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New Synthesis of 5-Carboxy-2'-deoxyuridine and Its Incorporation into Synthetic Oligonucleotides

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New Synthesis of 5-Carboxy-2'-deoxyuridine and Its Incorporation into Synthetic Oligonucleotides

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ABSTRACT

5-Carboxy-2'-deoxyuridine is a methyl oxidation product of thymidine. It can be formed by the menadione-mediated photosensitization of thymidine in aerated aqueous solution. Here in we present a new four-step synthesis of the 5-carboxy-2'-deoxyuridine phosphoramidite building block based on the alkaline hydrolysis of 5-trifluoromethyl-2'-deoxyuridine. The phosphoramidite derivative has been incorporated at defined sites into oligonucleotides using the solid phase synthesis approach.

5-Carboxy-2'-deoxyuridine (CdU) is an oxidized lesion of thymidine that can be formed by menadione-mediated photosensitization of thymidine.^[1] Therefore, it is important to evaluate the possible biological effects of CdU. For assessing the structural and biological features of this lesion, the use of site-specific modified oligonucleotides appears to be a good approach. Recently, a synthesis has been

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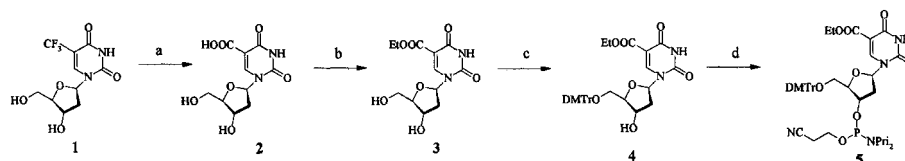


Figure 1. a: CF₃dT, H₂O, NaOH pH10, 37 °C, 36 h, 90% ; b: HOBT, DCCI, Bu₃N, EtOH anhydre, 15 h, 75% ; c: DMTrCl, DMAP, pyridine, 23 h, 55% ; d: 2-Cyanoethyl-diisopropyl-chlorophosphoramidite, DIEA, CH₂Cl₂, 3 h under argon atmosphere, quantitative yield.

described.^[2] Up to now no biological data have been reported in the litterature. Here in we report a new method to synthesize 5-Carboxy-2'-deoxyuridine-containing oligonucleotides.

The phosphoramidite derivative of the protected ethyl ester of 5-carboxy-2'-deoxyuridine **5** was prepared from 5-trifluoromethyl-2'-deoxyuridine **1** in 4 steps (Fig. 1). Thus, alkaline hydrolysis of commercially available 5-trifluoromethyl-2'-deoxyuridine **1**^[3] followed by neutralization with 1M HCl and desalting on a cation exchange Dowex resin gave 5-carboxy-2'-deoxyuridine **2**.^a The carboxyle function was then esterificated by an ethyl group as described.^[2] The last two steps are standard dimethoxytritylation and phosphitylation.

Using compound **5**, 5-carboxy-2'-deoxyuridine-containing oligonucleotides were synthesized using an automated DNA synthesizer according to the standard β-cyanoethyl phosphoramidite chemistry. After deprotection with 0.1M NaOH at room temperature for 24 h, the oligonucleotides were purified using electrophoresis on denaturing polyacrylamide gel containing 8M urea and then desalted.

The synthetic oligonucleotides were characterized by ESI-MS and MALDI-TOF MS analyses. The enzymatic digestion of the oligonucleotides followed by HPLC-MS/MS analysis has been performed, confirming the presence and the integrity of 5-Carboxy-2'-deoxyuridine into the oligonucleotides.^b

^a**2**: ESI-MS (negative mode): $[M - H]^- = 270.9 \pm 0.1$ Da. ¹H NMR (200 MHz, D₂O) δ 9.01 (s, 1H, H-6); 6.33 (t, 1H, H-1'); 4.55 (m, 1H, H-3'); 4.17 (m, 1H, H-4'); 3.98 and 3.85 (m, 2H, H-5' and H-5''); 2.62 and 2.48 (m, 2H, H-2' and H-2''). **3**: ESI-MS (positive mode): $[M + H]^+ = 301.1 \pm 0.1$ Da, $[M + Na]^+ = 323.2 \pm 0.1$ Da. **4**: ESI-MS (positive mode): $[M + H]^+ = 624.9 \pm 0.1$ Da, $[M + Na]^+ = 646.9 \pm 0.1$ Da. **5**: ESI-MS (positive mode): $[M + H]^+ = 803.8 \pm 0.1$ Da, $[M + Na]^+ = 825.8 \pm 0.1$ Da.

^bThe following 22-mer-(5-carboxy-2'-deoxyuridine-containing)-oligonucleotide was synthesized: 5' CAC TTC GGA XCG TGA CTG ATC T 3', with X = CdU. Purity was checked by ESI-MS analysis, MALDI-TOFF MS analysis (calculated MW = 6731.4 Da, found MW (negative mode) = 6731.1 Da) and enzymatic digestion followed by HPLC-MS/MS analysis. All the analyses confirm the purity of the oligonucleotide together as well as the presence and the integrity of CdU **2**.

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