

Synthesis of a Novel 2'-Deoxyuridine Derivative Bearing a Cyanomethoxycarbonylmethyl Group at C-5 Position and Its Use for Versatile Post-Synthetic Functionalization of Oligodeoxyribonucleotides

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Received 20 February 1998; revised 26 March 1998; accepted 27 March 1998

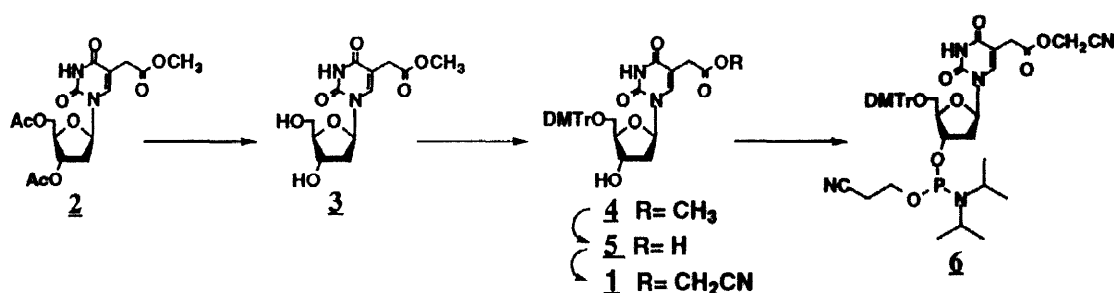
Abstract: A novel 2'-deoxyuridine derivative bearing a cyanomethoxycarbonylmethyl group at the C-5 position (**1**) was synthesized, and its use was examined as a convertible nucleoside for the versatile post-synthesis functionalization of oligodeoxyribonucleotides (ODNs). The ODNs containing **1** reacted with primary monoamines such as heptylamine, histamine, and tyramine as well as di- and polyamines under mild conditions, giving the corresponding derivatized ODNs. © 1998 Elsevier Science Ltd. All rights reserved.

Modified oligonucleotides (ODNs) with functional molecules such as fluorophores, enzymes, biotin, catalysts, etc., have attracted great interest in recent years as an inhibitor of specific gene expression and as a probe for the detection and biophysical studies of nucleic acids.¹ In most cases, the synthesis of modified ODNs is accomplished by incorporation of a nucleoside bearing a modified base. Modification of the nucleoside base has been generally performed prior to the preparation of the ODN. This pre-synthetic modification necessitates the preparation of a variety of modified monomers, some of which require multi-step synthesis, and the individual synthesis of a series of the modified ODNs takes much time. On the other hand, post-synthetic modification of ODNs, in which various functional groups are introduced into the ODN after DNA synthesis, enables the preparation of a series of modified ODNs at one time.

The C-5 position of pyrimidine in the ODNs is not involved in hydrogen bonding and faces out of the major groove when duplexes are formed with the complementary oligonucleotides. Therefore, the C-5 position of 2'-deoxyuridine is an appropriate site for the introduction of a functional molecule into ODNs either by a pre- or post-synthetic method. Matsuda *et al.* reported the synthesis of ODNs containing 5-methoxycarbonyl- or 5-trifluoroethoxycarbonyl-2'-deoxypyrimidine and post-synthesis derivatization of the ODNs by the reaction with ethylenediamine or hexamethylenediamine, furnishing the corresponding ODNs carrying the amino-linkers at the C-5 position.² The amino-linker was further utilized for the preparation of functionalized ODNs by an appropriate amine substitution reaction with a functional molecule such as an intercalator. Previously, we reported a facile synthesis of a 5-methoxycarbonylmethyl-2'-deoxyuridine from arabinosaminooxazoline,³ and incorporation of this nucleoside into ODNs which react with polyamines giving the corresponding amine-modified ODNs.⁴ Reactivity of 5-methoxycarbonyl, 5-trifluoroethoxycarbonyl, and 5-methoxycarbonylmethyl groups with monoamines, however, is low. Therefore, these methods are not applicable for the direct synthesis of functionalized ODNs by a single-step reaction with monoamine-bearing functional molecules. We have explored a reactive 2'-deoxyuridine derivative which can be used as a convertible monomer unit for the modified ODNs synthesis. We have found that 5'-O-(4,4'-dimethoxytrityl)-5-cyanomethoxycarbonylmethyl-2'-deoxyuridine (**1**) can be introduced into ODN under the conventional DNA synthesis conditions and the resulting ODN on a solid support reacts easily with monoamines as well as di- and polyamines under mild conditions,

