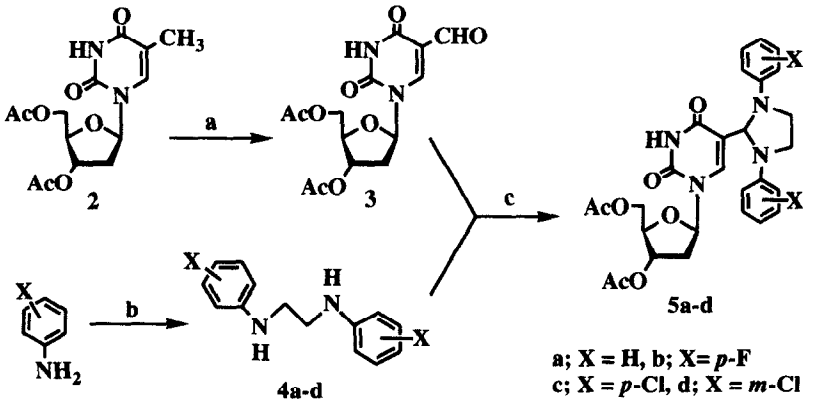
Chemical properties of 5-formyluridine have been studied under aqueous alkaline conditions. Armstrong *et al.* reported that 5-formyluridine was unstable under aqueous alkaline conditions and underwent anomerization.[5] It was also reported that **1** was unstable under conditions preparing its 5'-phosphate with phosphorus oxychloride in trialkyl phosphates.[6] We have also observed that **1** was unstable even at pH 8[7] and gave a complex mixture upon treatment with concentrated NH_4OH by HPLC analysis (data not shown). From these data, the formyl group in **1** would not be stable under conditions used for synthesis of oligonucleotides on a DNA synthesizer. Therefore, the formyl group of **1** should be protected to be incorporated into oligonucleotides. The protecting group must be stable not only in basic conditions, such as NH_4OH treatment, but also in acidic conditions used for deprotections of the DMTr group in cycles of DNA synthesis on a DNA synthesizer. Moreover, it must be removed at the final deprotection of the DMTr group. In this

communication, we describe a new protecting group for the formyl group of **1** and synthesis of a DNA hexamer, TT1TTT.

Scheme 1[a]



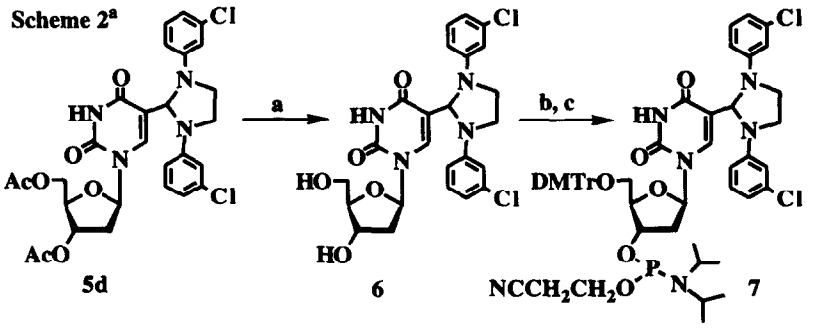
[a]a) $K_2S_2O_8$, $CuSO_4 \cdot 5H_2O$, 2,6-lutidine, aqueous 50% CH_3CN, 65 °C; b) 1,2-dibromoethane, EtOH, reflux; c) CSA, DMF, room temperature.

5-Formyl-2'-deoxyuridine (**1**) has been synthesized by a classical condensation method using 5-benzyloxymethyluracil or 5-formyluracil dimethyl acetal in a multi-step procedure[8] and by oxidation of the 5-methyl group of a thymidine derivative by bromine under photolysis[9] followed by hydrolysis. We adopted Ikeda's method with slight modifications.[10] 3',5'-Di-*O*-acetylthymidine (**2**) was heated with potassium peroxysulfate ($K_2S_2O_8$) in the presence of $CuSO_4$[11] and 2,6-lutidine in aqueous CH_3CN to give 3',5'-di-*O*-acetyl-5-formyl-2'-deoxyuridine (**3**) in 52% yield, which can be converted to a dimethyl acetal derivative followed by deacetylation by NaOMe and acidification with Dowex-50 (H^+) affording **1** in 92% from **3**.[8c]

The protection of the formyl group was next examined.[12] We adopted 1,3-diphenylimidazolidine derivatives, which could be obtained from the reaction of **1** or **3** with *N,N'*-diphenylethylenediamine derivatives.[13,14] Although this protecting group would be stable in basic conditions and can be removed by acid catalysts, the rate of hydrolysis under acidic conditions may be controlled by changing the basicity of the amino group by introduction of electron-withdrawing groups at the phenyl ring. Substituted *N,N'*-diphenylethylenediamine derivatives **4b-d** were prepared according to reference procedures with modifications.[13,15] Reactions of **3** with **4a-d** in DMF in the presence of DL-camphorsulfonic acid (CSA) afforded the desired protected nucleosides **5a-d** in good yields.[16] Stability of **5a-d** in 3% trichloroacetic acid in CH_2Cl_2 at room temperature was examined. However, none of the derivatives was stable under these conditions. Under 1% dichloroacetic acid in CH_2Cl_2, **5d** had sufficient stability (stable more than 5 min by TLC analysis). Additionally, the imidazolidine group was able to be hydrolyzed in aqueous 80% AcOH solution overnight at room temperature. Therefore, **5d** was converted into nucleoside 3'-phosphoramidite **7** as shown in Scheme 2 which was used for oligonucleotide synthesis.