giving the various modified ODNs. Here, we report the synthesis of **1** and its use for the post-synthesis modification of the ODNs with monoamines with functional groups, as well as polyamines.

Synthesis of a derivative of **1** for ODN synthesis is outlined in Scheme 1. 5-Methoxycarbonylmethyl-3',5'-di-O-acetyl-2'-deoxyuridine (**2**) was prepared from arabinosaminooxazoline by the method described previously.³ Deacetylation of **2** in methanol solution in the presence of sodium methoxide at room temperature for 1 hr afforded a 2'-deoxyuridine derivative **3** in 80 % yield. The 5'-hydroxyl group of **3** was protected with a dimethoxytrityl group by the conventional method giving **4** in 91 % yield. The methoxycarbonylmethyl group in **4** (0.55 g, 0.91 mmol) was hydrolyzed in the reaction mixture containing THF (2 ml) and 1.0 N lithium hydroxide solution (10 ml) at room temperature for 1 hr. The resulting carboxylic acid **5** was extracted with



Scheme 1. Synthesis of 5-cyanomethoxycarbonylmethyl-2'-deoxyuridine derivative for modified ODN synthesis

dichloromethane after adjusting the pH of the aqueous solution to 4.0 and used for cyanomethyl esterification without further purification. To a solution of **5** in hexamethylphosphortriamide (30 ml), bromoacetonitrile (0.13 g, 1.1 mmol) and potassium carbonate (0.15 g, 1.1 mmol) were added and the reaction mixture was stirred at room temperature for 6 hr. The nucleoside **1**⁵ was obtained in 70 % yield by usual work up. Longer reaction time or the use of chloroacetonitrile and triethylamine instead of bromoacetonitrile and potassium carbonate promoted the cyanomethylation at the N-3 position of the uridine moiety, resulting in low yield of **1**. The cyanomethoxycarbonylmethyl group in **1** is stable under the conventional DNA synthesis condition of phosphoramidite chemistry, and **1** can be stored in a desiccator for a long period at room temperature. The nucleoside **1** was phosphitilated according to a standard procedure to give **6** in 86 % yield.

Oligodeoxyribonucleotides were prepared by using commercially available phosphoramidites and **6** on a DNA synthesizer. An acetyl group was used for the protection of the amino group of cytosine instead of a benzoyl group to prevent transamination to a cytosine residue of ODN.⁶ The coupling period for **6** was 60 sec used for the normal nucleoside phosphoramidite and the coupling yield was over 98 %. We at first examined the optimum condition for the introduction of a monoamine to the ODNs using heptylamine and the trimer XTT on CPG support, where X is the 2'-deoxyuridine derivative bearing a cyanomethoxycarbonylmethyl group. XTT on CPG was treated with heptylamine in THF for 1 hr at room temperature. The support was then treated with 28% ammonia solution at room temperature for 1 hr for the cleavage of the trimer from the support. The resulting trimer was analyzed by HPLC. The effect of the heptylamine concentration for the aminolysis of the cyanomethoxycarbonylmethyl group on the ODN is shown in Figure 2a. 0.6 M heptylamine solution was required for the complete aminolysis in the absence of a catalyst. Triazole worked as an acid-base catalyst and promoted the aminolysis of the active ester in the ODN with 0.2 M heptylamine as shown in Figure 2b, while imidazole, 4-dimethylaminopyridine and 1-methylimidazole had little effect on the aminolysis. Tetrazole, on the other hand, inhibited the aminolysis because of its acidic character.

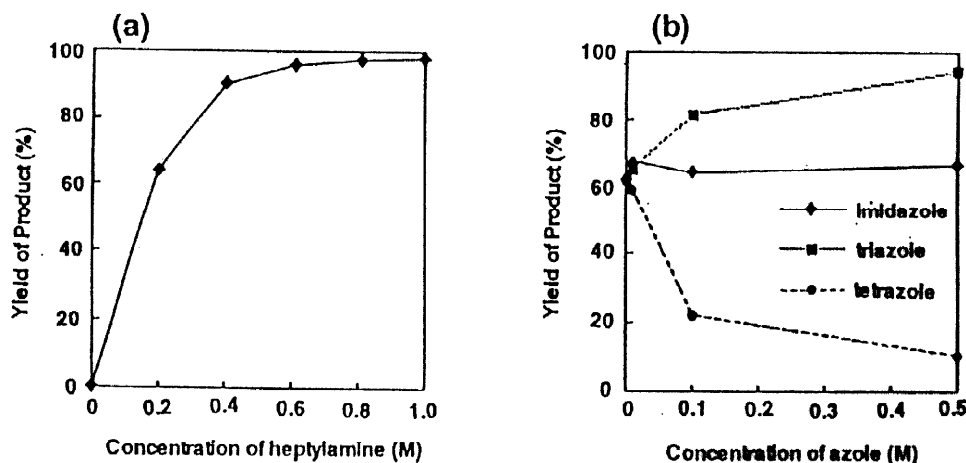
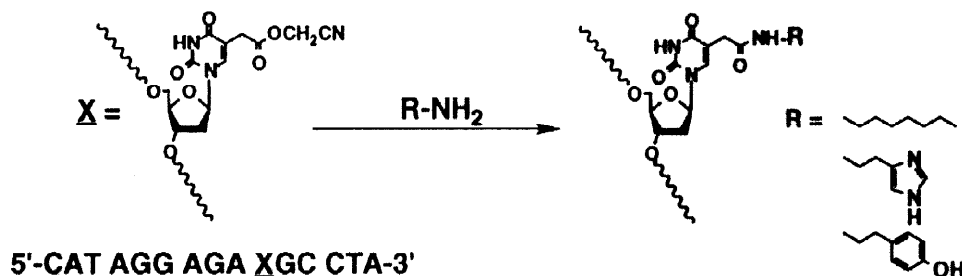


Figure 1. Effect of heptylamine concentration (a) and catalyst (b) on the introduction of heptylamine into the ODN on CPG support. (a) XTT on CPG support was treated with 0-1.0 M heptylamine in THF for 1 h at 20°C. (b) XTT on CPG was treated with 0.2 M heptylamine in THF in the presence of azoles as a catalyst under the condition described above.

The ODN 15mer, CATAGGAGAXGCCTA which is complementary to the *rev* region of HIV mRNA, on CPG support was treated with 1.0 M heptylamine in THF at room temperature overnight, then with ammonia solution for cleavage and deprotection and purified by C-18 HPLC. The HPLC of the ODN after deprotection of the dimethoxytrityl group with 20 % acetic acid at room temperature revealed the formation of ODN bearing a heptyl group as a single product. The presence of the derivatized nucleoside in the ODN was confirmed by enzyme digestion of the ODN with nuclease P1 and alkaline phosphatase, followed by HPLC analysis. The nucleoside composition analyzed by the HPLC is consistent with the desired ODN as shown in Table 1. We further conducted introduction of histamine or tyramine into the ODN 15mer. An imidazole pendant of histamine