The oligonucleotide, TT1TTT, was synthesized on a DNA synthesizer by the phosphoramidite method.[17] An average coupling yield of **7** was more than 95%. The fully protected oligonucleotide was treated with concentrated NH_4OH to give an oligonucleotide protected with the DMTr and the imidazolidine

groups, which was purified on a C-18 silica gel column with a linear gradient of CH_3CN on 0.1 M TEAA buffer. Before concentration of the mixture, de-salting by passing through a short C-18 column was necessary to prevent degradation. The de-salted oligomer was then treated overnight with aqueous 80% AcOH to remove the DMTr and the imidazolidine groups (Fig. 1a). The deprotected oligomer was purified by HPLC on a C-18 silica gel column with a linear gradient of CH_3CN in 0.1 M TEAA buffer and then desalted as above to give TT1TTT (2.5 OD units at λmax 254 nm from a 1 μmole scale synthesis). The oligonucleotide showed a single peak by HPLC with a C-18 column (Fig. 1b).



[a]a) NH_3/MeOH, room temperature, 83%; b) DMTrCl, pyridine, room temperature; c) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylamine, pyridine, 69% from **6**.

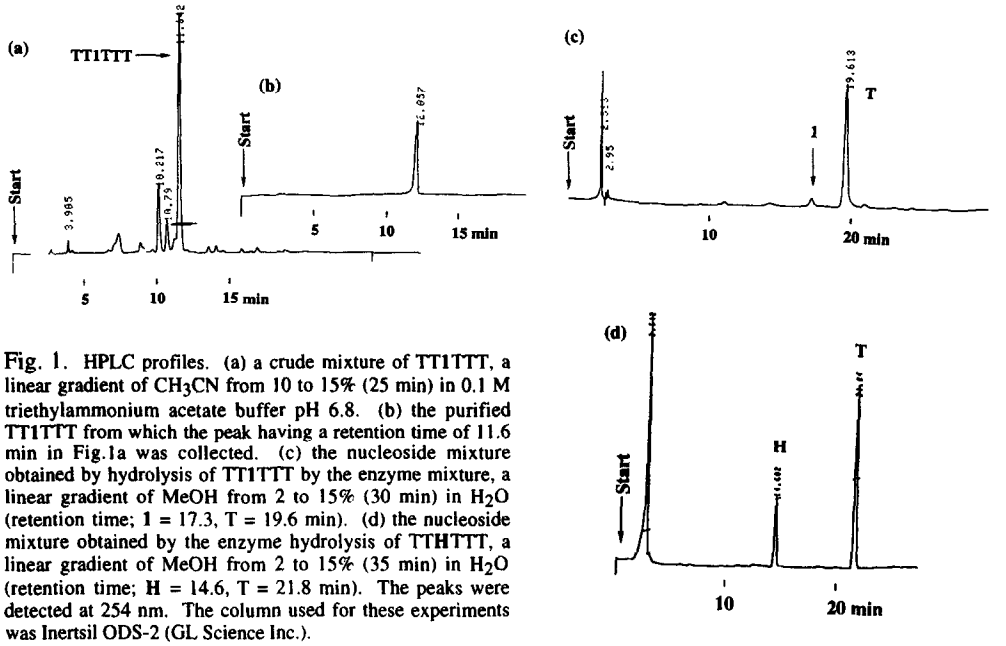


Fig. 1. HPLC profiles. (a) a crude mixture of TT1TTT, a linear gradient of CH_3CN from 10 to 15% (25 min) in 0.1 M triethylammonium acetate buffer pH 6.8. (b) the purified TT1TTT from which the peak having a retention time of 11.6 min in Fig.1a was collected. (c) the nucleoside mixture obtained by hydrolysis of TT1TTT by the enzyme mixture, a linear gradient of MeOH from 2 to 15% (30 min) in H_2O (retention time; **1** = 17.3, T = 19.6 min). (d) the nucleoside mixture obtained by the enzyme hydrolysis of TTHTTT, a linear gradient of MeOH from 2 to 15% (35 min) in H_2O (retention time; **H** = 14.6, T = 21.8 min). The peaks were detected at 254 nm. The column used for these experiments was Inertsil ODS-2 (GL Science Inc.).

To confirm the presence of **1** in the oligomer, it was completely hydrolyzed by a mixture of venom phosphodiesterase and alkaline phosphatase[18] to the corresponding nucleosides. As shown in Fig. 1c, a peak

area corresponding to **1** was too small to be expected. A part of **1** might react with these enzymes during the hydrolysis. Therefore, before the complete hydrolysis, **1** in the oligomer was reduced by $NaBH_4$ to afford an oligodeoxynucleotide containing 5-hydroxymethyl-2'-deoxyuridine (**H**). Complete hydrolysis of the oligomer containing **H** under the same conditions gave peaks corresponding to **H** and thymidine in a ratio of 1:5 analyzed by HPLC as shown in Fig. 1d. This experiment clearly showed that the unstable 5-formyl-2'-deoxyuridine can be incorporated into the oligodeoxynucleotide using the new protecting group. Synthesis of more complicated oligodeoxynucleotides and their properties are now under investigation and we will report shortly.

References and Notes

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16. A mixture of **3** (170 mg, 0.5 mmol), **4d** (210 mg, 0.75 mmol), and CSA (5 mg) in DMF (5 mL) was stirred for 12 h at room temperature. The reaction mixture was diluted with AcOEt. The whole was washed with aqueous sat. $NaHCO_3$ and H_2O, and dried (Na_2SO_4) and concentrated. The residue was purified on a silica gel column to give **5d** (254 mg, 84% as a solid): EI-MS *m/z* 602 (M^+-1); UV λmax (MeOH) 261 nm. *Anal.* Calcd for $C_{28}H_{28}Cl_2N_4O_7$: C, 55.73; H, 4.68; N, 9.28. Found: C, 55.74; H, 4.80; N, 9.10.
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