Scheme 2. Introduction of functional amines into ODNs containing cyanomethyl ester at C-5 position of 2'-deoxyuridine derivative

attached to the ODN is expected to catalyze cleavage of the complementary target RNA,⁷ and tyramine in the ODN can be labeled with ¹²⁵I by a conventional chloramine-T method.⁸ Introduction of histamine into the ODN was achieved by treatment of the ODN on CPG with 1.0 M histamine in THF at room temperature overnight. For the reaction of the ODN with tyramine, the ODN on the support was treated with saturated tyramine in *N,N*-dimethylacetamide (DMAc) in the presence of 2.0 M triazole for 5 days, as tyramine has poor solubility in organic solvent and poor reactivity. Deprotection and purification of the derivatized ODN as described above gave the ODN functionalized with histamine or tyramine as a sole product. The nucleoside compositions of the ODNs analyzed by HPLC after enzyme digestion of the ODNs are also shown in Table 1. The ODN bearing tyramine was further labeled with ¹²⁵I, giving a possible probe for *rev*-mRNA of HIV.

Table 1. Yields and nucleoside compositions of the functionalized ODN by post-synthesis derivatization

Amines	Concentration (M)	Isolated yield (OD)	Retention time of HPLC (min)	Nucleoside composition ^f A : G : C : T : X ^{amine}
5'-d-CATAGGAGAXGCCTA				
Heptylamine	1.0	57 ^a	28.3	5.0 : 4.3 : 2.8 : 2.0 : 1.0
Histamine	1.0	54 ^a	21.9	4.8 : 3.9 : 3.0 : 2.3 : 0.9
Tyramine	0.5	4.3 ^b	18.1	5.0 : 3.7 : 3.0 : 2.2 : 1.0
5'-d-CGCXTCTTCCTGCCA				
tris(aminoethyl)amine	0.5	8.1 ^b	19.2	0.8 : 1.9 : 7.5 : 3.8 : 1.0
pentamethylenediamine	1.0	6.0 ^b	17.9	0.8 : 1.7 : 7.3 : 4.3 : 1.0

The reaction was carried out at 20°C for 1 day in THF without catalyst, except for the reaction with tyramine in which the reaction was carried out at 20°C for 5 days in DMAc in the presence of 2.0 M triazole. a. The reaction was carried out in a 1 µmol scale.

b. The reaction was carried out in a 0.2 µmol scale. c. The modified nucleosides X^{amine} were confirmed by comparison of retention time of HPLC with those of the authentic samples prepared from 1 and the corresponding amines.

Similarly, treatment of the ODN15mer, CGGXTCTTCCTGCCA, with tris(aminoethyl)amine or pentamethylenediamine in THF gave the modified ODN bearing the corresponding polyamine at the C-5 position of the uracil component. The derivatized ODNs with several functional molecules are listed in Table 1. When ODN bearing a methoxycarbonylmethyl group at the C-5 position of deoxyuridine was used as a control, no introduction of heptylamine, histamine or tyramine into the ODN could be accomplished under the same conditions, although di- and polyamines could be introduced easily. Thus, the use of a deoxyuridine derivative bearing a cyanomethoxycarbonylmethyl group as a convertible monomer enables versatile post-synthesis derivatization of the ODNs with various functional molecules having a primary amine.

In conclusion, a 2'-deoxyuridine derivative bearing a reactive cyanomethoxycarbonylmethyl group at the C-5 position was synthesized and incorporated into ODNs, which can be derivatized to a number of modified ODNs by reaction with various functional amines under mild conditions simultaneously.

Acknowledgment. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture, Japan.

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5. 1; mp 94-97 °C. ¹H-NMR(CDCl₃) δ 2.41-2.60(4H, m, H2' and 5-CH₂-), 3.48(2H, qq, H5'), 3.80((6H, s, OCH₃), 4.07(1H, q, H4'), 4.60(2H, s, OCH₂CN), 4.65(1H, q, H3'), 6.47(1H, t, H1'), 6.82-7.35(13H, m, DMTr) and 7.86(1H, s, H6). ESI-Mass m/z 626.4 (M-H⁺, 626.7 Calcd for C₃₄H₃₂N₃O₉).
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